12th BALKAN CONGRESS OF HUMAN GENETICS
8th NATIONAL CONFERENCE FOR RARE DISEASES

8-10 September 2017
Grand Hotel Plovdiv, Bulgaria

CONFERENCE PROCEEDINGS

PLATINUM SPONSOR

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КОГАТО НОВИТЕ ТЕХНОЛОГИИ СРЕЩНАТ ГОЛЕМИТЕ ИДЕИ

Illumina е американска компания, чиято дейност е съсредоточена в новогенерационните решения в сферите на геномните проучвания, онкологията, репродуктивното здраве, геномика на комплексните заболявания, микробиалната геномика, агригеномика, съдебна медицина и др. Днес компанията е световен лидер в една индустрия, която е в пресечната точка на биологията и технологията. На най-основно ниво, дава се възможност да се прочитат и разбират генетични вариации. Illumina се стреми да направи своите решения все по-прости, по-достъпни, и винаги надеждни. В резултат на това, открития, които бяха немислими преди няколко години вече стават рутинни и проправят своя път в лечението на пациента.

Развитието на новогенерационното (NGS) портфолио в сферата на онкологията помага на Illumina да прави революция в онкогеномиката. Нашите NGS и микрочипови технологии са сред най-надеждните в света, осигуряват отлично качество и възпроизводими резултати.

Новогенерационните и микрочипови технологии представляват златен стандарт в качеството по цял свят и представляват ~ 90% от данните получени при секвениране. Иновативни, интуитивни, и напълно интегрирани, нашите решения дават възможност на всяка лаборатория да води напред в кривата на бързо развиващата се персонализирана медицина.

ЕЛТА 90 М е ексклузивен представител за България , Румъния, Сърбия и Македония на Illumina Inc.

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CONFERENCE PROCEEDINGS

Under the auspices of

Comittee on Healthcare to the 44th National Assembly of the Republic of Bulgaria

12-ТИ БАЛКАНСКИ КОНГРЕС ПО ГЕНЕТИКА НА ЧОВЕКА
8-МА НАЦИОНАЛНА КОНФЕРЕНЦИЯ ЗА РЕДКИ БОЛЕСТИ

8-10 септември 2017 г.
Гранд Хотел Пловдив

СБОРНИК
С ПРЕЗЕНТАЦИИ И ПОСТЕРИ

ПОД ПАТРОНАЖА НА

Комисия по здравеопазване към 44-то Народно събрание

ПЛАТИНЕН СПОНСОР

ЗЛАТЕН СПОНСОР

СРЕБЪРНИ СПОНСОРИ

МЕДИЕН ПАРТНЬОР

ISSN 1314-3581

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Organizer

Bulgarian Society of Human Genetics and Bulgarian Association for the Promotion of Education and Science

12th Balkan congress of Human Genetics and 8th National Conference for Rare diseases is under the auspices of Comittee on Healthcare to the 44th National Assembly of the Republic of Bulgaria

Committee

Corr. Member, Prof. Draga Toncheva, MD, PhD, Conference co-chair
Prof. Rumen Stefanov, MD, PhD, Conference co-chair
Assoc. Prof. Lyudmila Angelova, MD, PhD, Conference vice-chair
Vladimir Tomov, MSc, Conference vice-chair
Assoc. Prof. Vili Stoyanova, MD, PhD, Conference secretary
Evelina Popova, MSc, Conference secretary

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Rada Staneva
Dragomira Nikolova
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Aleksandra Ivanova
Anita Bozhikova
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Elisaveta Naumova	Lilyana Grozdanova	Radka Tincheva	Veselina Goranova
		Radka Stoeva	Vyara Shumnalieva

Dear colleagues,

Welcome you to 12th Balkan Congress of Human Genetics and to 8th National Conference on Rare Diseases and Orphan Drugs, from September 8th – 11th in Plovdiv, Bulgaria.

12th Balkan Congress of Human Genetics will present the newest achievements in the field of dysmorphology genetics, neurogenetics, reproductive genetics, prenatal genetics, cancer genetics and precision medicine. Highlights also include the current developments in omics technologies and their application in everyday medicine. We welcome you to share your outstanding research, new ideas and scientific experience!

8th National Conference for Rare Diseases and Orphan Drugs will present innovations and trends in personalized and genomic medicine. This joint event is an excellent occasion for new contacts at regional and European level. 2017 is a year of new horizons for rare diseases. 24 reference networks for rare and complex diseases are now officially operating within the European Union. These are new opportunities for improved diagnosis, treatment and follow up of patients with rare diseases. These are new prospects for integrative approach and international cooperation in Bulgaria, the Balkans and Europe.

Plovdiv is one of the cities with richest history in Europe. The city is a place, where history, culture and tradition meet science, technology and innovation. This makes it a perfect home for the 12th Balkan Congress of Human Genetics and the 8th National Conference for Rare Diseases and Orphan Drugs. Plovdiv is located in the heart of Bulgaria and is well known for its Roman theater and picturesque old town with narrow cobblestone streets and historical architecture that will send you back in time! You will be able to experience rich culture, traditional arts and world renowned cuisine and wines. The city is also hosting an International Technical Fair known for the presentation of innovations and new technologies, which is why it is considered the largest technology meeting in the Balkans.

We hope to make together the 12th Balkan Congress of Human Genetics and the 8th National Conference for Rare Diseases and Orphan Drugs a highly inspirational meeting that should not be missed.

Corr. Member, prof. Draga Toncheva, MD, PhD
Prof. Rumen Stefanov, MD, PhD

CONTENT

OPENING SESSION

Moderators: Draga Toncheva, Rumen Stefanov

- **Development of genomic medicine in Bulgaria**
  D. Toncheva

- **State-of-art of rare disease policy in Bulgaria and the EU**
  R. Stefanov

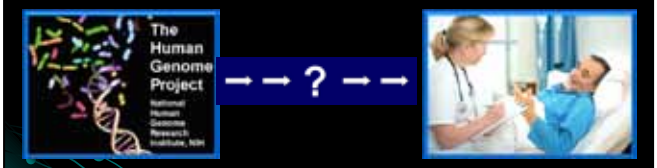
- **Rare diseases and genomic medicine – patients' perspective**
  V. Tomov

- **Stem cell application for osteoarthritis in the knee joint**
  D. Primorac

DEVELOPMENT OF GENOMIC MEDICINE IN BULGARIA

Draga Toncheva

The Path to Genomic Medicine



NHGRI – new vision for GM from base pairs to bedside.

Beyond the HGP

Human Genome Sequenced for the First Time by the Human Genome Project

1

Five Domains of Genomic Research

Structure of Genomes

Biology of Genomes

Biology of Diseases

Science of Medicine

1

Effectiveness of Healthcare

Genomic Medicine

An emerging medical discipline that use genomic information about the individual as part of their clinical care (e.g., for diagnosis or therapeutic decision)

Cost of sequencing

Human Genome Sequenced for the First Time by the Human Genome Project

1

Cost of sequencing a human genome has been reduced nearly 1 Million-Fold

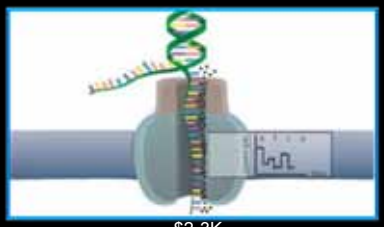
2

How quickly we can sequence genomes

Human Genome Project (1st Sequence)

~$ 1B
~6-8 years

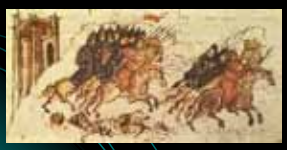
Today

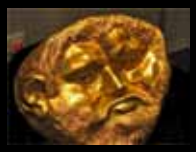


~$2-3K
~3 days

D.Nesheva

Thracians and Proto-Bulgarians





Beyond the HGP

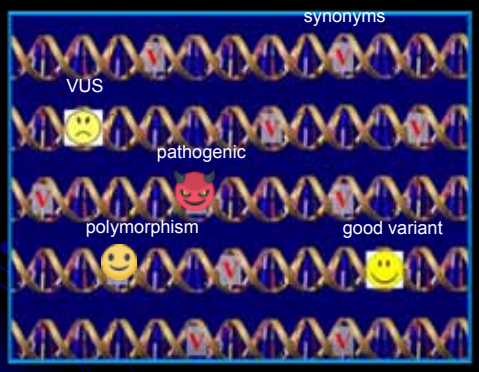
1. Human Genome Sequenced for the First Time by the Human Genome Project
2. Cost of sequencing a Human Genome Reduced Nearly 1 Million-Fold
3. Tens of thousands of HG have already been sequenced

- ❑ 2500 genomes
- ❑ 26 populations
- ❑ 90 million sites in the human genome
- ❑ 3 billion human genetic variations

ARTICLE

A global reference for human genetic variation

Genetic Variants (SNPs)



Sena Karachanak

Bulgarians vs European and other populations

tagSNPs

10 million

500,000

Your Genome by the number

- ❑ 6B nucleotides
- ❑ 3-5M SNPs
- ❑ 150K - not in databases
- ❑ 60 not in either parent

Beyond the HGP

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4. Advances in Understanding How the Human Genome Functions

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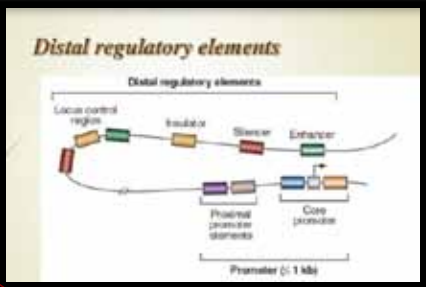
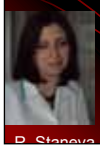
3. Tens of Thousands of Human Genomes Sequenced
4. Profound Advances in Understanding How the Human Genome Functions
5. Significant Advances in Unraveling the Genomic Bases of Human Disease

Common Diseases

Non-coding Functional Sequences





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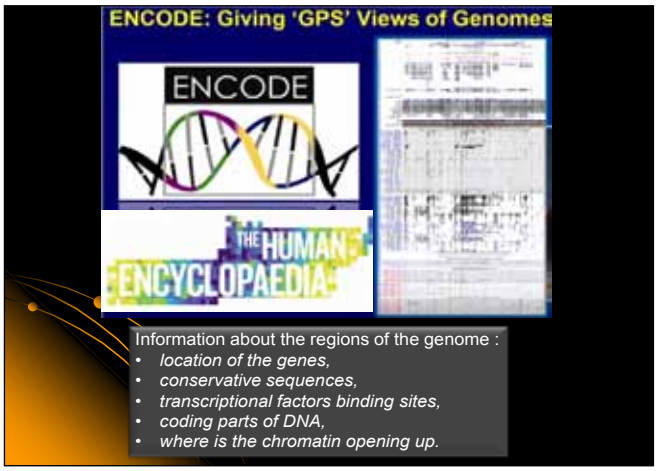
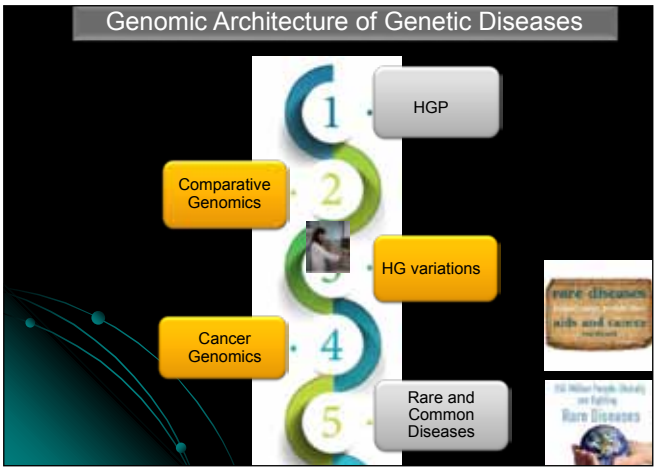
5. Significant Advances in Unraveling the Genomic Bases of Human Disease
6. Genomic Medicine is coming into focus



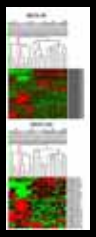
Genomic Architecture of Genetic Diseases



"Hot Areas" in Genomic Medicine

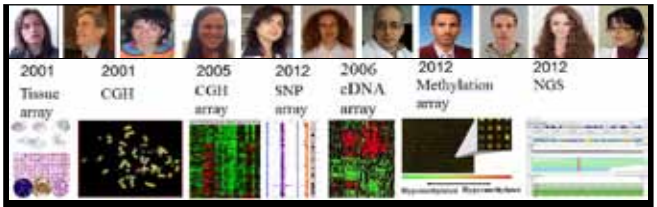
Cancer Genomics

D. Nikolova



Rare Diseases

EURORDIS
Rare Diseases Europe



"Hot Areas" in Genomic Medicine

Genomics of Pregnancy

S. Hadjidekova

L. Balabanski

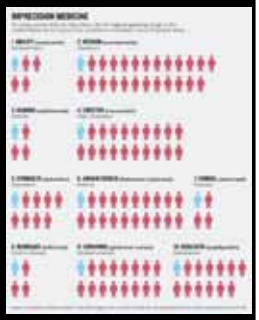
"Hot Areas" in Genomic Medicine

Cancer Genomics

Pharmacogenomics

O. Boyanova

Z.Hammoudeh



- The person in blue is the person where the medicine works.
- The people in red are people where the medicine doesn't work

"Hot Areas" in Genomic Medicine

Cancer Genomics

Pharmacogenomics

Rare Genetic Diseases Diagnostics

Genomics of Pregnancy

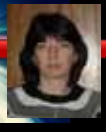
Clinical Genomics Informational System

"Hot Areas" in Genomic Medicine

Cancer Genomics

Pharmacogenomics

Rare Genetic Diseases



R. Vazharova

7,000 rare diseases → 4,300 genes

2014 Precision Medicine Initiative

Precision Medicine

Today: most medical care are based on expected response of the average patient

Tomorrow: medical care will be based on individual genomic, environmental and lifestyle differences that enable more precise ways to prevent and treat diseases

J. Koeva

PRECISIONMEDICINE
ANEWERA

R. Staneva

- ❑ > 1 million U.S. volunteers - they are going to be partners
- ❑ Participants to share genomics data, lifestyle information, biological samples
- ❑ New model for doing science

STATE-OF-ART OF RARE DISEASE POLICYIN BULGARIA AND THE EU

Rumen Stefanov

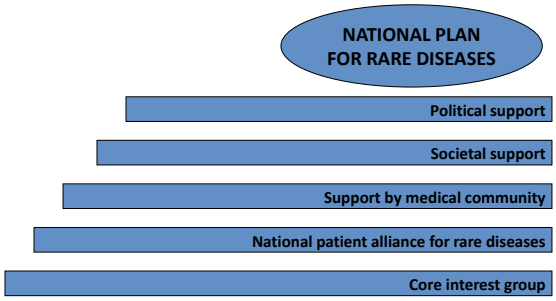
EU RARE DISEASE POLICY BODIES

- **On rare disease policy: EU Commission Expert Group on Rare Diseases**
- **On ERNs: Board of Member States on ERNs**
- **On research and development: International Rare Disease Research Consortium (IRDiRC) – a joint activity with NIH (USA)**

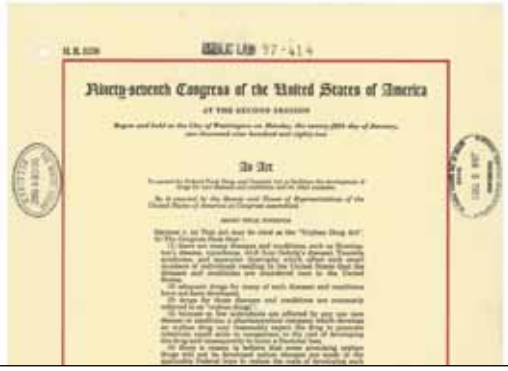
RARE DISEASES IN THE BALKAN REGION: MANY COUNTRIES, COMMON PROBLEMS

- **National health systems facing new challenges**
  - Transition to market economy
  - European adhesion and integration
  - Civil society engagement
- **New rare disease realities**
  - Increased visibility of rare disease patients
  - Improved awareness of medical professionals
  - Right to equal right to adequate and quality health care

BUT SUCCESS DEPENDS ON LOCAL EFFORTS



THE BEGINNING OF RARE DISEASE POLICY – ORPHAN DRUG ACT OF 1983


Ninety-seventh Congress of the United States of America

BUT SUCCESS DEPENDS ON LOCAL EFFORTS

NATIONAL PLAN FOR RARE DISEASES

- Bulgaria, Greece, Croatia, Slovenia, Bosnia and Herzegovina, Macedonia and Montenegro: **all have adopted a national plan/programme/strategy for rare diseases**
- Denmark, Finland, Norway, Sweden: **no officially adopted national plan/programme/strategy for rare diseases**
- A Bulgarian rare disease patient: "I do not care if we have a national plan. Denmark does not have a national plan. I want the quality of health care as in Denmark!"

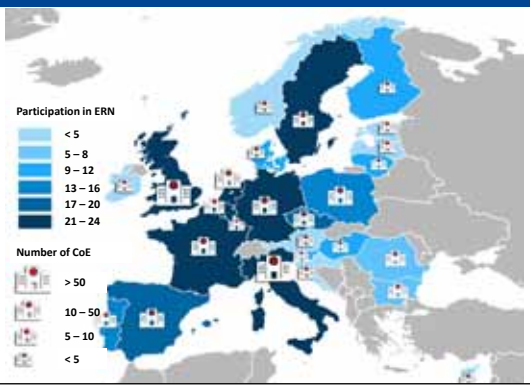
MILESTONES IN THE EU RARE DISEASE POLICY

- **Regulation (EC/141/2000) of the European Parliament and of the Council on orphan medicinal products**
- **Communication on rare diseases: Europe's challenges COM (2008) 679**
- **Recommendation on an action in the field of rare diseases (2009/C 151/02)**
- **Directive (2011/24/EU) of the European Parliament and of the Council on the application of patients' rights in cross-border healthcare**
- **Implementation report on the Commission Communication and Council Recommendation on rare diseases COM(2014) 548**

NEWEST EU RARE DISEASE POLICY DEVELOPMENT

- **On 15 December 2016 ERN Board of Member States approved 24 ERNs**

NEWEST EU RARE DISEASE POLICY DEVELOPMENT



TRANSPOSITION OF EU DIRECTIVE ON CROSS-BORDER HEALTHCARE

December 19, 2013

RARE DISEASES IN THE BALKAN REGION: MANY COUNTRIES, COMMON PROBLEMS

- **Responding to rare diseases patient needs and expectations in 2017**
  - Improved access to innovative therapies
  - Improved access to multidisciplinary care

ORDINANCE 16 ON THE CENTRES OF EXPERTISE FOR RARE DISEASES

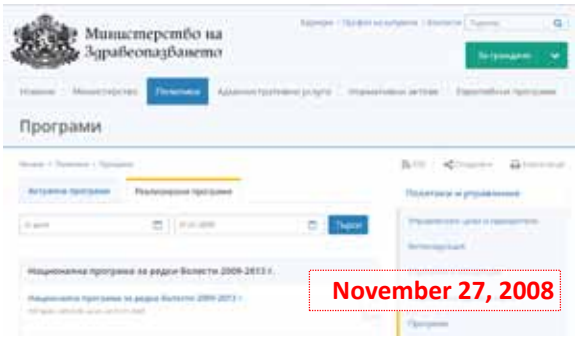
July 30, 2014

LOCAL EXPERIENCE FROM BULGARIA

COMMISSION ON RARE DISEASES

February 13, 2015

ADOPTION OF NATIONAL PROGRAMME FOR RARE DISEASES (2009-2013)



November 27, 2008

COMMISSION ON RARE DISEASES

- **Commission on rare diseases is permanently established at the Ministry of Health:**
  - **4 representatives from the Ministry of Health**
  - **7 medical professionals**
  - **2 patient representatives**
- **Functions:**
  - **to define a List of rare diseases**
  - **to recommend on the official designation of centres of expertise**
  - **to evaluate the activities of the National registry for rare diseases**
  - **to evaluate the activities of the designated centres of expertise**
  - **to advise on the prevention, diagnosis, treatment, follow-up and rehabilitation of rare diseases**
  - **to collaborate with the Commission, Member States, European and international organisations**

COMMISSION ON RARE DISEASES

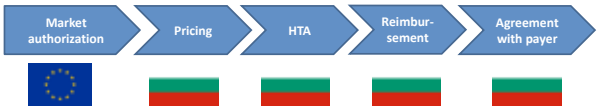
NATIONAL REGISTRY OF RARE DISEASES

- National Centre for Public Health establishes and manages a National registry for rare diseases.
- Management procedures and research methodologies are to be set by the Commision on rare diseases.
- Minimal data set:
  - ID number (date of birth and sex are included in it)
  - address
  - main diagnosis (ICD 10 and ORPHA codes)
  - concomitant diagnosis
- Centres of expertise send anonymised data to the National registry twice a year.

LIST OF RARE DISEASES

- List of rare diseases is established and used for:
  - designation of centres of expertise and reference networks
  - management of the National registry for rare diseases
  - planning medical services for rare diseases
  - European and international collaboration
- First version of 119 conditions in November 2015
- 15 conditions added to the List in March 2016
- 1 condition added to the List in April 2017

ACCESS TO ORPHAN DRUGS IN BULGARIA



- Pricing: National Council for Pricing and Reimbursement (international reference pricing)
- HTA: Commission for HTA (specific provisions for orphan drug assessment)
- Reimbursement decision: National Council for Pricing and Reimbursement (level of reimbursement, reimbursement settings)
- Payer: National Health Insurance Fund (mandatory rebates – 10% at least)

CENTRES OF RARE DISEASES

ACCESS TO ORPHAN DRUGS IN BULGARIA

- Before 2011
  - Payer: Ministry of Health
  - Annual fixed budget for rare disease medicinal therapies
  - Market access to only 22 out 61 orphan drugs available in 2011
  - Mean time from market authorization to a positive reimbursement decision (± SD) – 43 ± 29 months
- After 2011
  - Payer: National Health Insurance Fund
  - Rare disease medicinal therapies represent 7.8% of NHIF total drug budget in 2014

PARTICIPATION IN ERN

- 6 centres participating in 5 ERN by March 2017
  - National Hematology Hospital, Sofia (Rare anemias and coagulopathies)
  - St Marina University Hospital, Varna (Rare anemias and coagulopathies)
  - St Marina University Hospital, Varna (Rare pediatric endocrinology diseases)
  - Alexandrovska University Hospital, Sofia (Rare genetic and metabolic diseases)
  - Alexandrovska University Hospital, Sofia (Primary immunodeficiencies)
  - Alexandrovska University Hospital, Sofia (Fabry disease)
  - St Naum University Hospital, Sofia (Dystonia)
  - St Naum University Hospital, Sofia (Huntington disease)
  - St Ivan Rilski University Hospital, Sofia (Rare metabolic diseases of liver)
  - National Cardiac Hospital, Sofia (Pulmonary arterial hypertension)
  - National Cardiac Hospital, Sofia (Congenital cardiac malformations)
  - Ivan Penchev University Endocrinology Hospital, Sofia (Acromegaly, Cushing disease, hypopituitarism)
  - Unversity Pediatric Hospital, Sofia (Rare pediatric diseases)

ACCESS: A MATTER OF POLICY OR FUNDING?

- Number of market approved orphan drugs by EMA in January 2017 (excluding those with expired orphan status): **93**
- Number of accessible (reimbursed) orphan drugs in January 2017:
- Greece: 45
- Slovenia: 36
- Romania: 26
- Bulgaria: 22
- Croatia: 15
- Serbia: 6
- Montenegro: 0

Study by Pejcic A, Jakovljevic M, Iskrov G and Stefanov R (2017)

RARE DISEASES AND GENOMIC MEDICINE – PATIENTS`PERSPECTIVE

Vladimir Tomov

NATIONAL ALLIANCE OF PEOPLE WITH RARE DISEASES

ESSENTIAL SEQUENCE
THE ONLY POSSIBILE DIAGNOSIS FOR SOME RARE DISEASES

THE EXPLORATION OF HUMAN GENOME IS THE KEY TO SUCCESSFUL TREATMENT
THE IDENTIFICATION OF MUTATION IN PATIENTS WITH GENETIC DISEASE IS IMPACT ON THE TOTAL HEALING PROCESS.

PROPHYLAXIA

EARLY DIAGNOSTICS

TARGET THERAPY

NATIONAL ALLIANCE OF PEOPLE WITH RARE DISEASES

FROM GAUCHER ASSOCIATION TO EURASIA ALLIANCE FOR RARE DISEASES

IN 1999 THE PARENTS OF CHILDREN WITH GAUCHER DISEASE REGISTERED ASSOCIATION BY RECOMMENDATION OF EUROPEAN GAUCHER ALLIANCE

IN 2007 WAS THE FIRST NATIONAL CONFERENCE REGISTRATION OF NAPRD

IN 2009 APPEARED THE CONFEDERATION OF HEALTH PROTEKTION

IN 2012 IN MOSCOW WAS THE FIRST CONFERENCE OF EURO-ASIAN ALLIANCE FOR RARE DISEASES

NATIONAL ALLIANCE OF PEOPLE WITH RARE DISEASES

PROPHYLAXIA

DETERMINATION OF RISK IN PREGNANCY

FAMILIES WITH CHILDREN AFFECTED BY RARE DISEASE CREATED THE SECOND CHILD AFTER THEY HAVE OBTAINED POSSIBILITIES FOR GENETIC TESTING

LIKE GAUCHER DISEASE, MUCOPOLISAHARIDOSIS, TALASEMIA, HEMOPHILIA, BALOSIS EPIDERMOLYSIS, CF

NATIONAL ALLIANCE OF PEOPLE WITH RARE DISEASES

RARE DISEASES DEFINICION

ACCORDING TO BULGARIAN HEALTH ACT – A DISEASE THAT AFFECTED LESS THEN ONE HUMAN AT 2000 PEOPLE IS RARE

THERE ARE MORE THEN 7 THOUSAND NOZOLOGICAL UNITS DEFINED AS RARE

85% OF RARE DISEASES ARE GENETIC

ONLY 5% OF RARE DISEASES ARE TREATABLE

NATIONAL ALLIANCE OF PEOPLE WITH RARE DISEASES

THE EARLY DIAGNOSIS

ARE DETERMINING THE BENEFICIARY BEFORE DEALING WITH THE DISEASE.

IMPORTANT FOR PATIENTS WITH LATER DEVELOPMENT OF DISEASE

LIKE HUNTINGTON DISEASE , FAMILY AMILOID POLYNEVROPATHY

NATIONAL ALLIANCE OF PEOPLE WITH RARE DISEASES

HEREDITARY RERE DISEASES IN NATIONAL ALLIANCE

Angioedema, Arthrogryposis, Ataxia,
Gaucher disease, Fabry disease, Wilson disease,
Huntington's disease, Thalassemia, Hemophilia,
Epidermolysis bullosa, Porphyria,
Familial amyloid polyneuropathy, CF, Aniridia,
Growth hormone deficy, Mucopolisaharidosis, Neuro-muscular diseases, Niemann Pick

NATIONAL ALLIANCE OF PEOPLE WITH RARE DISEASES

THE TARGET THERAPY

GIVES THE POSSIBILITY OF USING NEW MEDICINES IN DISEASES FOR WHICH SYMPTOMATIC TREATMENT IS POSSIBLE METHOD TO THE MOMENT.

THERE IS A POSSIBILITY OF USING TARGET THERAPY IN CYSTIC DISEASE FIBROSIS POSSIBLE ONLY FOR A CERTAIN TYPE OF MUTATION

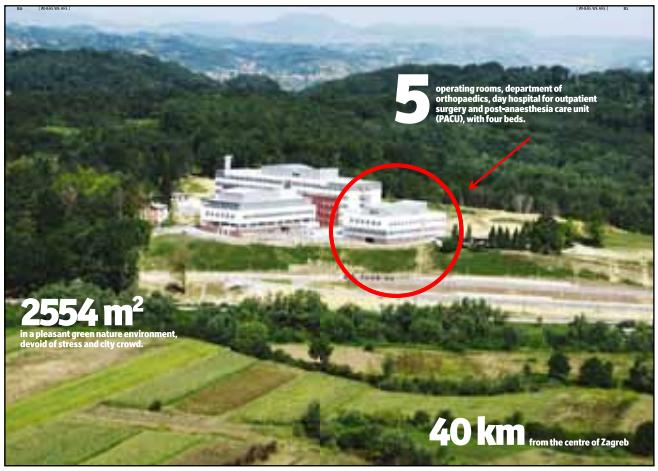
IN GENOMIC SEQUENCING A TREATMENT FOR CEREBELLAR ATAXIA CAN BE DETERMINATED

THROUGH DETERMINATION OF THE MUTATION IT CAN BE APPLIED A TREATMENT IN DUCHENNE MUSCULAR DYSTROPHY-DISEASE

BY INJECTING A PARTICULAR VIRUS IT MAY BE CAUSED A MUTATION IN A GENE DAMAGE THAT NORMALIZES IT AND LEAD TO A COMPLETE CURE

STEM CELL APPLICATION FOR OSTEOARTHRITIS IN THE KNEE JOINT

Dragan Primorac



DP
The Office of Dragan Primorac

"We can't solve problems by using the same kind of thinking we used when we created them."
Albert Einstein

"Insanity: doing the same thing over and over again and expecting different results."
Albert Einstein

TRANSLATION MEDICINE

BASIC SCIENCE ⟷ CLINICAL MEDICINE

Since February, 2014.....

The Leading Hospitals of the World

St. Catherine
ORTHOPEDICS, SURGERY, INTERNAL MEDICINE, NEUROLOGY AND PHYSICAL MEDICINE AND REHABILITATION SPECIALITY HOSPITAL
THE LEADING HOSPITALS OF THE WORLD

Personalised medicine approach in orthopedics in St. Catherine Hospital

CLINICAL CENTER OF EXCELLENCE FOR SPORTS MEDICINE AND A THE OFFICAL HOSPITAL OF THE CROATIAN OLYMPIC COMMITTEE

Personalised medicine refers to a medical model using characterization of individuals' phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring **the right therapeutic strategy for the right person at the right time,** and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention.
Horizon 2020 Advisory Group

ISABS

BIOGLOBE

CROATIAN PERSONALISED MEDICINE PROGRAM

GENOS

St. Catherine

MAYO CLINIC

St. Catherine SPECIALTY HOSPITAL

ISABS

UNIVERSITY OF NEW HAVEN

GENOS

PENNSTATE

21

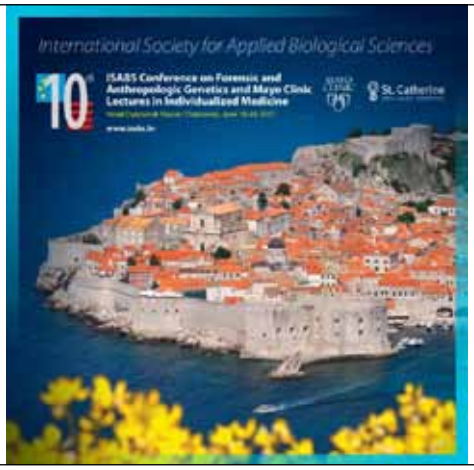
PERSONALIZED MEDICINE APPROACH

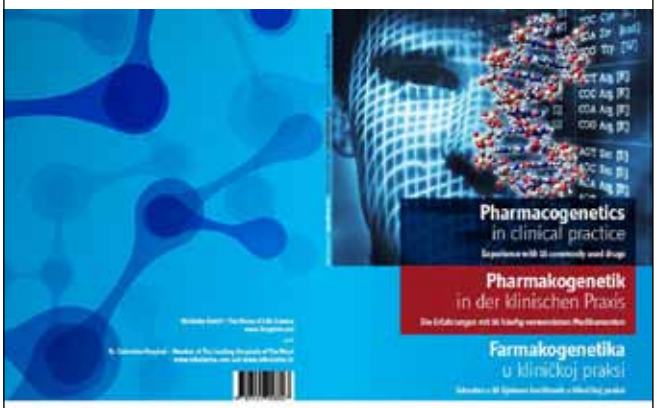
A two-level risk reducing model

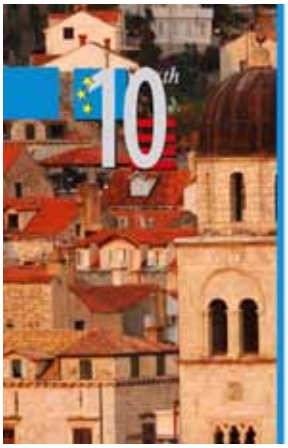
Molecular diagnostics
TESTING GENETIC PREDISPOSITION TO DISEASE

Pharmacogenetic testing
TESTING GENETIC PREDISPOSTION TO ADVERSE DRUG REACTION

Treatment according to PERSONALIZED MEDICINE PHILOSOPHY







Topics for 10th ISABS Conference

Genomics of Individualized medicine:
Biomarker discovery
Epigenomics
Microbiome
Clinomics
Pharmacogenomics

Forensic Genetics:
Next Generation Sequencing (NGS) in Forensics
Advancements in Forensic DNA Routine
DNA Investigative Intelligence

Anthroplogy Genetics:
Ancient DNA
Migration history
Genetic adaptation

TRANSLATION MEDICINE

BASIC SCIENCE ⟷ CLINICAL MEDICINE

ROBERT HUBER,
The Nobel Prize in Chemistry
1998

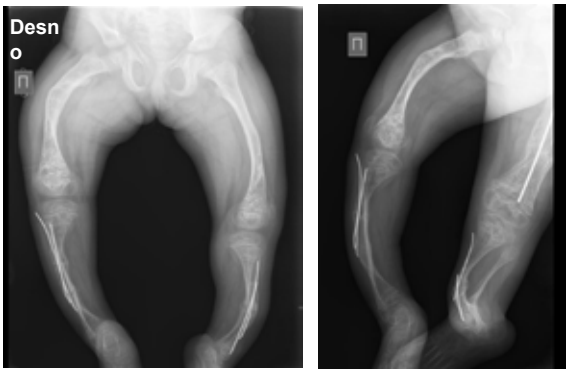
ROBERT HUBER,
The Nobel Prize in Physiology and Medicine
2008

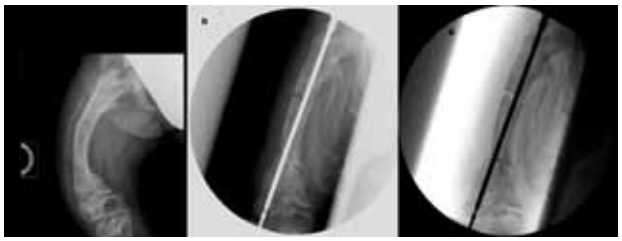
ADA YONATH,
The Nobel Prize in Chemistry
2009

St. Catherine

CLINICAL CENTER OF EXCELLENCE FOR OSTEOGENESIS IMPERFECTA

Elizaveta Lisova 10 years old girl from Moscow.

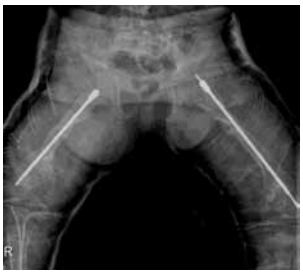


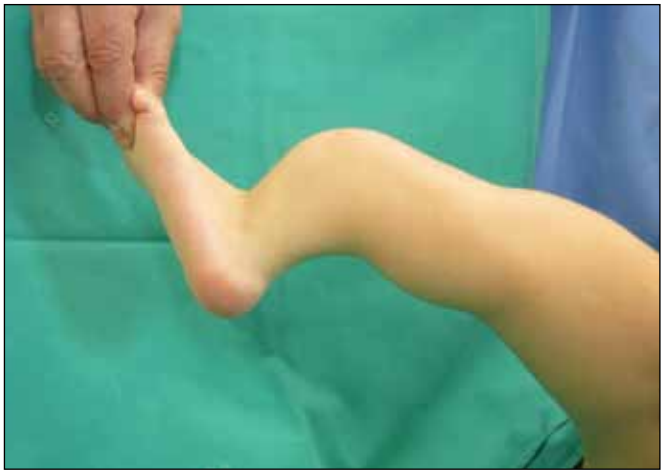


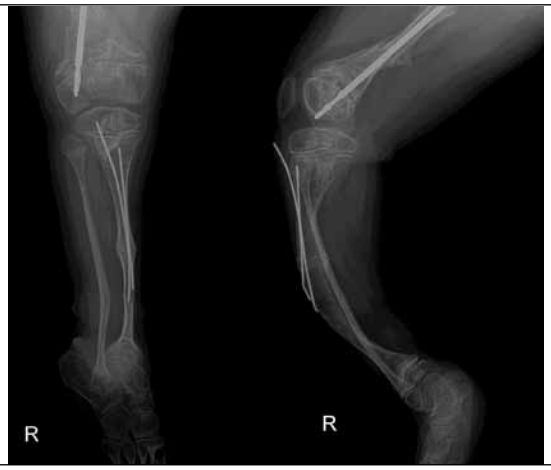
COL1A1 gene	Genotype	Expected phenotype
NGS analysis of all coding exons and flanking intron regions; MLPA-analysis	c.2235+1G>A; G/A heterozygous, c.104-441G>T; G/T heterozygous	Osteogenesis imperfecta type 3 / 1

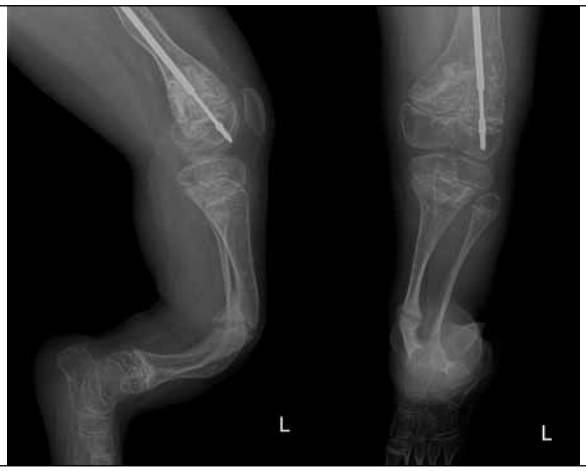
COL1A2 gene	Genotype	Expected phenotype
NGS analysis of all coding exons and flanking intron regions; MLPA-analysis	normal	--

Sequence variants without pathological relevance have been documented but they are not reported here.





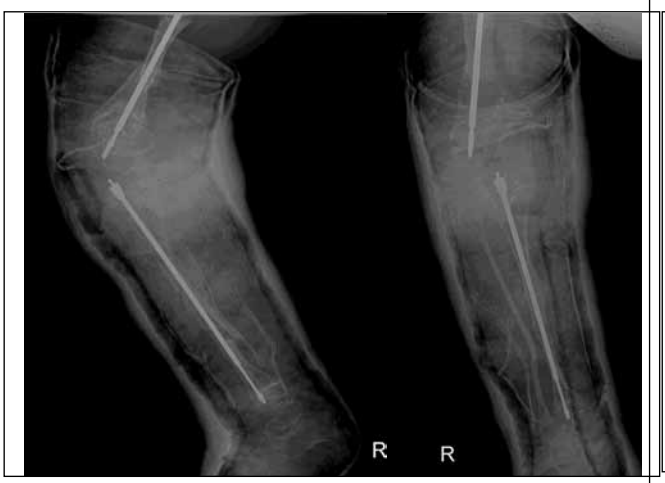




Osteoarthritis (OA)

Osteoarthritis (OA) is a disease **of the total joint**, not just the articular cartilage.

The Osteoarthritis Research Society International Disease State Working Group defined OA as *"**A progressive disease representing the failed repair of joint damage that, in the preponderance of cases, has been triggered by abnormal intra-articular stress**."* It is important to remember that all tissues of the joint are involved, including not only the articular cartilage, but also the subchondral bone, ligaments, periarticular structures, and menisci, when present. The results of the OA process are cartilage degradation and bone remodeling; these features are associated with the development of symptoms of pain, stiffness, and functional disability.

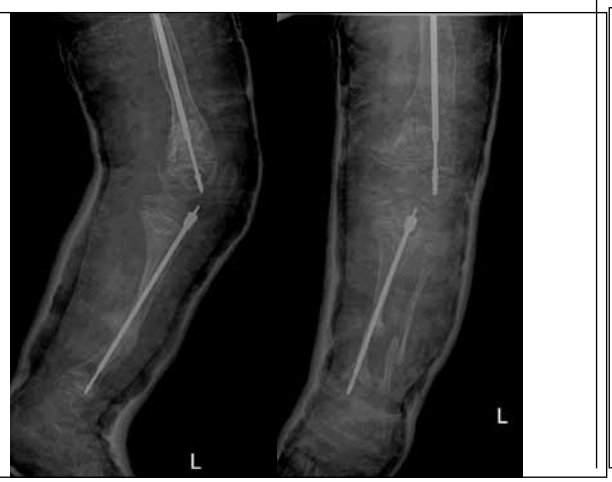


In the United Kingdom, the lifetime risk of developing symptomatic knee and hip OA is estimated to be **44.7% and 25.3%**, respectively. The economic cost of OA is also particularly high, resulting from decreased quality of life, hospitalizations and loss of productivity.

Worldwide estimates indicate that 9.6% of men and 18% of women 60 years have symptomatic OA.

In the United States, the annual medical care expenditures for OA were estimated at **$185.5 billion** (in 2007 dollars), of which $149.4 billion were insurer expenditures and $36.1 billion were paid directly by the patients (*Medicographia. 2013;35:197-202)*

From 2013- 2015, an estimated **54.4 million US adults** annually had ever been told by a doctor that they had some form of arthritis. In the Europe the number of patients with osteoarthritis (OA) is even higher.



Half of the world's population aged 65 years or older has OA, which is the most prevalent disorder of articulating joints in humans.

UN World Population Ageing publication (2015) estimated that today approximately 900 million people is older than 60 years. Therefore, according to Scientific Reports **more than 400 million people today** is having some kind of OI while in 2030 estimation is that **700 million people** Worldwide will have OI *(Scientific Reports (2016) doi:10.1038/srep24393)*

Ten leading causes od disability globally among persons aged 60 years or over, by sex (2012)

	Females	YLDs* per 100,000 people	Males	YLDs* per 100,000 people
1	Unipolar depressive disorders	1 465	Other hearing loss	1 670
2	Other hearing loss	1 427	Back and neck pain	1 530
3	Back and neck pain	1 413	Falls	1 347
4	Alzheimer's disease and other dementias	1 295	Chronic obstructive pulmonary disease	1 276
5	Osteoarthritis	1 201	Diabetes mellitus	1 121
6	Chronic obstructive pulmonary disease	1 200	Refractive errors	902
7	Diabetes mellitus	1 143	Unipolar depressive disorders	883
8	Refractive errors	1 066	Alzheimer's disease and other dementias	850
9	Falls	998	Hyperplasia of prostate	840
10	Cataracts	756	Osteoarthritis	739

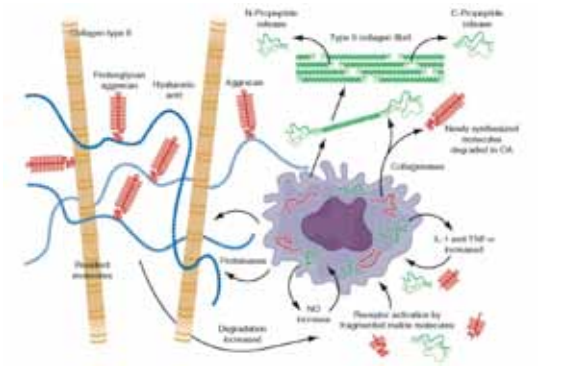
Data source: WHO (2014). *Global Health Estimates.*
*YLDs = Years of life lost due to disability.

Cartilage does not contain blood vessels (it is ***avascular***) or nerves (it is ***aneural***). Nutrition is supplied to the chondrocytes by **diffusion**.

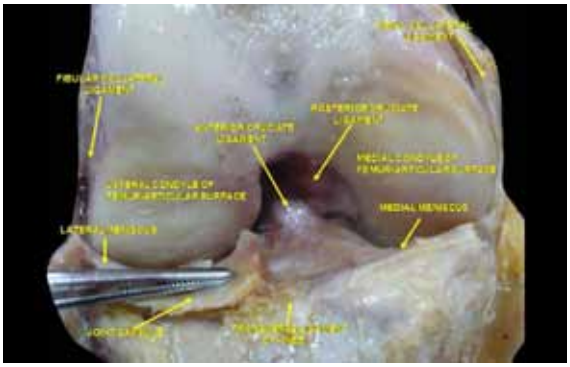
The compression of the articular cartilage or flexion of the elastic cartilage generates fluid flow, which assists diffusion of nutrients to the chondrocytes.

Compared to other connective tissues, cartilage has a very slow turnover of its extracellular matrix and does not repair.

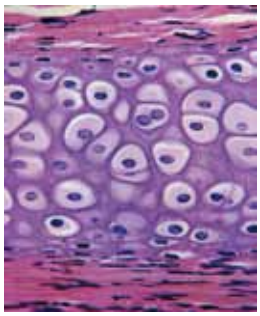
- The anterior cruciate ligament prevents the femur from sliding backward on the tibia (or the tibia sliding forward on the femur).
- The posterior cruciate ligament prevents the femur from sliding forward on the tibia (or the tibia from sliding backward on the femur).
- The medial and lateral collateral ligaments prevent the femur from sliding side to side.



Ann Rheum Dis 2002;**61**:ii78-ii81 doi:10.1136/ard.61.suppl_2.ii78



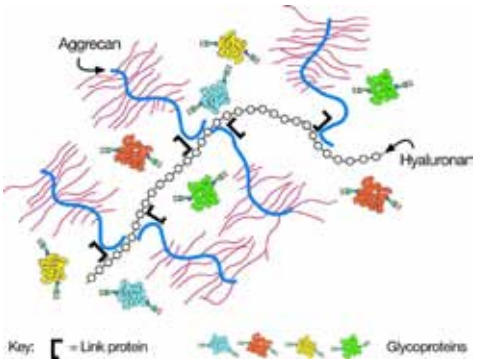
What about Proteoglycans?



Photomicrograph of HYALINE CARTILAGE. Chondrocytes are located in matrix lacunae, and most belong to isogenous groups. The upper and lower parts of the figure show the perichondrium stained pink. Note the gradual differentiation of cells from the perichondrium into chondrocytes.

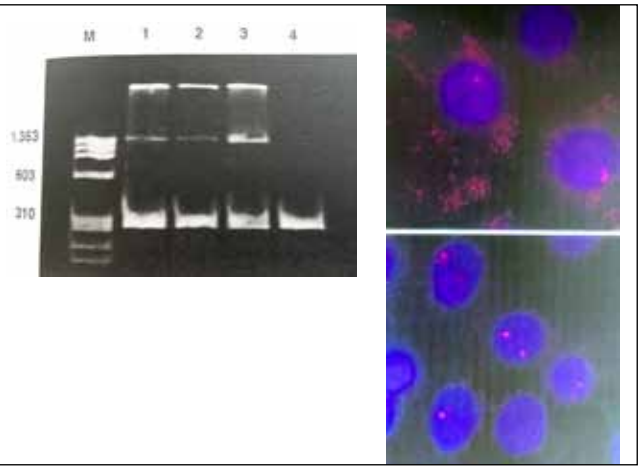
Glycosaminoglycans are highly polar and attract water. They are therefore useful to the body as a lubricant or as a shock absorber.

The large cartilage CS proteoglycan (aggrecan) forms an aggregate with hyaluronan and link protein

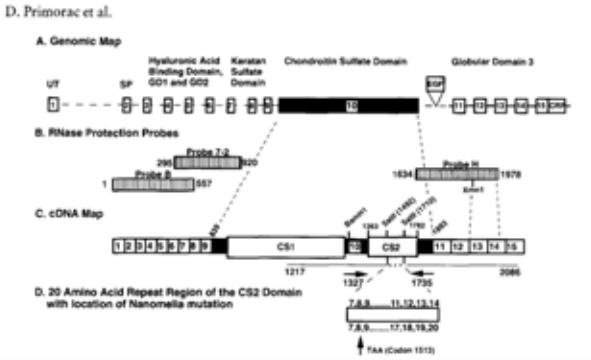


Essentials of Glycobiology Second Edition

Chapter 16, Figure 1



Map of the Agreecan Gene and cDNA



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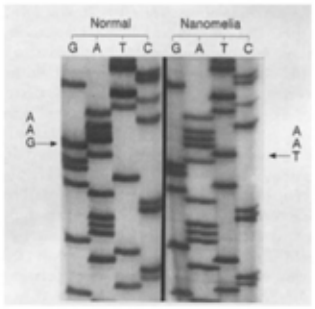
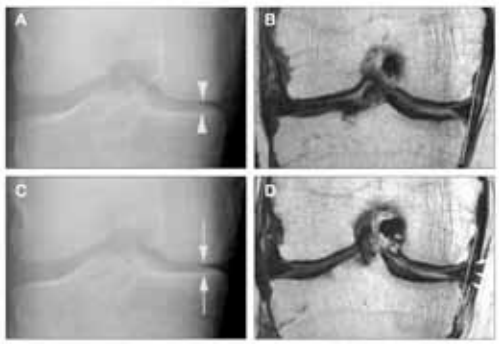


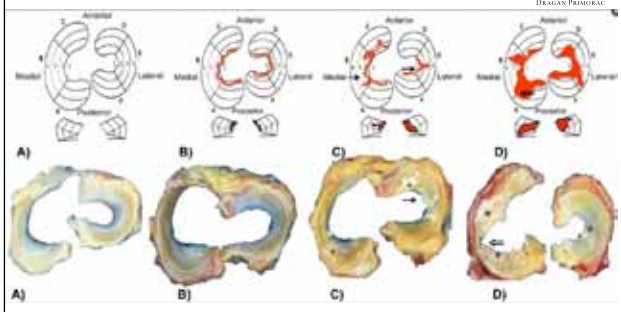
Fig. 4. DNA sequencing for the *Sst*II fragment within the CS2 domain that harbored the nanomelic mutation. The PCR derived fragment was subcloned into Bluescript KS$^+$ and sequenced from the vector primers.



(A) Baseline anterior-posterior radiograph showing doubtful joint space narrowing in the medial tibiofemoral compartment (arrowheads). (B) Follow-up image at 24 months showing **a definite increase in joint space loss medially** (arrows).(C) Corresponding baseline intermediate-weighted magnetic resonance imaging (MRI) showing normal cartilaginous tibiofemoral surface and no relevant meniscal extrusion. (D) MRI at 24 months **showing definite meniscal extrusion** (arrows) likely to be responsible for the joint space narrowing on the radiograph. Guermazi A at al. Mediographia. 2013;35 (2):164-171.

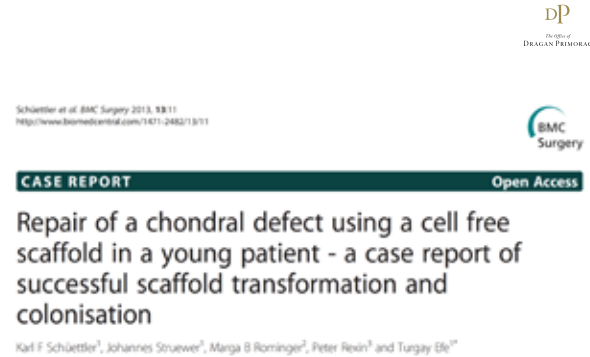
Nanomelia

A meniscal transplant



A) Macroscopic assessment: Grade 1: Normal intact menisci, attached at both ends with sharp inner borders, no tibial or femoral surface changes. B) Grade 2: Fraying at inner borders, tibial or femoral surface fibrillation, no tears. C) Grade 3: Partial substance tears (→), fraying, tibial or femoral side fibrillations. D) Grade 4: Full/complete substance tears, loss of tissue (⇐), tissue maceration. *Calcium deposition is marked in addition to the grade. Red marks in b – d indicate the degeneration pattern. Every location (A/F) posterior, (B/E) middle, (C/D) anterior can be given a grade 1–4.

Osteoarthritis Cartilage . 2011 September ; 19(9): 1132–1141. doi:10.1016/j.joca.2011.05.008.



Schüttler et al. BMC Surgery 2013, 13:11
http://www.biomedcentral.com/1471-2482/13/11

BMC Surgery

CASE REPORT Open Access

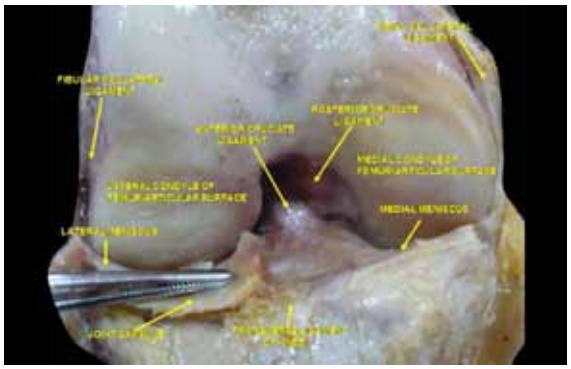
Repair of a chondral defect using a cell free scaffold in a young patient - a case report of successful scaffold transformation and colonisation

Karl F Schüttler[1], Johannes Struewer[2], Marga B Rominger[2], Peter Rexin[3] and Turgay Efe[1*]

The first arthroscopic meniscal transplantation in the Region

Medial meniscus

Arthroscopic meniscal transplants improve knee symptoms and knee function and **allow return to play in soccer players, regardless of the degree of chondral lesion**. Alentorn-Geli E, Vazquez RS, Diaz PA, Cusco X, Cugat R. Arthroscopic meniscal transplants in soccer players: outcomes at 2- to 5- year follow-up. Clinic J Sport Med 2010;20(5):340-3.



Cartilage Regeneration System
with biological implants

Cartilage Regeneration System
with biological implants

ARTHRO.KINETICS
REGENERATING JOINT MOBILITY

CLINICAL CENTER OF EXCELLENCE FOR:
Croatia, Slovenia, Hungary, Romania, Bulgaria, Ukraine, Bosnia and Herzegovina, Serbia, Monte Negro, Macedonia, Albania

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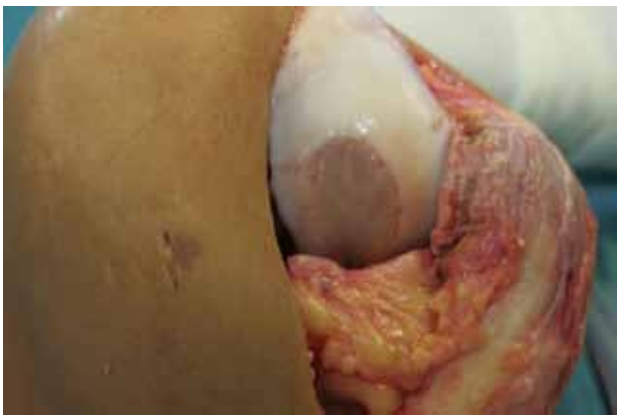
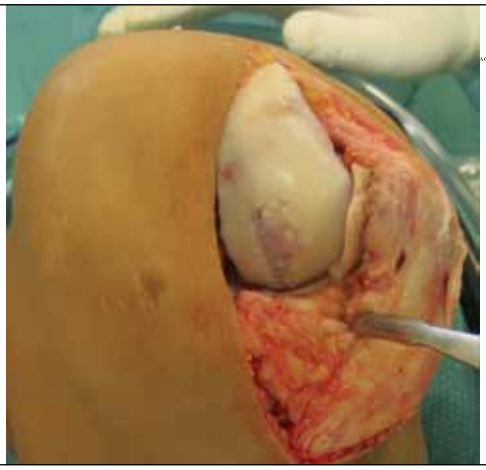
CaReS-1S

CaReS®-1S is a technology of cartilage regeneration providing all benefits of autologous chondrocyte implantation (ACI) without requiring an additional intervention for biopsy (autologous chondrocyte immigration, ACIM).

CaReS®-1S is an implant used for the treatment of full-layer, localized small cartilage defects of up to 8 cm^2.

CaReS®-1S **fills the defect zone completely and is colonized by cells migrating from the surrounding tissue leading to the formation of hyaline-like regenerated cartilage tissue**.



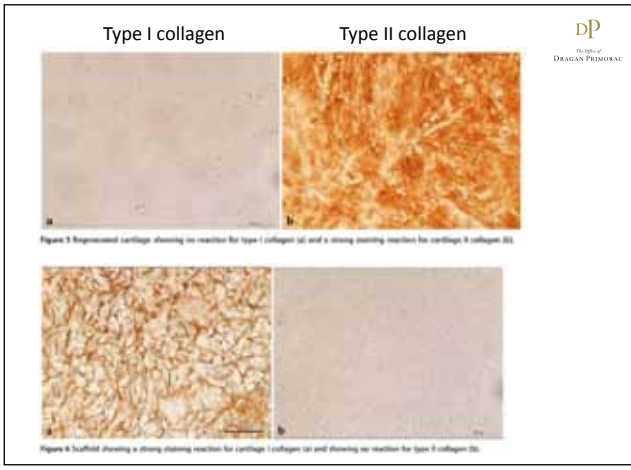


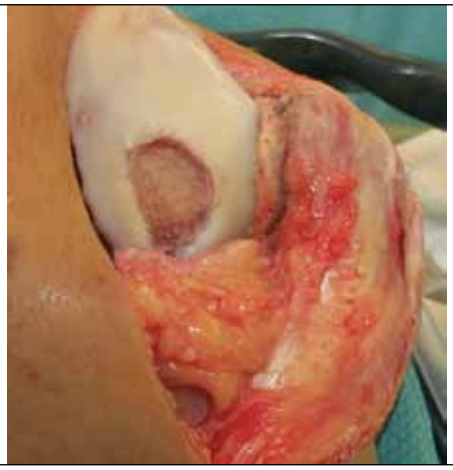
International Orthopaedics (SICOT)
DOI 10.1007/s00264-015-2695-9

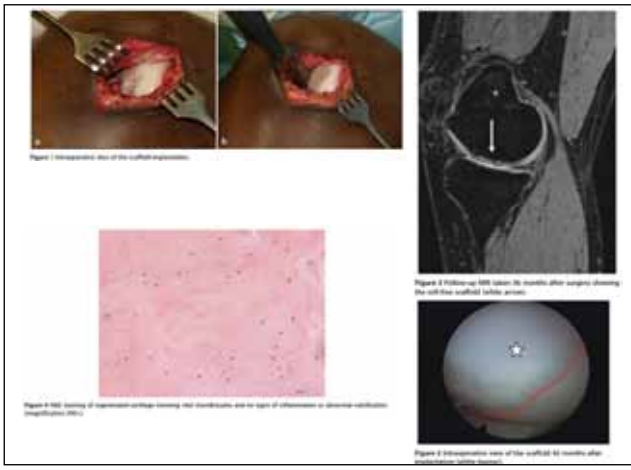
ORIGINAL PAPER

Short-term follow up after implantation of a cell-free collagen type I matrix for the treatment of large cartilage defects of the knee

Philip P. Roessler · Bernhard Pfister · Markus Gesslein · Jens Figiel · Thomas J. Heyse · Christian Colcuc · Olaf Lorbach · Turgay Efe · Karl F Schüttler

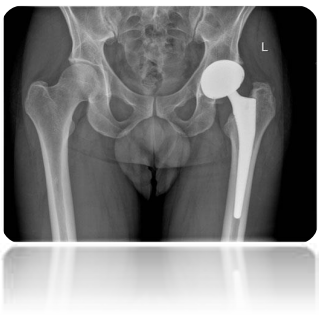






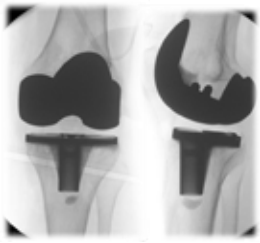
Hip arthroplasty

- Primary total hip arthroplasty
- Anterolateral (Watson Jones) and lateral (Hardinge) approach
- Implants:
  - DePuy Corail stem
  - DePuy Pinnacle acetabulum
  - Metal/ polyethylene
  - ceramics



Knee arthroplasty

• **Primary and revision cases**
Medial parapatellar and midvastus approach)
• **Implants:**
Zimmer Nexgen highflex knee
DePuy PFC Sigma



Platelet-rich plasma (PRP) and bone marrow concentrate (BMC)

-**PRP** is plasma from the patient's own blood in which the platelet concentration is higher than baseline value. The basis for using PRP is that in contains growth factors, cytokines, chemokines and other mediators which are currently thought to accelerate the natural healing process postinjections. **Neutrophils** are thought to impede tissue healing by increasing inflammation and may not be desired in PRP therapies. Recent data indicate that removing **RBCs** from PRP is most beneficial to treating musculoskeletal pathology. **RBC are having a negative effect on chondrocytes (recurrent hemarthrosis, associated with hemophilia has been associated with arthritic changes in the knee joint**.

-**BMC** contains MSCs, hematopoetic stem cells, platelets (containing growth factors) and cytokines. The anti-inflamatory and immunomodulatory properties of bone marrow medicinal signaling can facilitate the regeneration of tissue.

Stem cells

The stem cells are unspecialized cells that can undergo self-renewal and differentiation into particular specialized type of cells.

They can be: **adult stem cells**, **embryonic stem cells (ESCs)** and **induced pluripotent stem cells (iPSCs)** that are derived from reprogramming adult cells back to an ESC-like state. Unfortunately with ESCs and IPSCs is the significant chance to develop teratoma.

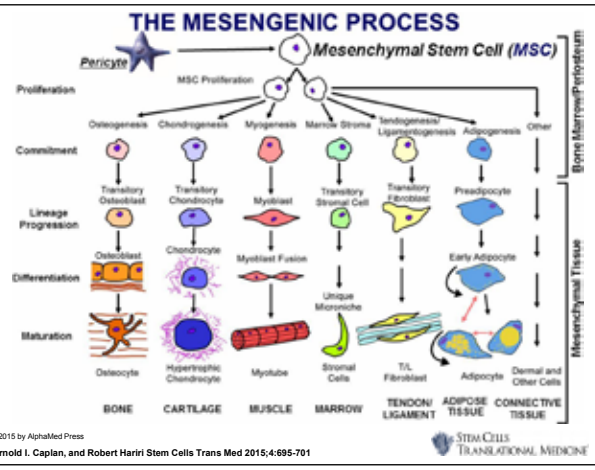
Mesenchymal stromal cells (MSCs) are multipotent cells derived from bone marrow or adipose tissue that can defferiantiate in adipocytes, osteocytes and chondrocytes. Those cells are **immunoprivileged** (having no major histocompatibility complex (MHC) clas II antigen expression on the MSC cell surface. In addition, MSCs **cause immunosupression by downregulating T cell responses**. MSCs **are capable of crossing the blood-brain barrier, quickly culture expand ex vivo.**

MESENCHYMAL STEM CELLS

Mesenchymal stem cells are defined as multipotent and self-renewable cells with the ability in vitro to adhere to plastic and to differentiate into multiple lineages, including osteogenic, chondrogenic and adipogenic ones

Mesenchymal stem cells (MSCs) are heterogenous population of progenitor cells expressing a pattern of characteristic, but not specific, surface markers, including CD73, CD90, and CD105, **but lacking the expression of hematopoietic markers** CD34, CD45, CD14 or CD11, CD79a or CD19, and HLA class II (International Society for Cellular Therapy).

MSCs could participate in tissue repair, not only differentiating into cells of the target issue, **but also releasing several factors**, contributing to restorative processes, including angiogenetic process. Indeed, the secreted trophic factors, participate to tissue rescue through **pro-angiogenic** and **anti-fibrotic** mechanisms, **anti-inflammatory** and **immunomodulatory** properties, **anti-apoptotic and antimicrobial** characteristics .



Adipose stem cells (ASCs)

To date, human adult adipose tissue may be the best suitable alternative source of MSCs. Adipose stem cells (ASCs) can be largely extracted from subcutaneous human adult adipose tissue. A large number of studies show that adipose tissue contains a biologically and clinically interesting heterogeneous cell population called **stromal vascular fraction (SVF).** The SVF may be employed directly or cultured for selection and expansion of an adherent population, so called adipose derived stem cells (ASCs).

1 g of adipose tissue contains 500 times more pluripotent cells than 1 g of bone marrow aspirate.

HBMSCS vs ASC

HBMSCS (BONE MARROW MARROW STROMAL CELLS)

- Invasive and painful procedure
- Increase the degree of viral infection
- Significant decline in cell viability and differentiation with donor age

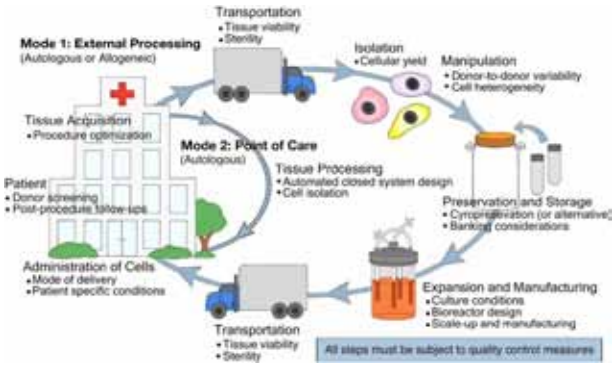
ASC (ADIPOSE TISSUE)

- Abundant subcutaneous fat
- 1 g of adipose tissue contains 500 times more pluripotent cells than 1g of bone marrow aspirate
- High yield upon isolation and a greater proliferative rate in culture
- Non-invasive
- Minimal discomfort for the patient
- Decreased risk of viral infection due to presence of natural antimicrobial (**cathelicidin**)-effective against both viruses and bacteria

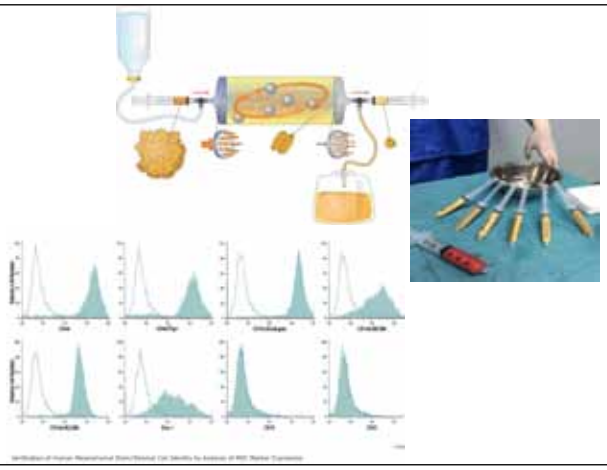
Whenever people agree with me I always feel I must be wrong."
- Oscar Wilde

The first treatment of knee osteoarthritis with autologous mesenchymal stem cells in SEE
(November 28, 2015)

The steps of adipose stem cell therapy.



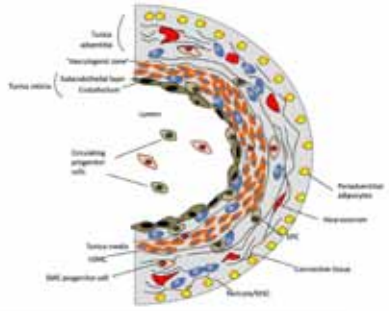
Rachel C. Nordberg, and Elizabeth G. Loboa Stem Cells Trans Med 2015;4:974-979



Sv. Katarina

CLINICAL CENTER OF EXCELLENCE
FOR

LIPOGEMS



The heterogeneity of the distribution of vascular resident progenitor/stem cells across the vessel wall. Various subsets of resident progenitor/stem cells have been recognized in the vascular wall. **Endothelial progenitor cells (EPCs)** reside in the so-called 'vasculogenic zone' which is located in the inner layer of the adventitia. In addition, EPCs can be found in the subendothelial layer. The adventitial 'vasculogenic' zone is also enriched with **pericytes**, **mesenchymal stem cells (MSCs)** and **smooth muscle cell (SMC) progenitors**. MSCs and pericytes have been identified in other mural layers while a so-called 'side cell population' capable of differentiating into endothelial cells and vascular SMCs was observed in the tunica media. *Bobryshev Yuri V, Orekhov Alexander N. , Chistiakov Dimitry A. Vascular stem/progenitor cells: current status of the problem. Cell and Tissue Research. 2015;362:1-7.*

A New Nonenzymatic Method and Device to Obtain a Fat Tissue Derivative Highly Enriched in Pericyte-Like Elements by Mild Mechanical Forces From Human Lipoaspirates

Francesca Bianchi,*† Margherita Maioli,*‡ Erika Leonardi,‖ Elena Olivi,*†
Gianandrea Pasquinelli,¶ Sabrina Valente,¶ Armando J. Mendez,‖ Camillo Ricordi,‖
Mirco Raffaini,# Carlo Tremolada,# and Carlo Ventura*†

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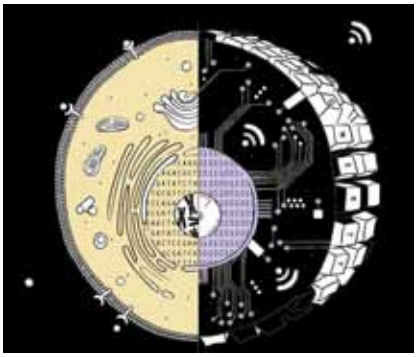
†Cardiovascular Department, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy
‡Department of Biomedical Sciences, University of Sassari, Sassari, Italy
‖Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, FL, USA
¶Surgical Pathology Unit, Department of Hematology, Oncology and Clinical Pathology, University of Bologna, Bologna, Italy
#Esthetic Image, Diabetes Research Institute [DRI], Fontevedra, Milan, Italy

A eukaryotic cell is a continuum of components for **sensing the external environment and translating these signals through signalling pathways with both positive and negative gates.**

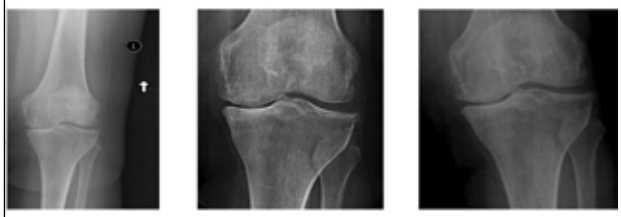


Arnold I. Caplan, and Robert Hariri Stem Cells Trans Med 2015;4:695-701

TREATMENT OF KNEE OSTEOARTHRITIS WITH MICROFRAGMENTED FAT (LIPOGEMS™)

K. Slynarski[1], A. Krzesniak[1], C. Tremolada[2], A. Caplan[3]
[1]Lekmed Hospital, Warsaw, Poland; [2]Image Medical Spa, Milan, Italy; [3]Case Western Reserve University, Department of Biology, Cleveland, USA.

World Congress of International Cartilage Repair Society (2015, Chicago, U.S.)



Weight bearing AP view X-rays.
Left) before Lipogems¨injection; Middle) 6 months after Lipogems¨injection; Right) 12 months after Lipogems¨injection.

At 6 months, from the X-rays it appeared that the intra articular gap increased, thus suggesting cartilage regeneration. The reduction of joint space narrowing remained also after 12 months.

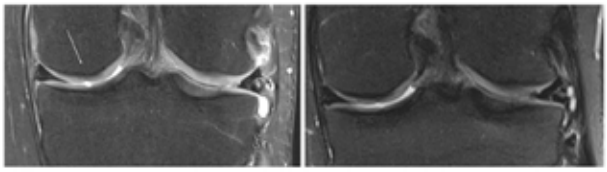
STUDY DESIGN

•Plain X-rays (AP standing and LL knee projections) were performed for each patient. **Kellgren Lawrence classification** was used to assess grade of knee osteoarthritis (OA),

•Full length weight bearing (FLWB) X-rays in the standing position were performed in order to measure limb alignment,

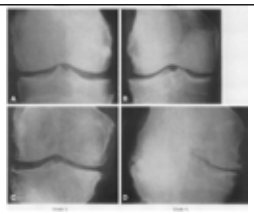
•The severity of early OA in the study cohort was determined according to MRI by an experienced musculoskeletal radiologist using the scoring system introduced by the *International Cartilage Research Society* based on a modified *Outerbridge system* divided into 5 stages according to cartilage lesion size and depth as well as the appearance of the surrounding subchondral bone,

•Visual Analogue Scale for pain assessment was performed in each patient.



Left) MRI before Lipogems¨ injection showing cartilage defect on medial compartment (see arrow); **Right)** MRI 5 months after Lipogems¨ injection showing cartilage defect healed.

The Kellgren and Lawrence (K&L) classification

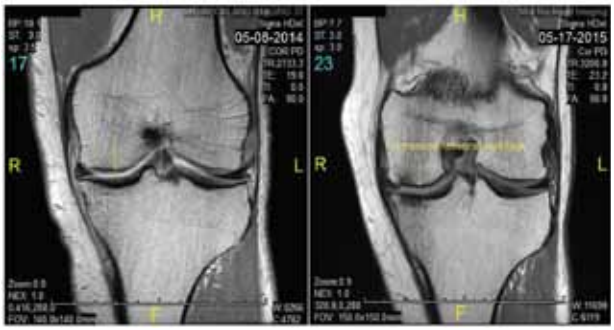


Grade 0: no radiographic features of OA are present
Grade 1: doubtful joint space narrowing (JSN) and possible osteophytic lipping
Grade 2: definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph
Grade 3: multiple osteophytes, definite JSN, sclerosis, possible bony deformity
Grade 4: large osteophytes, marked JSN, severe sclerosis and definite bony deformity

Radiographs of the knee presented in the original Kellgren-Lawrence article. (**A**) Representative knee radiograph of KL classification Grade 1, which demonstrates doubtful narrowing of the joint space with possible osteophyte formation. (**B**) Representative knee radiograph of KL classification Grade 2, which demonstrates possible narrowing of the joint space with definite osteophyte formation. (**C**) Representative knee radiograph of KL classification Grade 3, which demonstrates definite narrowing joint space, moderate osteophyte formation, some sclerosis, and possible deformity of bony ends. (**D** Representative knee radiograph of KL classification Grade 4, which demonstrates large osteophyte formation, severe narrowing of the joint space with marked sclerosis, and definite deformity of bone ends. Reprinted with permission from Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis.* 1957;16:494–502.

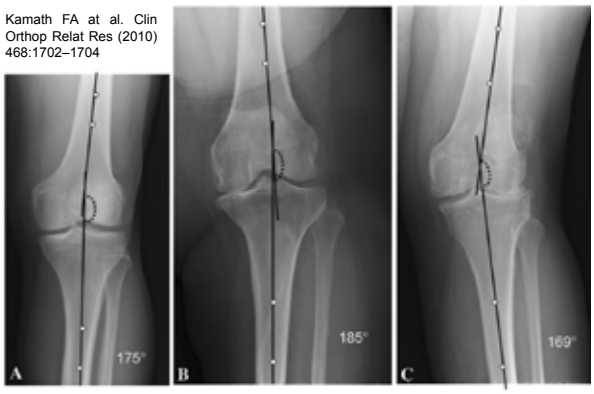
Non-Responsive Knee Pain with Osteoarthritis and Concurrent Meniscal Disease Treated With Autologous Micro-Fragmented Adipose Tissue Under Continuous Ultrasound Guidance

R.Striano, H. Chen, N. Bilbool, K. Azatullah, J. Hilado, K. Horan



Left) MRI prior to treatment revealing medial compartment degeneration (arrow). Right) MRI 6 months after treatment with widening of the joint space and improved signal and thickness of the cartilaginous tissue (yellow arrow). MRI prior to treatment reveals a thinning articular cartilage measured by Radiologist to be 0.75mm. MRI taken 6 months post treatment reveals an improved thickened articular cartilage measured by Radiologist at 1.5mm of cartilage. Meniscus changes are not comparable in the 6 month post treatment MRI as the patient underwent arthroscopic surgery of the meniscus following the original pre-treatment MRI.

Kamath FA at al. Clin Orthop Relat Res (2010) 468:1702–1704

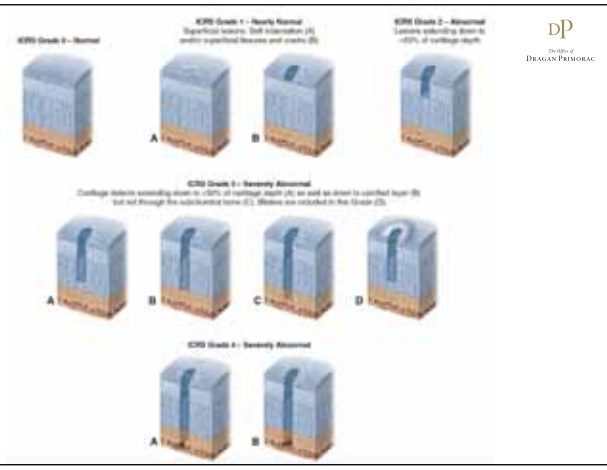


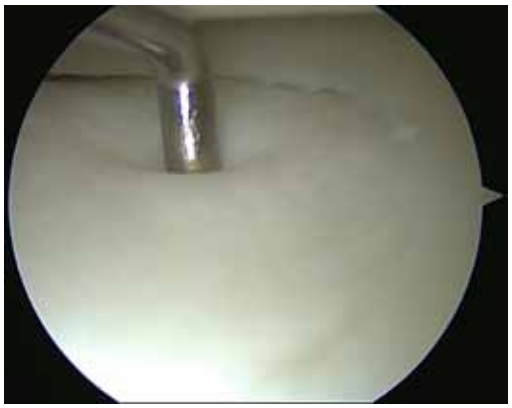
Standing AP knee radiographs show (A) normal, (B) varus, and (C) valgus femorotibial alignment as defined by a standardized 360 classification system

Study design

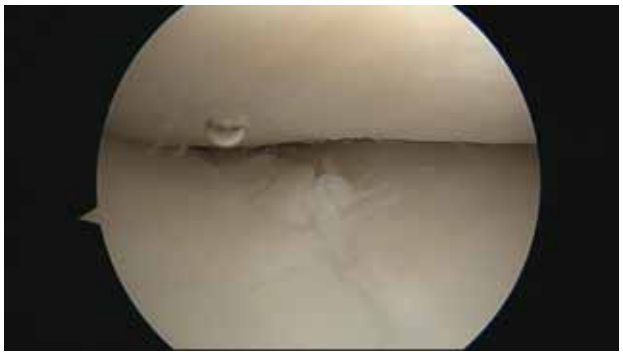
In this study, we measured outcomes of autologous adipose derived mesenchymal stem cells (Ad-MSCs) therapy in 17 patients (32 knees) at baseline (M0), 3 months (M3), 6 months (M6) and 12 months (M12).

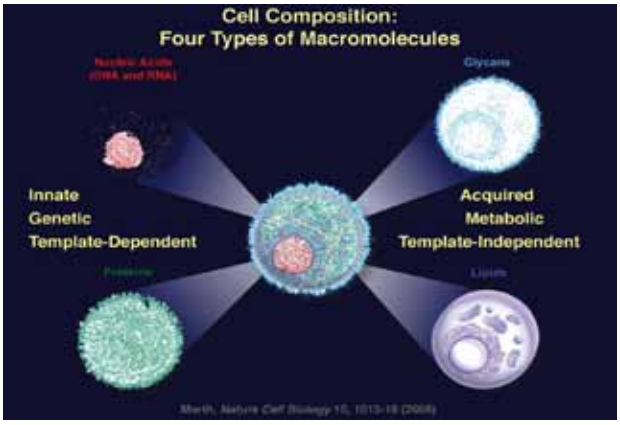
Patients with primary knee OA who satisfied the inclusion criteria (radiological Kellgren Lawrence grade II-IV; onset of symptoms of the index knee 6 or more months ago; ability to follow the instructions of the study; age 40-85.

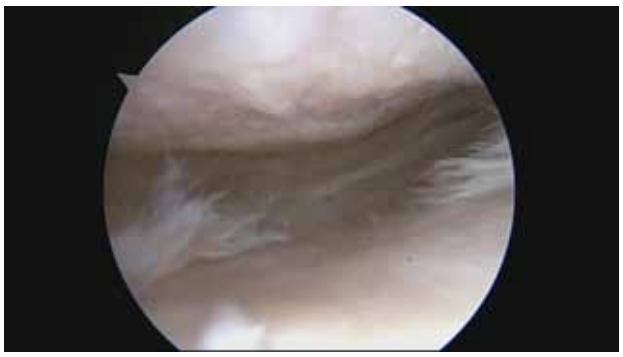




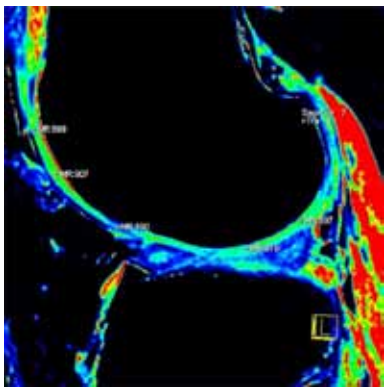
- Blood collections for a serum CRP measurement and plasma blood collection for immunoglobulin G (IgG) glycan analyses were taken preoperatively and postoperatively. After releasing and labeling of N-glycans, the composition of the IgG glycome isolated from plasma was determined by UPLC analysis.
- Blood plasma and synovial fluid samples were taken preoperatively and postoperatively for immunoglobulin G (IgG) glycan analyses. After releasing and labeling of N-glycans, the composition of the IgG glycome isolated from plasma was determined by UPLC analysis.







- Each subject received *gadolinium diethylene triamine penta-acetic acid*. The dGEMRIC index was analyzed on seven different articular facets: the medial and lateral femoral condyle, femoral trochlea, medial and lateral tibial condyle and both patellar facets, before the intra-articular application of stem cells and in any subsequent MR examination at three, nine and twelve months after the intra-articular application of stem cells.
- Within-individual variation was defined on the basis of the dGEMRIC error rates, which were based on previously published papers and indicated that the mean difference per region of interest between the two T1Gd measurements ranging from 3.7% to 6.8%. Based on this, we defined the arbitrary change of 15% in subsequent measurements as clinically relevant, and considered this as the liminal value (on the basis of two standard deviations from the estimated error rate).



Color-coded T1-weighted MR image showing delayed gadolinium-enhanced MRI of cartilage (dGeMRIC map of femoro-tibial cartilage)

dGEMRIC
(Delayed gadolinium-enhanced MRI of cartilage)

- Recent technique where an MRI scan dedicated to detecting early cartilage breakdown is performed.
- GAG (glycosaminoglycan) molecules are vital to the integrity of cartilage, maintaining water molecules within cartilage and therefore the tensile strength.
- **As both GAG molecules and gadolinium are negatively charged, gadolinium will penetrate articular cartilage in an inversely proportional manner to the GAG concentration;**
- That is to say, the lower the GAG concentration (lesser negative charge) the more the negatively charged gadolinium will penetrate the cartilage, relative to areas of higher GAG concentration.

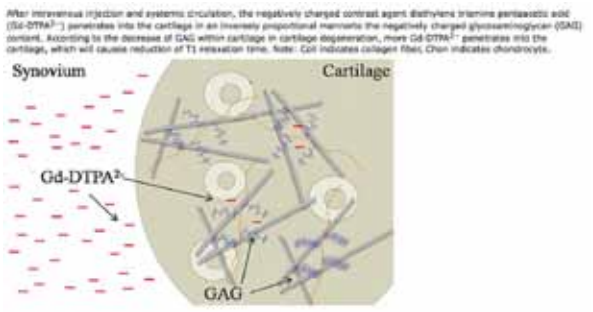
Patient Mr. Želimir Šikonja

He was admitted to St. Catherine Hospital several times because of a constant pain in his knees that significantly limits his daily activities.

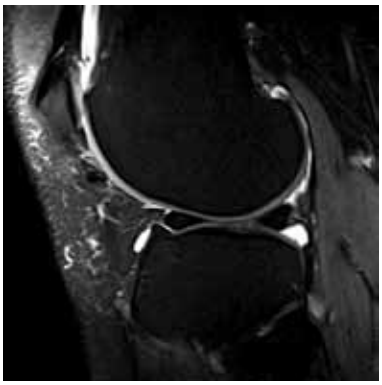
On 15.2.2016 he was admitted once again to our hospital where clinical examination, MRI + dGEMRIC MRI, Glycan analysis (both from blood and synovial fluid) were done. Observed VAS Score was 3/7. On the same day, AdMSCs were applied.

He also came to out hospital for two additional evaluations (30.6.2016 and 5.9.2016).

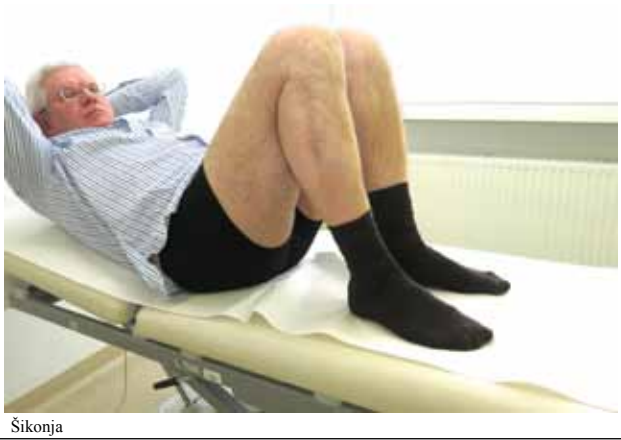
On 31.1.2017 he came to St. Catherine Hospital once again when clinical examination, MRI + dGEMRIC MRI, Glycan analysis (both from blood and synovial fluid) were done.
Observed VAS Score was 0/3.



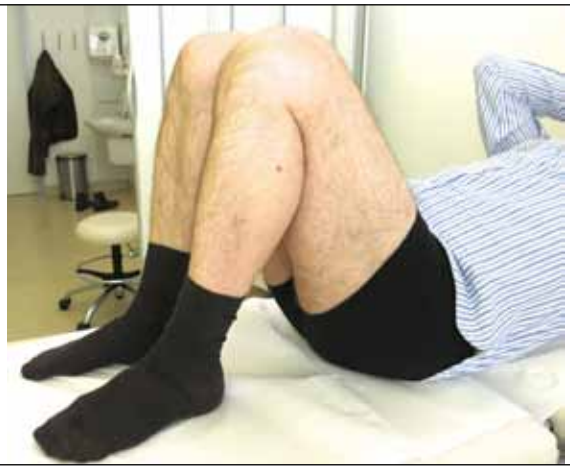
BITTERSOHL, Bernd. Delayed gadolinium-enhanced magnetic resonance imaging of hip joint cartilage (dGEMRIC): pearls and pitfalls. **Orthopedic Reviews**, [S.l.], v. 3, n. 2, p. e11, oct. 2011. ISSN 2035-8164. Available at: http://www.pagepress.org/journals/index.php/or/article/view/or.2011.e11/5530

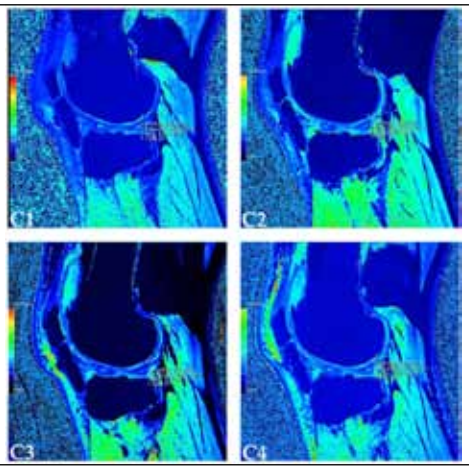


Sagital intermediate-weighted fat-saturated MR image



Šikonja

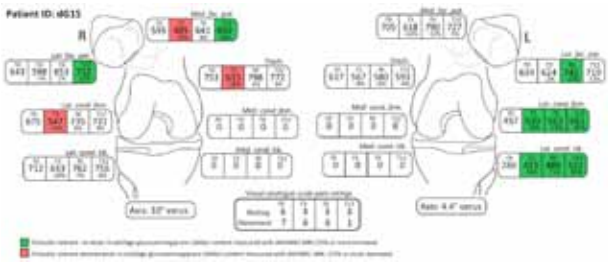


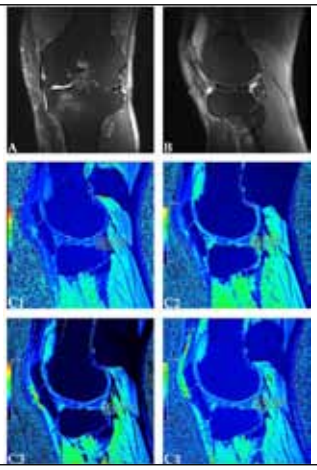


MR images with the dGEMRIC index values at four time points (T0: baseline, T3: three months after Ad-MSC application; T6: six months after Ad-MSC application; T12: twelve months after Ad-MSC application) (C1-C4). The scheme of the dGEMRIC index with different joint facets throughout the study period at T0, T3, T6 and T12 combined with VAS scale ratings in T0, T3, T6, T12 (D).

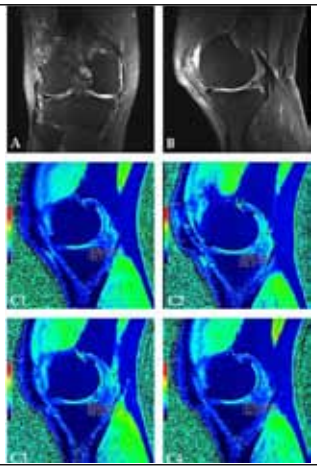
Basic clinical comparison across the follow-up

	Initial (M0)	First follow-up (M3)	Second follow-up (M6)	Third follow-up (M12)	P* (M0-M3)	P* (M0-M6)	P* (M0-M12)
CRP; mean±SD (min-max)	6.54±7.83 (1-20.3)	-	3.86±3.71 (0.6-12)	5.17±5.83 (0.6-23.1)	-	0.158	0.330
Visual analogue scale pain rating **(VAS),** resting; mean±SD (min-max)	3.94±2.56 (0-8)	1.24±1.48 (0-4)	1.17±1.62 (0-5)	0.56±1.2 (0-4)	0.001	<0.001	<0.001
Visual analogue scale pain rating **(VAS),** movement; mean±SD (min-max)	7.33±1.72 (4-10)	3.82±2.07 (1-7)	3.67±2.03 (0-7)	3.17±1.98 (0-7)	<0.001	<0.001	<0.001





(Patient dG15) Coronal (A) and sagittal (B) MR images show complete loss of articular cartilage of the medial femoral and tibial condyles (ICRS grade IV chondromalacia), thinning and shallow fissures of the articular cartilage in the lateral femoral and tibial condyles (ICRS grade IV chondromalacia), edge osteophytes and joint effusion. MR images with the dGEMRIC index values at four time points (T0: baseline, T3: three months after Ad-MSC application; T6: six months after Ad-MSC application; T12: twelve months after Ad-MSC application) (C1-C4). The scheme of the dGEMRIC index with different joint facets throughout the study period at T0, T3, T6 and T12 combined with VAS scale ratings in T0, T3, T6, T12 (D).



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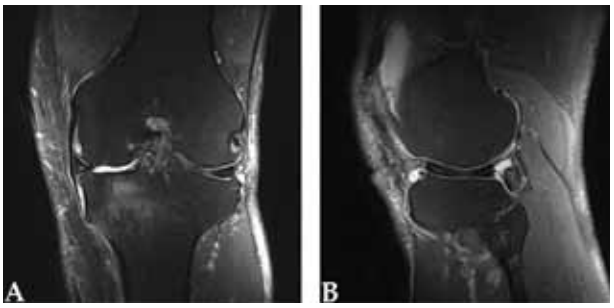

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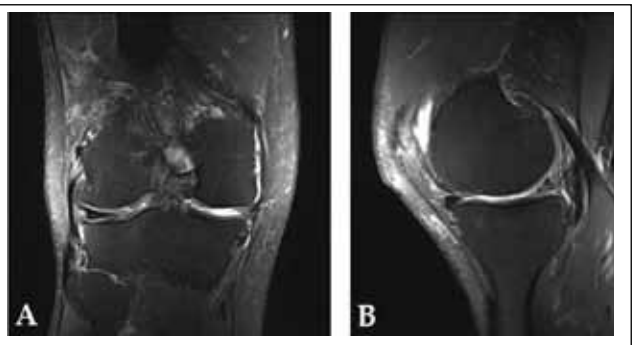

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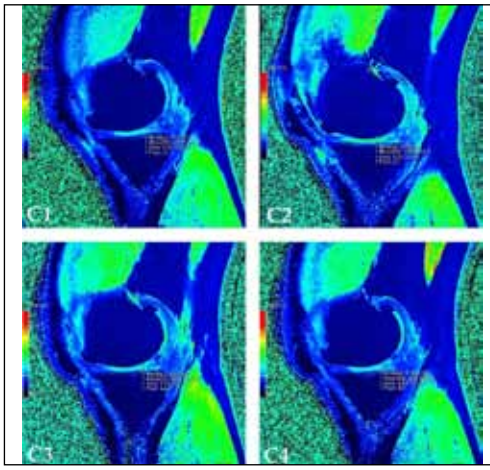

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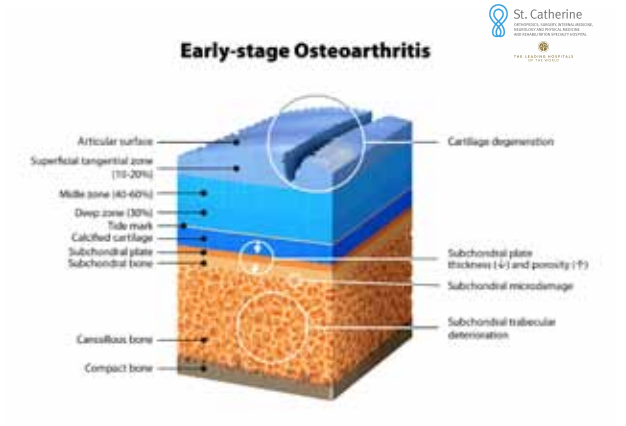
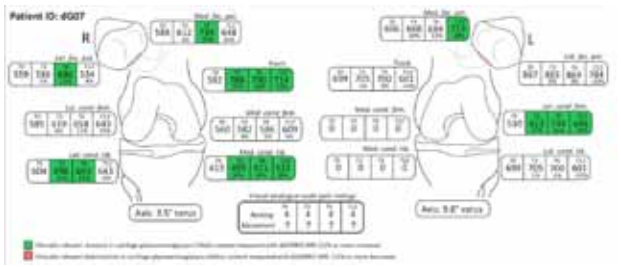
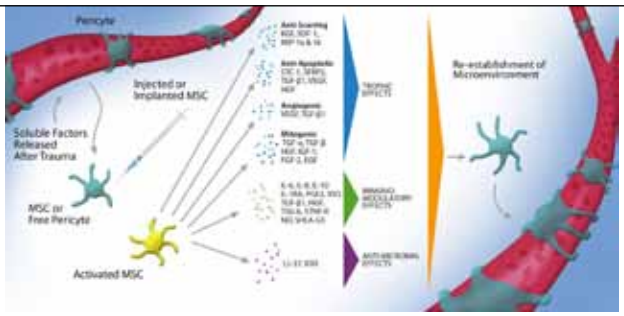
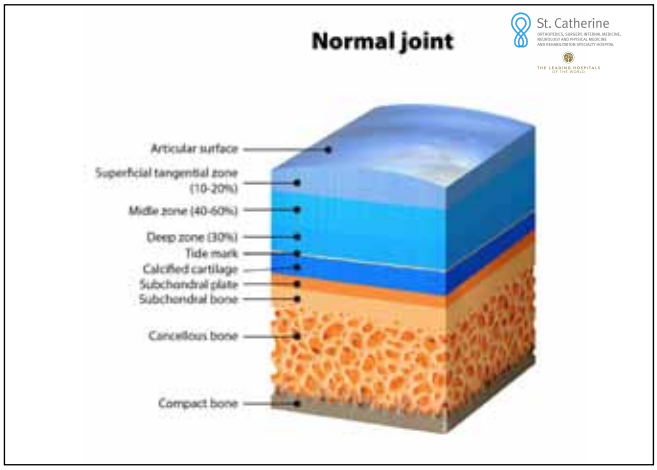






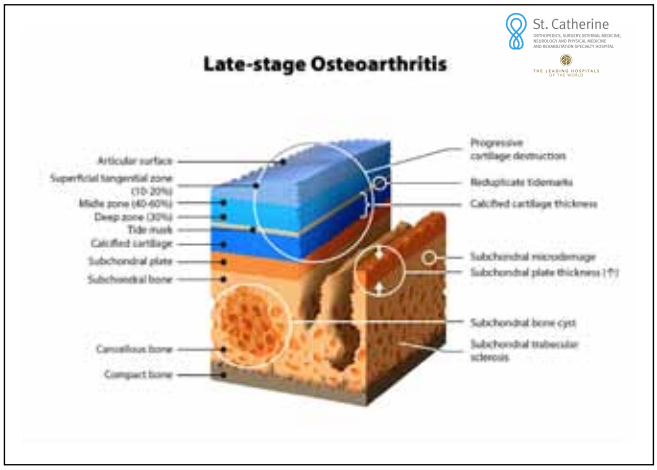
Matthew B Murphy, Kathryn Moncivais, Arnold I Caplan *Experimental & Molecular Medicine* (2013) **45**, e54; doi:10.1038/emm.2013.94 (**modified illustration**)



From these promising *in vitro* results, it may be speculated that an injectable autologous biologically-active scaffold (lipoaspirate), employed intra-articularly, may:

1. Become a fibrous tissue that provides mechanical support for the load on the damaged cartilage;
2. Induce host chondrocytes to proliferate and produce ECM;
3. Provide cells at the site of injury, which could regenerate or repair the damaged or missing cartilage.

Bosetti M, Borrone A, Follenzi A, Messaggio F, Tremolada C, Cannas M.Human lipoaspirate as autologous injectable active scaffold for one-step repair of cartilage defects. Cell Transplantation, The Regenerative Medicine Journal. In press.



St. Catherine

Prof. Dragan Primorac, M.D.,Ph.D.
Global Ambassador of The Penn State University
President of the Board of Trustees - St. Catherine Hospital

Eberly College of Science, The Pennsylvania State University, USA
The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, USA
Xi'an Jiaotong University, College of medicine and forensics, China

University of Rijeka, Medical School
University of Split, Medical School
University of Osijek, Medical School

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draganprimorac.org

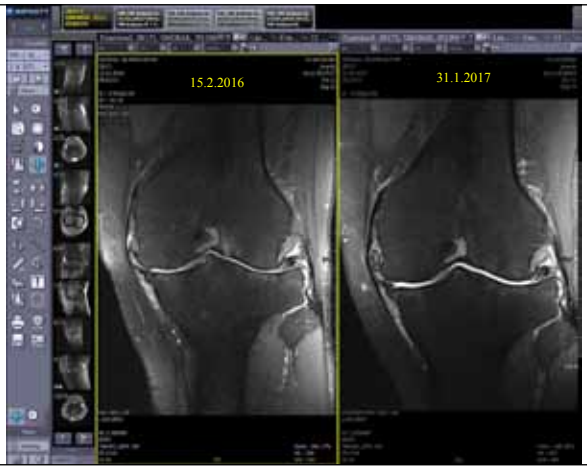
DP
The Office of Dragan Primorac

The International Cartilage Repair Society has set up an arthroscopic grading system by which cartilage defects can be ranked:

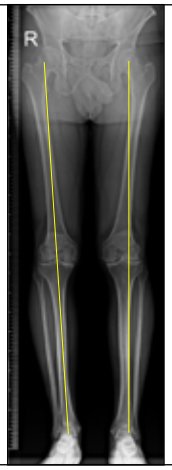
grade 0: (normal) healthy cartilage
grade 1: the cartilage has a soft spot, blisters, or superficial wear
grade 2: minor tears of less than one-half the thickness of the cartilage layer
grade 3: lesions have deep crevices of more than one-half the thickness of the cartilage layer
grade 4: the cartilage tear is full thickness and exposes the underlying (subchondral) bone

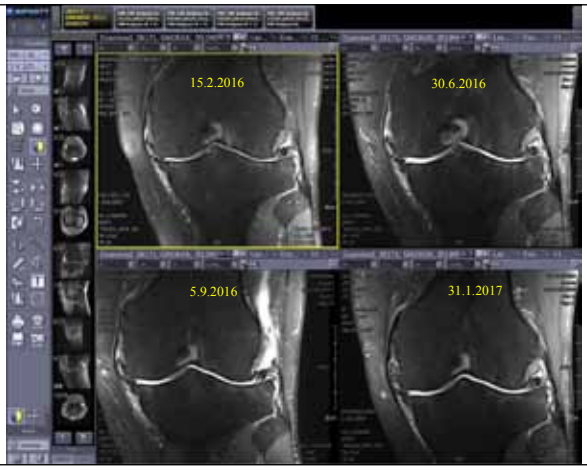
St. Catherine
ORTHOPEDICS, SURGERY, INTERNAL MEDICINE, NEUROLOGY AND PHYSICAL MEDICINE AND REHABILITATION SPECIALTY HOSPITAL
THE LEADING HOSPITALS OF THE WORLD

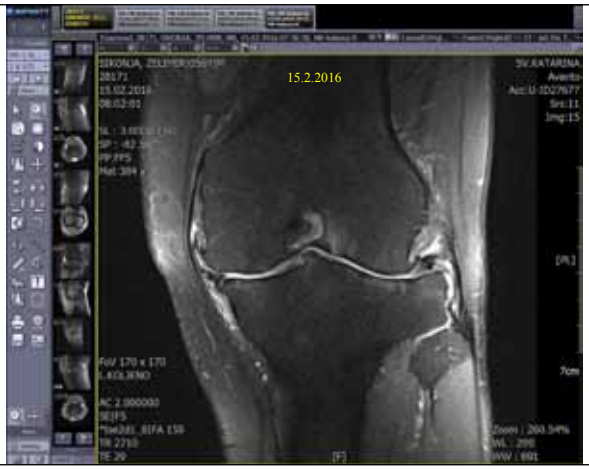
Personalized medicine approach
in
St. Catherine Hospital



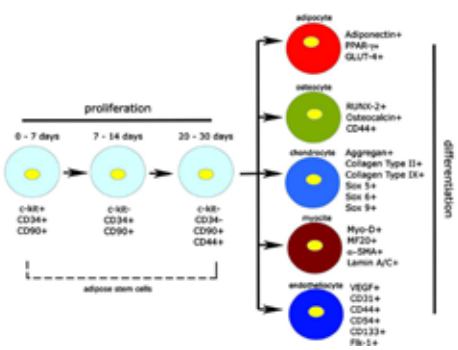
Patient Mr. Želimir Šikonja



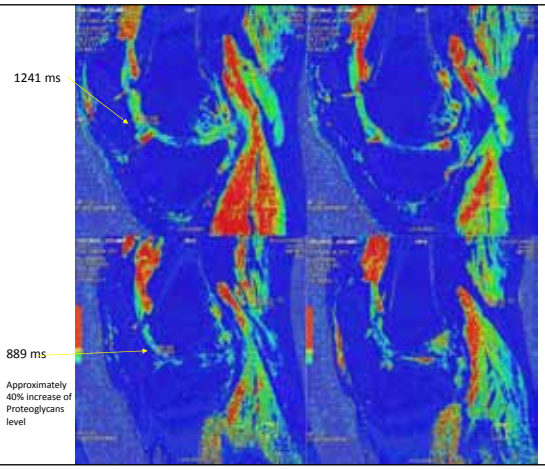


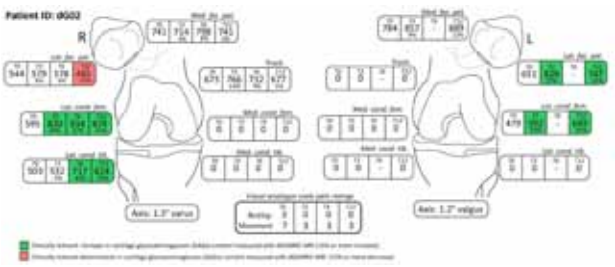


Schematic representation of ASCs proliferation and differentiation potency

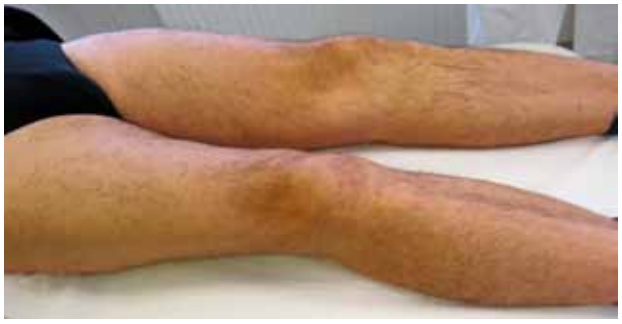


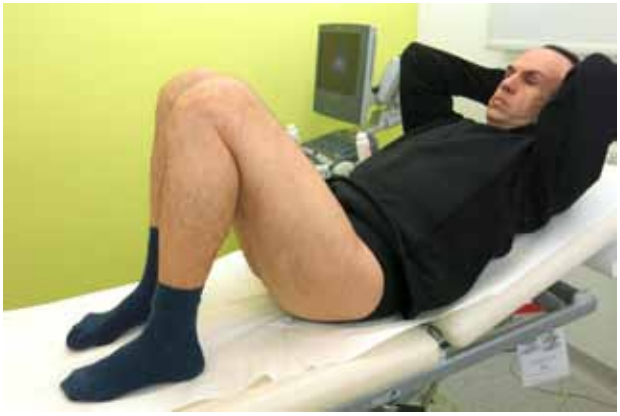
Published in *Tissue Engineering Part B: Reviews*. December 2015, Online Publication Date: June 16, 2015, 21(6): 572-584.
DOI: 10.1089/ten.teb.2014.0608



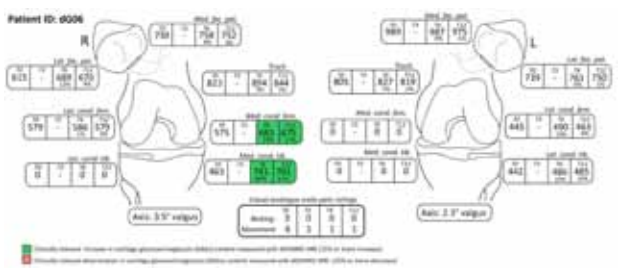


Šikonja





Hrkač



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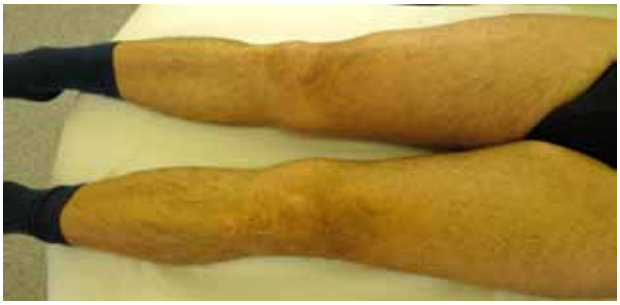
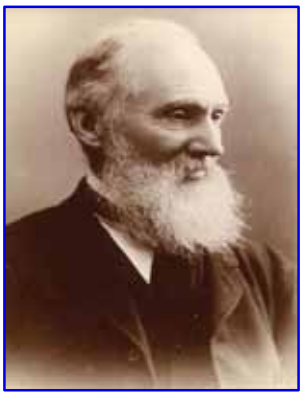


Sv. Katarina
SPECIJALNA BOLNICA ZA ORTOPEDIJU, KIRURGIJU, INTERNU MEDICINU, NEUROLOGIJU, FIZIKALNU MEDICINU I REHABILITACIJU
THE LEADING HOSPITALS OF THE WORLD

DP
Dragan Primorac

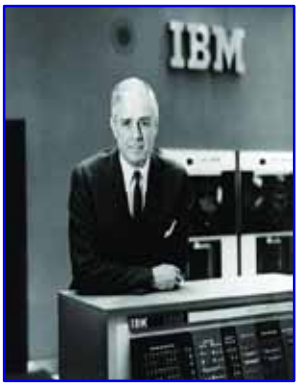
Pursuant to Horizon 2020 Advisory Group, personalised medicine has been defined as **"a medical model using characterization of individuals' phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention".**



"X-rays will prove to be a hoax."

Lord Kelvin
President of the Royal Society, 1883.

Personalised medicine refers to a medical model using characterization of individuals' phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring **the right therapeutic strategy for the right person at the right time,** and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention. Horizon 2020 Advisory Group



"I think there is a world market for maybe five computers."

Thomas Watson, Chairman of IBM, 1943

PRACTICE OF MEDICINE TOMORROW

- Personalized or individualized medicine
  - Prevent disease
  - Early diagnostics
  - Apply therapy appropriate to the patient
  - Screen family members for risk

President Barack Obama dedicated $215 million in his upcoming 2016 budget to a research initiative aimed at helping doctors develop personalized medical treatments for their patients.

INTERNATIONAL CONSORTIUM OF PERSONALIZED MEDICINE (ICPerMed)
(Supported by European Commission)

(Founded, on October 6, 2015)

(ICPerMed)

Personalized medicine:
THE FUTURE IS NOW

CROATIAN COMPETITIVENESS CLUSTER FOR PERSONALIZED MEDICINE
(With support of the Agency for Investments and Competitiveness)

(Founded on November 11, 2015)

Challenges:

1.Developing Awareness and Empowerment,
2.Integrating Big Data and ICT Solutions,
3.Translating Basic to Clinical Research and Beyond
4.Bringing Innovation to the Market
5.Shaping Sustainable Healthcare

PerMed Consortium

MAYO CLINIC

PERSONALIZED MEDICINE CONCEPT

The Biomarker Discovery Program captures genetic information from cells and analyzes it, searching for genetic patterns to help physicians make more precise diagnoses and prescribe more effective, individualized treatments.

The Microbiome Program explores the genetic code of the body's microorganisms, using the latest techniques to profile an individual's microbiome to detect, prevent and diagnose infections and other diseases.

The Pharmacogenomics Program investigates how variations in genes affect response to medications, thereby using a patient's genetic profile to predict a drug's efficacy, guide dosage and improve patient safety.

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"The best way to predict your future is to create it."

Abraham Lincoln

MAYO CLINIC

PERSONALIZED MEDICINE CONCEPT

Genomic sequencing is a process for analyzing a sample of DNA taken from your blood. In the lab, technicians extract DNA and prepare it for sequencing.

The Clinomics Program quickly moves discoveries from the research lab to the clinical setting, with practical, cost-efficient genomic tests for diagnosing and treating patients.

The Epigenomics Program investigates the role of the epigenome, examines which factors act on individual genes, and how certain changes in the epigenome affect our health.

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"We can't solve problems by using the same kind of thinking we used when we created them."
Albert Einstein

"Insanity: doing the same thing over and over again and expecting different results."
Albert Einstein

BEST HOSPITALS
U.S.News & WORLD REPORT
RANKINGS

U.S. NEWS HOSPITALS RANKINGS AND RATINGS (2015)

1.Mayo Clinic
2.Cleveland Clinic
3.Massachusetts General Hospital

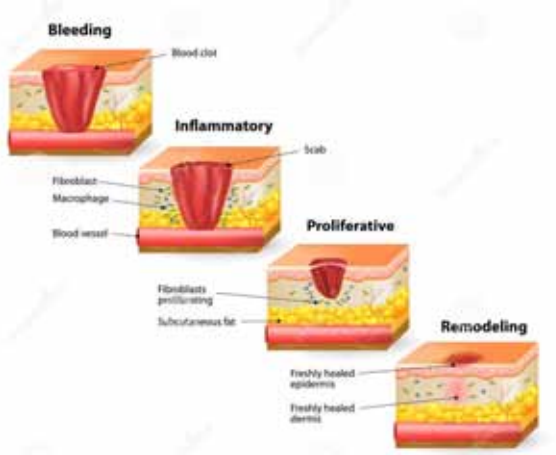
REGENERATIVE MEDICINE

Is the greatest chalange in medicine in the history of human kind.

The idea of treating disease with cells is an antique one. As far back as 3000 years ago, skin grafting is beleived to be performed in India.

The world's first sucessful kidney transplant happened in 1954, folowed with first bone marrow transplant in 1956.

Tissue Engineering

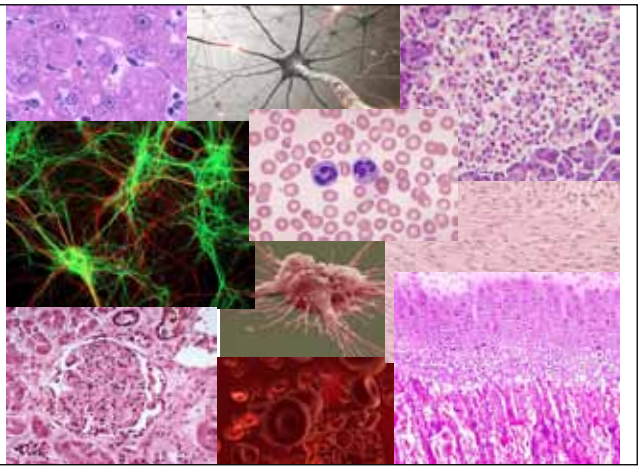


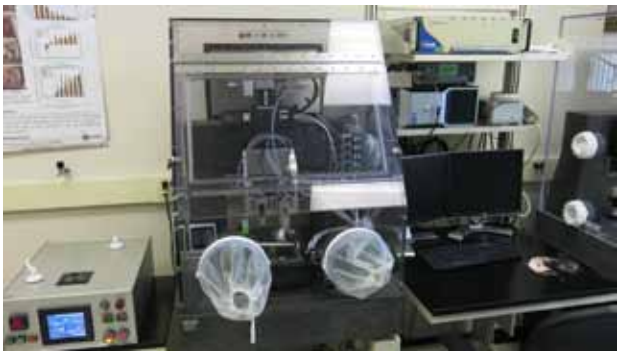
"Average person" (30 years old, 70 kg in weight, 1.7 m in height, 1.85 m2 in width of body surface) has approximately 3.72 x 10^{13} cells, or 37.2 trillion cells.

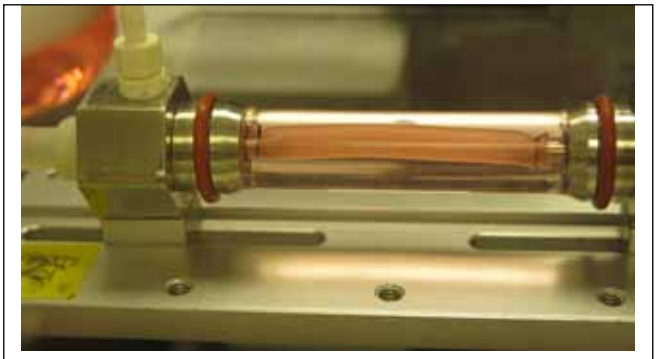
Eva Bianconi et all. An estimation of the number of cells in the human body. Annals of Human Biology. Volume 40, Issue 6, 2013

Anthony Atala (The Wake Forest Institute for Regenerative Medicine, Wake Forest University, Winston-Salem, NC, USA)

Growing human organs







St Catherine's Hospital is among 9% of top European Hospitals that won FP-7 project

St. Catherine

GENOS

SEVENTH FRAMEWORK PROGRAMME

Multi-dimensional omics approach to stratification of patients with low back pain

Work programme topics addressed: **HEALTH.2013.2.2.1-5: Understanding and controlling pain. FP7-HEALTH-2013-INNOVATION-1**

Chronic low back pain (CLBP) is a pressing clinical problem

Acute low back pain is one of the most common reasons for adults to consult with a family physician and **the majority of people (87 % ili 435 million inhabitants in EU) will experience back pain at some point in their life**.

About 10-15% of these patients (65 million) develop chronic symptoms (defined as pain that persists 3 months or more).

In Europe, more than 40% of adults suffer from at least one episode of low back pain (LBP), with temporary inability to work (**200 million inhabitants**).

In Europe the economic burden of CLBP is estimated to be **1-2% of GDP**

Multi-dimensional omics approach to stratification of patients with low back pain

Participant no.	Participant organisation name	Country
1 OSM (Coordinator)	Fondazione IRCCS San Matteo Hospital	Italy
2 HOL	Hospital Oost-Limburg	Belgium
3 ST-CAT	St Catherine Hospital	Croatia
4 UNIPR	University of Parma	Italy
5 KCL	Kings College London	UK
6 HMGU	Helmholtz Center Munich	Germany
7 YuriA	Yuri Aulchenko	Netherlands
8 Genos	Genos Ltd	Croatia
9 IPRC	IP research consulting SASU	France
10 CPI	Carolinas Pain Institute	USA

Global medical tourism market

Report"***Medical Tourism Market - Global Industry Analysis, Size, Share, Growth, Trends and Forecast 2013 - 2019***". The global medical tourism market is US$10.5 billion in 2012 and it will reach a market value of US$32.5 billion by the end of 2019.

Participant number in this project	Participant short name	Estimated eligible costs (whole duration of the project) RTD / Innovation (A)	Demonstration (B)	Management (C)	Other (D)	Total A+B+C+D	Requested EU contribution
1	OSM	1,194,000.00	0.00	96,400.00	11,000.00	1,299,000.00	1,002,000.00
2	HOL	429,600.00	0.00	0.00	0.00	429,600.00	322,800.00
3	St-Cat	869,200.00	0.00	2,000.00	22,400.00	894,100.00	669,200.00
4	UNIPR	460,840.00	0.00	20,000.00	20,000.00	510,840.00	474,000.00
5	KCL	877,632.00	0.00	3,500.00	22,400.00	903,532.00	524,124.00
6	HMGU	686,152.00	0.00	4,200.00	0.00	690,352.00	702,364.00
7	YuriA	441,731.20	0.00	0.00	22,268.00	464,031.20	324,498.00
8	GENOS DOO	1,150,440.00	0.00	84,300.00	43,200.00	1,277,940.00	990,300.00
9	PHASTCOMM	470,200.00	0.00	3,500.00	0.00	473,700.00	669,000.00
10	CPI	190,180.00	0.00	3,500.00	22,400.00	216,080.00	161,020.00
TOTAL		7,470,536.20	0.00	226,900.00	191,268.00	7,888,736.20	5,998,006.00

St. Catherine hospital and private health care insurances

Croatia zdravstveno osiguranje

vanbreda

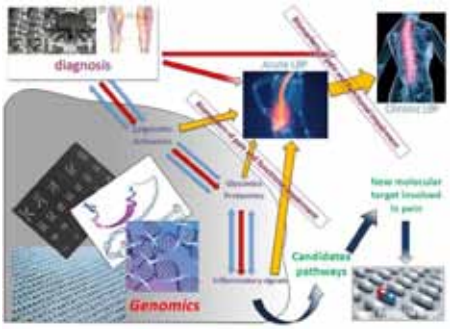
UNIQA

GBG Global Benefits Group

GRAWE

Project "Multi-dimensional omics approach to stratification of patients with low back pain" objectives:

- To perform a large retrospective study and **identify multiple "omics" biomarkers for stratification of patients with chronic LBP**,
- To validate **identified biomarkers for progression of acute to chronic LBP** in a prospective study,
- To validate **identified biomarkers and test their heritability** in a large twin cohort,
- To identify **pathways and relevant individual variations for generation, propagation and quenching** of pain



Availability to all who need our service

BEECHCRAFT KING AIR C90

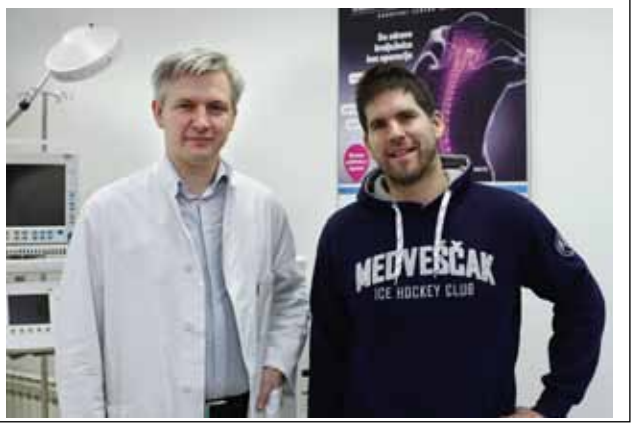
Not only a teaching hospital, the beautiful and very modern St. Catherine's Hospital is a European center of excellence for advanced imaging, orthopedics, spine surgery treatment of pain (pain management), and sports medicine. Most of the hospital physicians are forensic expert witnesses in the fields of orthopedics, neurosurgery, neurology, and spine surgery.

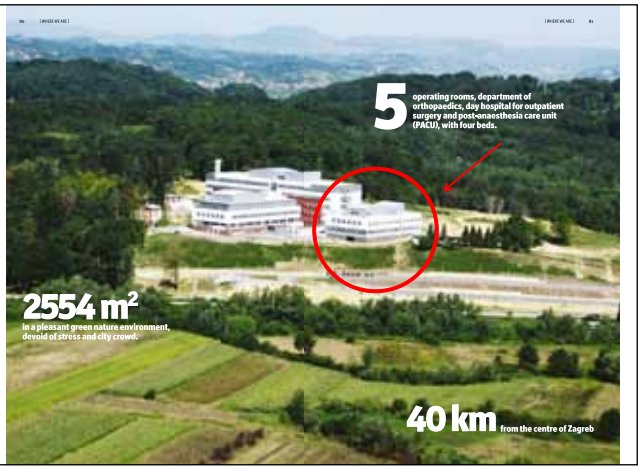
Academy News, September 2015.

Irish Patients' Association

"Thank you for the excellent care during my trip to St. Catherine's. I was very impressed with your professional services and staff, as well as St. Catherine's excellent facilities. The Disc Biacuplasty procedure went very well, and I was fully recovered in less than two days." **Donald Ward**, Ireland (April, 8.2014)

Canadian hokey player Mathieu Carle





"After the surgery at the St. Catherine Hospital, I feel great. I do not feel any pain and I hope for fast recovery, even faster than that we anticipate. Of course I wish immediately return to the ice, like an athlete I can say that is the most important! I'm still not completely relaxed, which is logical after spine surgery, but I was surprised how well I feel and that I can walk and sit! **Mathieu Carle, KHL Medveščak** (March 9, 2014)

St. Catherine Hospital is fascinating institution and just several days after PRP treatment for the very first time my elbow pain disappeared.
Garry Kasparov, chess Grandmaster and a former World Chess Champion

"It was our desire to have medical care for Croatian team players under one roof, and that's why we are pleased that we have found a partner who, like us, is striving to be the best in what he does. As our players achieve global results, the hospital of St. Catherine in a short time has become a center of excellence for sports medicine. As a former player, I know how the health and medical care are important to every football player and I have no doubt that this contract will guarantee our players the best possible service in this context . When we talking about the hospitals , all that I can say is that St. Catherine Hospital is Space Shuttle among hospitals**!"**

Davor Šuker, President of the Croatian Football Federation and former player of Real Madrid, Arsenal, West Ham United and 1860 Munich. During during the 1998 World Cup in France he won the Golden Boot by scoring 6 goals in 7 matches. (March 23, 2014)

US OPEN 2014

National Basketball Association
NBA trust us

Bogdan Bogdanović
(Brooklyn Nets)

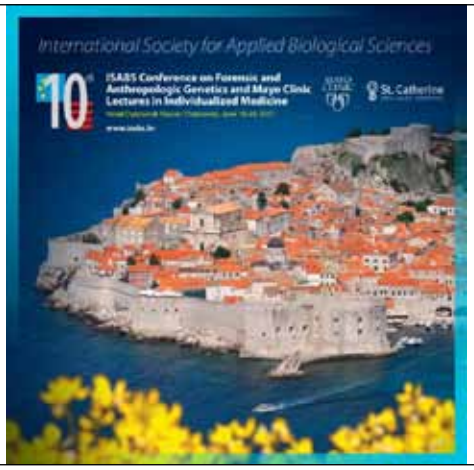
Maria Clara Salgado Rufino
(Brasilian Volleyball Team)

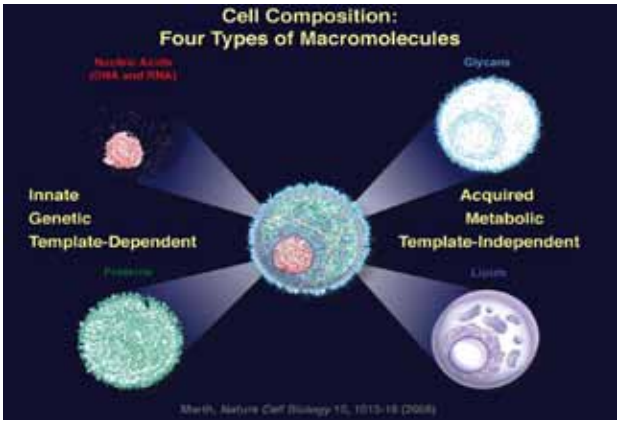
Ivan Rakitić
(Barcelona)

Since February, 2014.....

The Leading Hospitals of the World

Mario Mandžukić
(Juventus, Italy)



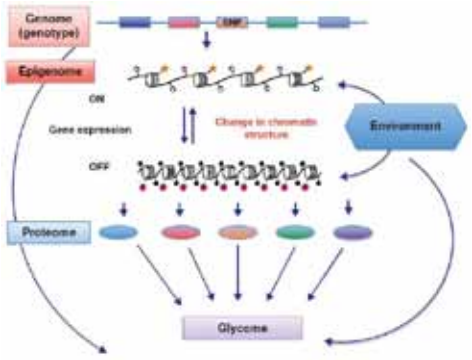


Transplantation experiments and deep sequencing suggest that inactivating mutations in *Pax5* promote leukemogenesis by creating an aberrant progenitor compartment that is susceptible to malignant transformation through accumulation of secondary *Jak3* mutations.

Thus, treatment of *Pax5*$^{+/-}$ leukemic cells with specific JAK1/3 (Janus kinase (JAK) family of tyrosine kinases involved in cytokine receptor-mediated intracellular signal transduction) inhibitors resulted in increased apoptosis. These results uncover the causal role of infection in pB-ALL development.

Alberto Martín-Lorenzo et al. Cancer Discov 2015;5:1328-1343

Genes an environment integrate at the level of protein glycosylation



Childhood B-cell precursor acute lymphoblastic leukemia (pB-ALL) is the most common cancer in childhood. The overall cure rate is excellent (approximately 90%); however, treatment is associated with severe toxic side effects and long-term sequelae, and 20% of children still relapse and may later succumb to their disease.

The role of epigenetics in personalized medicine

How infection can cause leukaemia?
Nature 526, 167 (October 8, 2015)

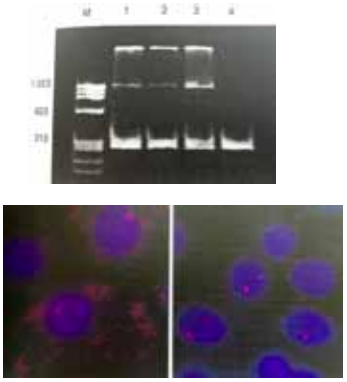
Children with precursor B-cell acute lymphoblastic leukemia often have mutations in the *PAX5* gene, which is involved in immune-cell development, but the mutations alone do not cause the disease.

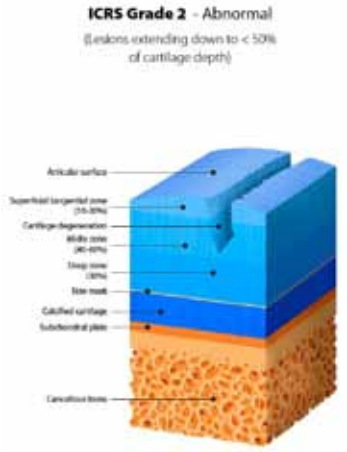
How infection can cause leukaemia?
Nature 526, 167 (October 8, 2015)

Arndt Borkhardt (University of Dusseldorf) and Isidro Sanchez Garcia (University of Salmanca) exposed mice with the **lymphoid transcription factor gene mutations - Pax5** mutations (Loss of function mutation) to common pathogens. The mice developed cancer B-cell precursor acute lymphoblastic leukemia (pB-ALL), whereas Pax5 mutant mice kept in a sterile environment did not.

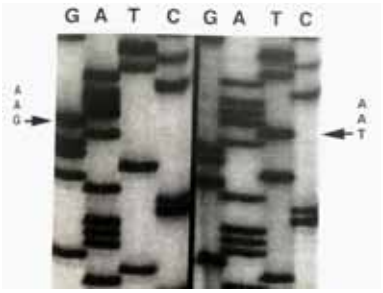
By sequencing **tumor DNA** from diseased mice, the team **found extra mutations (Jak3) probably caused by infection in genes encoding signaling proteins that help to regulate cell growth.**

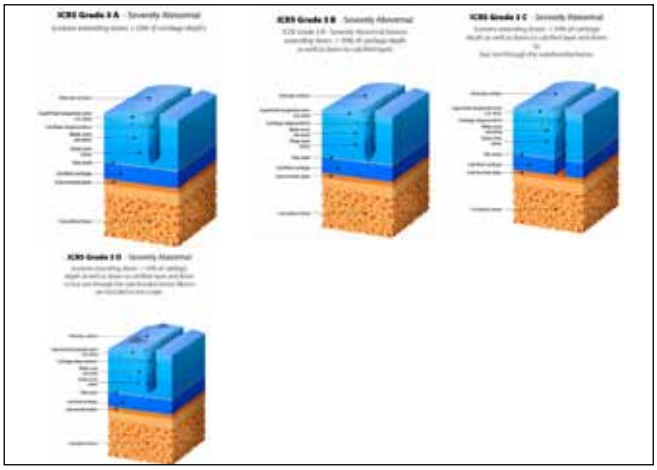
DP
The Office of Dragan Primorac

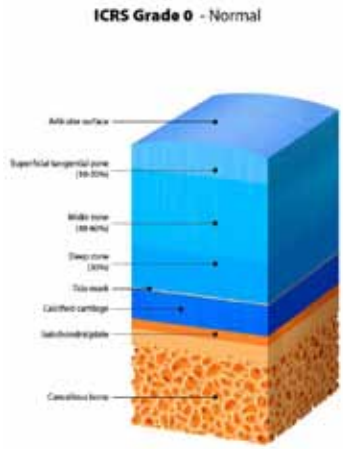


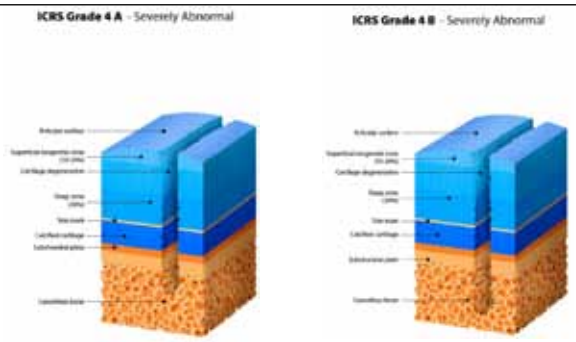


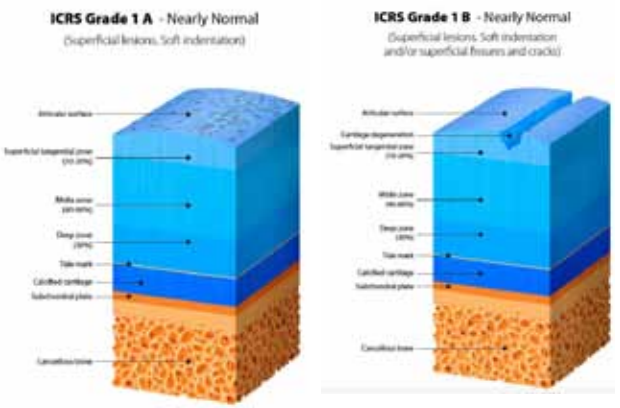
Nanomelia











Nobel Spirit round table debate, Dubrovnik, June 21, 2017

SESSION 1

Moderators: Draga Toncheva, Rumen Stefanov

- **Genomic medicine in health care systems: the time for change Kanay Yararbas Interpreting next generation sequencing and array data of patients with rare conditions**
  B. Peterlin

- **Interpreting next generation sequencing and array data of patients with rare conditions**
  K. Yararbas

- **Rare diseases in Turkey**
  U. Ozbek

- **Next generation clinical genetics: genotype first or phenotype first approach**
  K. Writzl

- **Next Generation Sequencing Introduction by Illumina**
  T. Zamfirov

GENOMIC MEDICINE IN HEALTH CARE SYSTEMS: THE TIME FOR CHANGE

Borut Peterlin

Challenges for implementing genomic medicine

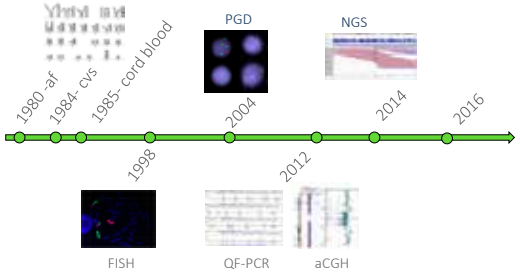
- Limited evidence of benefit/value
- Lack of standards for genomic aplications
- Lack of diagnostic guidelines
- Lack of institutional/clinican acceptance
- Limited access to expertise and testing
- Lack of reimbursement

Aims and scope of genetic services

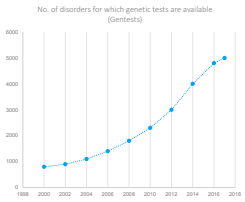
The aim of a genetic service is to respond to the needs of individuals and families who are threatened by genetic disease, particularly their wish to know whether or not they are at risk of developing or transmitting a disorder with a genetic component.

Provision of genetic services in Europe: current practices and issues
European Journal of Human Genetics (2003)

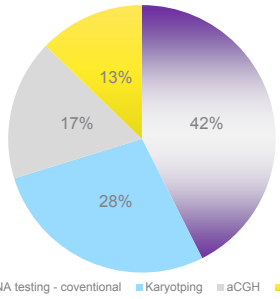
Implementation of genetic testing in Slovenia



Provision of genetic testing: challenge for health systems



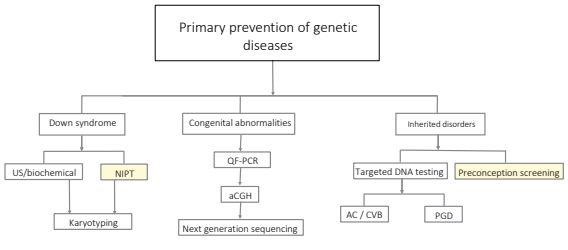
Genetic testing at CIMG



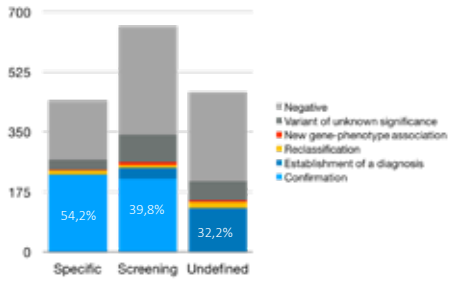
Access to genetic testing in EU

- Differences between the MS are huge in allowing / financing cross border testing
- Permission for cross border testing requires long processes in some MS
- Laboratories have difficulties in collecting payments from abroad

EUCERD Joint Action WP8



Diagnostic yield of NGS testing at CIMG

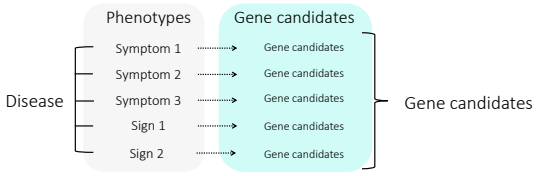


Clinical institute of Medical Genetics University medical centre Ljubljana

ORIGINAL RESEARCH ARTICLE Genetics inMedicine

Phenotype-driven gene target definition in clinical genome-wide sequencing data interpretation

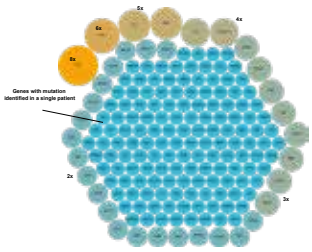
Maver Ales, MD[1], Lovrecic Luca[1], Volk Marija[1], Rudolf Gunsel[1], Writzl Karin[1], Blatnik Ana[1], Hodzic Alenka[1] and Borut Peterlin[1]



Clinical institute of Medical Genetics University medical centre Ljubljana

12th Balkan Congress of Human Genetics 8th National Conference for Rare Diseases

8-10 September 2017, Plovdiv, Bulgaria



Clinical institute of Medical Genetics University medical centre Ljubljana

ORIGINAL RESEARCH ARTICLE Genetics inMedicine

Comprehensive use of extended exome analysis improves diagnostic yield in rare disease: a retrospective survey in 1,059 cases

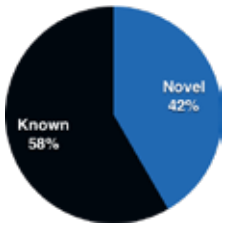
Gaber Bergant, MD[1], Ales Maver, MD, PhD[1], Luca Lovrecic, MD, PhD[1], Goran Čuturilo, MD, PhD[2,3], Alenka Hodzic, PhD[1] and Borut Peterlin, MD, PhD[1]

Clinical institute of Medical Genetics University medical centre Ljubljana

12th Balkan Congress of Human Genetics 8th National Conference for Rare Diseases

8-10 September 2017, Plovdiv, Bulgaria

Reported clinically relevant variants in
CIMG database (n=596 variants, submitted to ClinVar)



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Predictive factors for NGS yield

Predictor	NGS positive	NGS negative
Mean age of onset	18	34
Family history	11%	4%
Severity of disease	More severe	Less severe
Specific diagnosis	72%	65%

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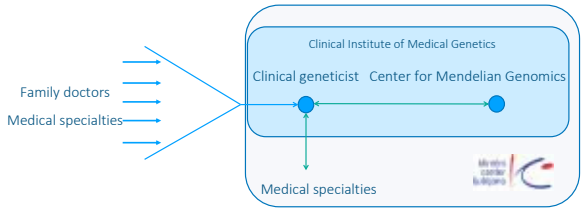
Clinical pathway for NGS testing at CIMG



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Conclusions – genomic technologies into health systems

- Improved access to genetic diagnosis
- Increased diagnostic efficacy
- Improved economic efficacy
- Competence and resources first

Clinical institute of Medical Genetics University medical centre Ljubljana

POLICY

Whole-genome sequencing in health care

Recommendations of the European Society of Human Genetics

EJHG Open

POLICY

Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening

EJHG Open

POLICY

Responsible implementation of expanded carrier screening

Conclusions – genomic technologies for 4P medicine

- 4 P medicine is not "wishful thinking"
- Public health services: opportunity / duty?

ACMG STATEMENT | Genetics inMedicine

Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics

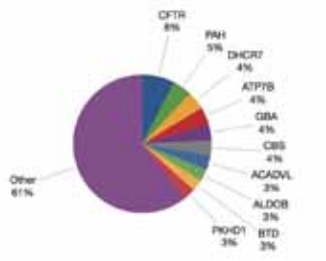
2.8 (5.8) %

Invitation

- Balkan genome variability platform
- Public genetic services for 4 P medicine

Prevalence of carriers in Slovenian population (186 genes for severe recessive disorders)

54%



12th Balkan Congress of Human Genetics

8th National Conference for Rare Diseases

8-10 September 2017, Plovdiv, Bulgaria

Acknowledgments

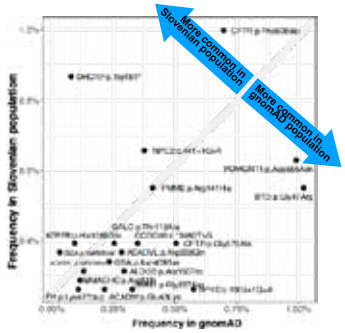
Center for Mendelian Genomics

Alenka Hodžić
Gaber Bergant
Lovro Vidmar
Aleš Maver

Clinical team - CIMG

Karin Writzl
Gorazd Rudolf
Marija Volk
Luca Lovrečić

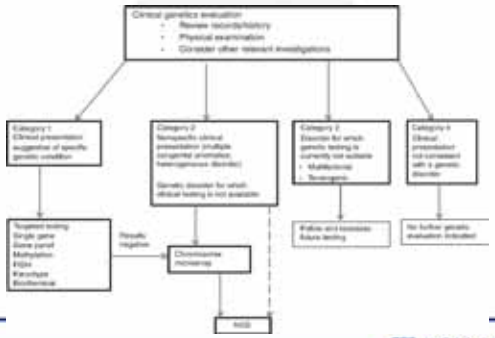
Slovenian population has a distinct profile of carrier frequency in comparison with world populations (gnomAD)



INTERPRETING NEXT GENERATION SEQUENCING AND ARRAY DATA OF PATIENTS WITH RARE CONDITIONS

Kanay Yararbaş, Yasemin Alanay, Uğur Özbek

How?



Shashi V 2014 Gen Med

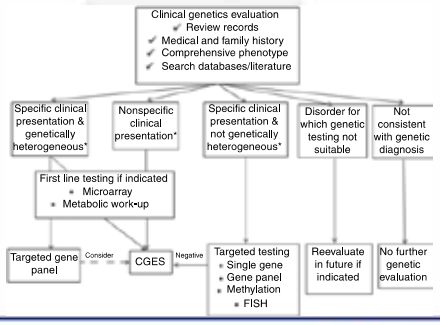
ACIBADEM

Topics

- 2 questions: Who and How?
- Array and NGS as combination test?
- Exome or panel testing: Which is effective?
- Rare conditions = Undiagnosed cases = Unknown variants: What to do?
- Clinical reporting

ACIBADEM

How?



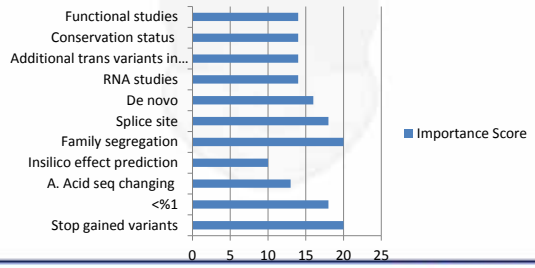
Genetics in medicine | Volume 18 | Number 11 | November 2016

ACIBADEM

2q: Who and How?

ACIBADEM

How?
Prediction of variant pathogenicity



Genetics in medicine, 2015: ACMG Standarts and Guidelines

ACIBADEM

Who could perform these tests?

- Competent lab: ISO 15189
- Competent tesing:
  - Proficiency testing, validation and verification, quality control procedures
  - External quality assessment
- Competent stuff:
  - Experience!
  - Team work: MDs and nonMDs. Technique and Clinic!

Ann Lab Med. 2017 Sep;37(5):365-370

ACIBADEM

Array Variants

- Size: 100Kb gain, 200Kb loss postnatal 750K postnatal diagnostic array
- Gene content
  - Morbid OMIM genes
- **Clinical relevance**
- De novo / familial segragation
- Enlarging variants
- Database search: Decipher etc
- …

Cytogenet Genome Res. 2011;135(3-4):212-21

ACIBADEM

Array and NGS as combination test?

Array vs WES

Array Karyotyping vs Exome Sequencing

- **Different applications have different requirements**: Copy number variants vs sequence variants
- Whole genome seq allows genome-wide CNV detection: 4000 dollars per genome!!

Physiol Genomics. 2013 Jan 7;45(1):1-16

Take home message

- Choose array for
  - ID,
  - DD,
  - ASD
  - Chromosome analysis indications like multiple congenital anomalies

Detecting CNV in Exome Seq

- Coverage problem.
- Technical failure.

Biomed Res Int. 2017;2017:7409598 doi: 10.1155/2017/7409598

Exome or panel testing: Which is effective?

Exome / SNP Genotyping Arrays

- Not effective, because WES:
  - getting cheaper.
  - Efficient.
- Whereas arrays:
  - Effective in GWAS.
  - Expression profiling.

PLoS One. 2016 Nov 15;11(11):e0166628

Panel testing – Targeted ANALYSİS

PROs

- Large panels >100 gene
- Narrow panels <100 gene
- Cost effective

CONs

- Needs phenotype to genotype commenting
- May not detect mixed phenotypes - >1 gene defects

AND GENOME WIDE ANALYSIS – WES vs WGS

– Largest panel of all	vs	full coverage of the genome
– Most efficient targeted analysis	vs	CNV analysis
– Cost effective	vs	NOT YET

Eur J Hum Genet. 2017 Feb;25(3):308-314

Genome-wide Scaling (WES or WGS)

- Useful in
  - heterogeneous conditions, mostly de novo changes might be present in the patients (like Autism Spectrum Disorders)
  - No diagnostic – patognomonic feature, indistinct phenotype
  - More than one phenotype in the same patient

Genet Med. 2015 Jun;17(6)

ACIBADEM

To report or NOT to report

- Generate standart reports: Follow ISO 15189 standarts of your own lab
- Consider (Sanger/MLPA-Q-PCR etc for array) confirmation testing
- Issue whether the reported result is confirming or consistent with the referring reason of a phenotype

J Mol Diagn. 2017 Jan;19(1):4-23

ACIBADEM

Rare conditions = Undiagnosed cases = Unknown variants: What to do?

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Classify and Report

- Class 1:
  - Not pathogenic
  - "Common" polymorphism and therefore not reported
- Class 2:
  - Unlikely to be pathogenic
  - Diagnosis not confirmed molecularly
- Class 3
  - Uncertain pathogenicity
  - Does not confirm or exclude diagnosis
- **Class 4**
  - **Likely to be pathogenic**
  - **Consistent with the diagnosis**
- **Class 5**
  - **Predicted to be pathogenic**
  - **This result confirms the diagnosis**

Dont report

??

Report

Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics

ACIBADEM

Orphan variants of Orphan diseases

Rare variant

- Unpublished
- May be published low freq variant!!
- <1%

Low-frequency variants

- Published
- Pathogenic or mostly unknown significance variants
- 1-5%

Both

- Were not included in previous technologies like custom genotyping arrays; and still NOT in custom sequencing panels
- Most are novel variants, millions are recorded, still emerging

Genome Biol. 2017 Apr 27;18(1):77

ACIBADEM

Thank you..

ACIBADEM

Clinical reporting

ACIBADEM

PRACTICAL AND ORGANIZATIONAL ISSUES OF RARE DISEASES. EXPERIENCE IN TURKEY

Uğur Özbek

How many Rare Diseases?

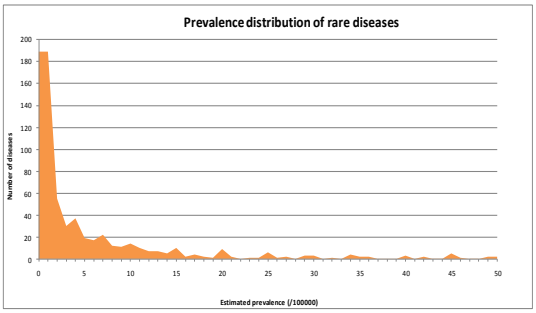
- Depends on the definition
  - Recognisable pattern of signs and symptoms
    - Clinical approach
  - with a unique mechanism
    - Physiopathological approach
  - with unique course and prognosis
    - Clinical evolution approach
  - with a unique response to intervention and treatment
  - with an established cause
    - Genetic origin
    - Environmental origin
- 5,954 distinct phenotypes in Orphanet
- 5-6 new diseases described per month

How many patients?

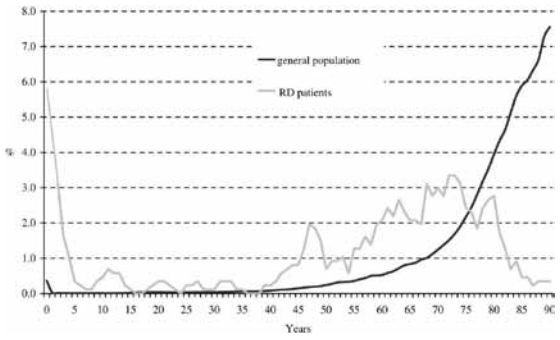
Prevalence of each disease ?

Mostly unknown
Possible sources:
Litterature
Registries

Distribution of prevalence rates



Age at death in patients with rare diseases and in the general population



Mazzucato *et al. Orphanet Journal of Rare Diseases* 2014 **9**:37

Customized Diagnostics

Genetic disease studied in research lab

↓

Candidate gene analyzed

↓

Mutations identified in patients (results relayed in some instances)

↓

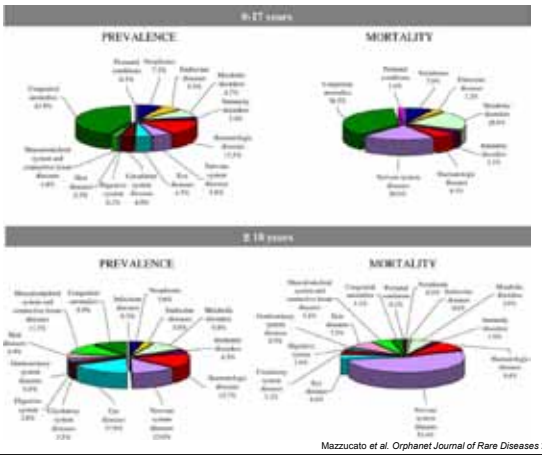
Family members request mutation testing/prenatal testing

↓

Research lab performs test

No test available

Research lab pursues other interest



Mazzucato *et al. Orphanet Journal of Rare Diseases* 2014 **9**:37

Customized Diagnostics

Genetic disease studied in research lab

↓

Mutation identified in patient sample

↓

Mutation confirmed in clinical lab on new sample from patient

(e.g. X-linked myotubular myopathy)

Patient sample received and DNA isolated in clinical lab

DNA aliquot stored in clinical lab

DNA aliquot given to research lab

Mutation confirmed in clinical lab ← Mutation identified in sample

(e.g. Lissencephaly)

Family members request mutation testing/prenatal testing → Clinical lab performs testing

Rare Disease vs Genetic Diseases

- All genetic diseases are rare
- Most rare diseases are genetic (80%) but not all

Customized Diagnostics

Once a disease mutation is identified in a patient, in a research setting, genetic testing for the patient and family members becomes simple

- Technically simple *(single PCR and sequencing)*
- Cost effective *(~$200)*
- Can easily be performed by a clinical laboratory
- Allows genetic testing including prenatal diagnosis to be performed under CLIA regulations

Rare diseases: a chalenge for testing services

Strategies for rare diseases

- **Organization and Duties of the Turkish Ministry of Health"**

- a) Maintaining consistencies in physical and mental well-being throughout the lifespans of individuals;
- b) Improving the equal public health status within the country;
- c) Struggling with threats posed by potentially hazardous factors towards public health;
- d) Regulating the principles related to the establishment, organization and duties of the MOH, with the intent to:
  - i) Plan each and every healthcare body solely under one authority;
  - ii) Ensure a standard supply of healthcare services from these bodies;
  - iii) Provide public access to healthcare services; and,
  - iv) Promote easy and appropriate public access to healthcare services.

- At present, the Turkish Ministry of Health (MOH) has not yet recognized a National Plan with reference to “rare diseases” and “orphan drugs”, as readily defined inside the European Union (EU).
- Health Services are given by MoH (State Universities, Universities, Private Hospitals and Organization) to all the citizens free of of charge in general.

Expert Centers

- These centres can accept referral patients from other centres/cities and state hospitals and are therefore described as ‘reference centres’. For these centres, the Ministry of Health and the social security system covers the invoices of non-private patients.

Funding of actions, regarding rare diseases

Funding of issues concerning RDs in Turkey

- *Prenatal screening- Ministry of Health*
- *Neonatal screening- Ministry of Health*
- *Diagnostic genetic services- Ministry of Health*
- *Treatment (clinical management)- Ministry of Health*
- *Rehabilitation- Ministry of Health*
- *Social care- Ministry of Health*
- *Orphan drugs*

National networking

- Turkey is planning to establish national networks for the prevention, surveillance, diagnosis and treatment of rare diseases. Projects to establish national centres of reference for rare diseases are expected to start by next year?! These centres will be part of the overall planning of healthcare in the country. The Ministry of Health and the different regional healthcare authorities will have to coordinate their approach and harmonise regional network activities

Provision of information for rare diseases

- *Turkish Orphanet website link is available*
- *No specific help line specificallly devoted to RDs*
- *The Orphanet list for RDs available in Turkish*

Research on rare diseases

- *Availability of research programme for RDs:*
  - *Turkish State Planning Organisation (DPT),*
  - *The Scientific and Technological Reseach Council of Turkey (TUBITAK)-*
  - *EU Framework Programmes-E-RARE*
  - *University Research Funds and Private Funds*

Expert Centers

- Though no centres of expertise for rare diseases currently exist, university hospitals and research centres are active in diagnosis and management of rare diseases, (such as: Hacettepe University Ankara) for metabolic and neuromuscular diseases, Istanbul University for neuromuscular diseases and Gazi University (Ankara) for metabolic diseases) with the necessary infrastructure for specialised care (i.e. inpatient beds and outpatients clinics, pathology services, genetic counselling units, genetic testing facilities for post and prenatal diagnosis ,biochemistry, physical therapy units, etc)

Prevention

- *Preimplantation and Prenatal screening available if there is a genetic or medical indication*
- *Nationwide neonatal screening for hypothyroidism, PKU, biotidinase deficiency; cystic fibrosis and thaleassemia*

Empowerment of patients organisations

- *Patients organizations are active for certain diseases although there is an immediate need to cover national rare disease organisation alliance.*
- *Support to the activities of patient organisations*
- *Representation and consultation of patient organisations*

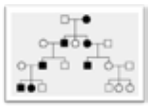
Specialised social services

- *Respite Care Services*
- *Therapeutic Recreational Programmes*
- *Services aimed at the integration of patients in daily life*

NEXT GENERATION CLINICAL GENETICS:GENOTYPE FIRST OR PHENOTYPE FIRST APPROACH

Karin Writzl

Clinical presentation



Familial inheritance pattern

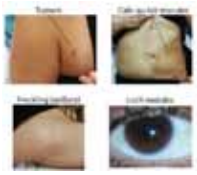
Gene-phenotype association

162200 NEUROFIBROMATOSIS, TYPE I;
61311 NF1

Genetic analysis / Variants' effect

Diagnostic process – traditional approach

Clinical presentation



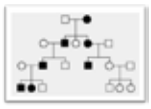
Traditional approach

Clinical presentation

Familial inheritance pattern

Gene-phenotype association

Genetic analysis / Variants' effect



Clinical presentation

Familial inheritance pattern

Traditional approach

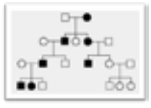
Clinical presentation

Familial inheritance pattern

Gene-phenotype association

Genetic analysis / Variants' effect

Reverse phenotyping



Clinical presentation

Familial inheritance pattern

Gene-phenotype association

NEUROFIBROMATOSIS, TYPE I (OMIM 162200)
NF1 (OMIM 61311)

At birth
37 weeks , C-section (hydronephrosis)
BW 2790 g (25P), BL 49 cm (25P), HC 36.5 cm (95P)

The first year of life
Tetralogy of Fallot
inguinal hernia
paracolic abscess (sigma resection)
intestinal neuronal dysplasia (histology)
hypothyroidism
bilateral pyelectasis

Motor developmental delay
Sat at 18 months
Walked at 7 years
4th class of regular school

Constipation, Hypermobility, Short stature

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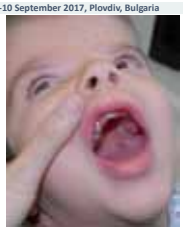
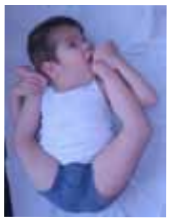
Hypertelorism
Coarse facial features
Full lips



Thickened gums

Striking hypermobility



Thickened eyelids

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Met918Thr in *RET* gene

Detected in a majority of cases with multiple endocrine neoplasia, type 2B (MEN2B)
MEN2B – a hereditary cancer syndrome, associated with early, childhood onset of medullary thyroid carcinoma (MTC)

Camacho et al. 2008

Marfanoid habitus

Donckier et al. 2008

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Diagnostic work-up
- Amino acid screen, growth hormone normal
- MPS not confirmed
- Skeletal X-ray – normal report

- Karyotype normal
- Array CGH – no pathogenic deletions, duplications

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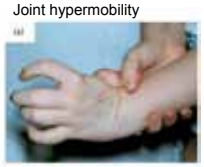
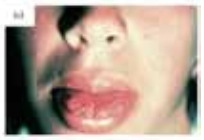
MEN2B has a rich clinical presentation in addition to cancer predisposition

Joint hypermobility





Full lips

Salpietro et al. Int J Endocrinol 2014.

Thickened eyelids

Wray et al. Ann Surg Oncol 2008.

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Diagnostic work-up
- Amino acid screen, growth hormone normal
- MPS excluded
- Skeletal X-ray – normal report

- Karyotype normal
- Array CGH – no pathogenic deletions, duplications
- Exome sequencing in the proband

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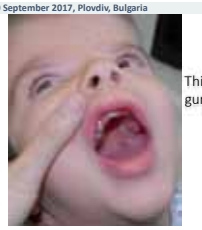
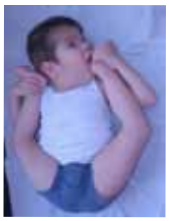
Hypertelorism
Coarse facial features
Full lips ✔



Thickened gums ✔

Striking hypermobility ✔



Thickened eyelids ✔

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Diagnostic work-up
- Exome sequencing in the proband

Gene	Variant	ExAC	Pathogenicity	ClinVar
RET	p.Met918Thr	NP	Damaging	Known pathogenic

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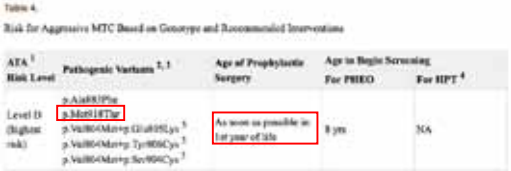
Met918Thr is a variant with one of the highest penetrance for thyroid carcinoma and warrants immediate intervention



ATA Risk Level	Pathogenic Variants	Age of Prophylactic Surgery	Age to Begin Screening: For PHEO	For HPT
Level D (highest risk)	p.Met918Thr p.Val804Met p.Val804Met p.Val804Met	As soon as possible in 1st year of life	8 yrs	NA

Marquard J, Eng C. Multiple Endocrine Neoplasia Type 2. 1999 Sep 27 [Updated 2015 Jun 25]. GeneReviews®

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Exome sequencing in critically ill neonates

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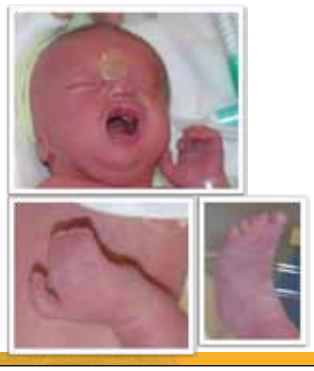
Case 1

- Cryptophtalmus
- Polysyndactyly
- Single hypoplastic kidney
- Genital anomalies
- Laryngeal stenosis



Fraser syndrome

FREM2
NM_207361.4:c.6727C>T
p.Arg2243X

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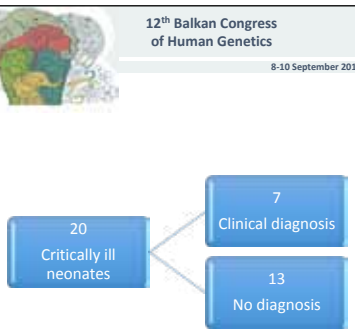

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Case 2

- Sacrococcygeal teratoma
- Hydronephrosis
- Ventricular septal defect

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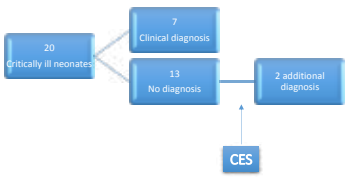
Exome sequencing in critically ill neonates



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Case 2

- Sacrococcygeal teratoma
- Hydronephrosis
- Ventricular septal defect

Schinzel Giedion syndrome

SETBP1
NM_015559.2:c.2614G>A
p.Gly872Arg

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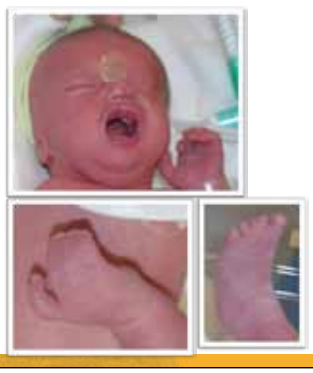
Case 1

- Cryptophtalmus
- Polysyndactyly
- Single hypoplastic kidney
- Genital anomalies
- Larygenal stenosis



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Case 3

- Severe hypotonia
- Generalized joint laxity

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Case 3

- Severe hypotonia
- Generalized joint laxity

Ehlers-Danlos syndrome (EDS), kyphoscoliotic form

PLOD1	NM_000302.3: c.1562G>A Trp521X	Heterozygote
PLOD1	8.9-KB DUP (EX10-16)	Heterozygote

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NICU – clinical diagnosis

Fraser syndrome
Schinzel – Gideon syndrome
Marfan syndrome
EDS – kyphoscoliotic form
Smith-Lemli-Opitz syndrome
Leigh syndrome

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Case 3

Prenatally:

- Mitral and tricuspid valve insufficiency

After the birth

- Bilateral spontaneous pneumothorax
- Crumpled ears
- Arachnodactyly

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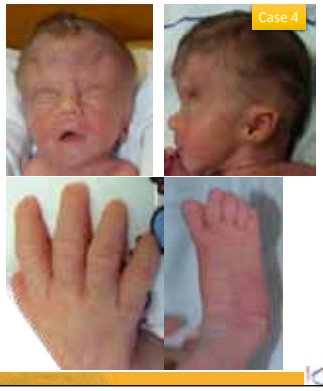
Case 4

- Family history: nil
- Pregnancy: gestational diabetes, IUGR
- Birth (sc at 34 weeks):
  - BW 1390g (0.4P)
  - BL 41 cm (0.4P)
  - HC 29 cm (9P)
- Abnormal shaped head with widely open sutures
- Small distal phalanges, absent nails
- Little subcutaneous fat, loose and wrinkled skin
- Umbilical hernia (small)
- Bilateral criptorchidism



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Case 3

Prenatally:

- Mitral and tricuspid valve insufficiency

After the birth

- Bilateral spontaneous pneumothorax
- Crumpled ears
- Arachnodactyly

Neonatal Marfan syndrome

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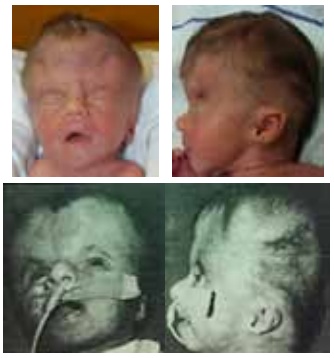
Case 4



Fontaine, J Genet Hum 1977;25:109

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Case 3

Prenatally:

- Mitral and tricuspid valve insufficiency

After the birth

- Bilateral spontaneous pneumothorax
- Crumpled ears
- Arachnodactyly

Neonatal Marfan syndrome

exome[hg19] 15q21.21(48,760,124-48,789,598)x1
Deletion of ex 19-38

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Case 4

•OMIM 612289: PROGEROID SYNDROME, CONGENITAL, PETTY TYPE

- prenatal / postnatal growth retardation
- generalized decreased sucutaneous fat
- bone dysplasia craniosynostosis
  wide fontanels
  small distal phalanges
- unusual face
- often early demise
- AD

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Case 4

Fontaine–Farriaux Syndrome: A Recognizable Craniosynostosis Syndrome With Nail, Skeletal, Abdominal, and Central Nervous System Anomalies

CLINICAL REPORT

American Journal of medical genetics

Petty Syndrome and Fontaine–Farriaux Syndrome: Delineation of a Single Syndrome

Stephen R. Braddock,[1*] Holly H. Ardinger,[2] Chun-Song Yang,[3] Bryce M. Paschal,[3] and Bryan D. Hall[4]

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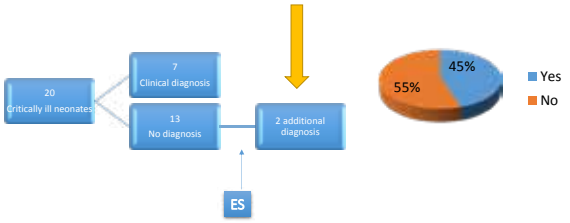
Exome sequencing in critically ill neonates

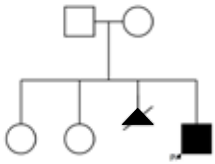


12th Balkan Congress of Human Genetics | 8th National Conference for Rare Diseases
8-10 September 2017, Plovdiv, Bulgaria

Case 4

12th Balkan Congress of Human Genetics | 8th National Conference for Rare Diseases
8-10 September 2017, Plovdiv, Bulgaria

Case 5



- Polyhydramnios
- Lower limb immobility

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Case 4

Writzl et al., submitted to AJHG

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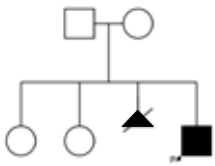
Case 5



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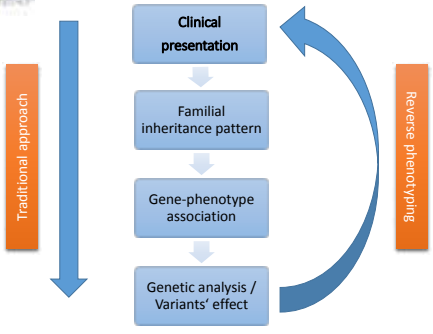

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Case 5

A 31Mb block of homozygosity on chromosome 15

A homozygous truncating variant in **gliomedin** gene in proband and fetus from previous pregnancy
(*GLDN*, p.Trp435X)

Implicated in development of Ranvier nodes in peripheral nerves

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Match-making

Case 5

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Case 5

Family with congenital arthrogryposis

REPORT

Mutations in *GLDN*, Encoding Gliomedin, a Critical Component of the Nodes of Ranvier, Are Responsible for Lethal Arthrogryposis

Survival among children with "Lethal" congenital contracture syndrome 11 caused by novel mutations in the gliomedin gene (*GLDN*)

Jennifer A. Wambach | Georg M. Stettner | Tobias B. Haack | Karin Writzl | Andreja Škofljanec | Alet Maver | Francina Munell

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Case 5

Clinical symptoms and physical findings

RESPIRATORY

MUSCULOSKELETAL

CONNECTIVE TISSUE

Genotype information

LIST OF GENES

GeneMatcher

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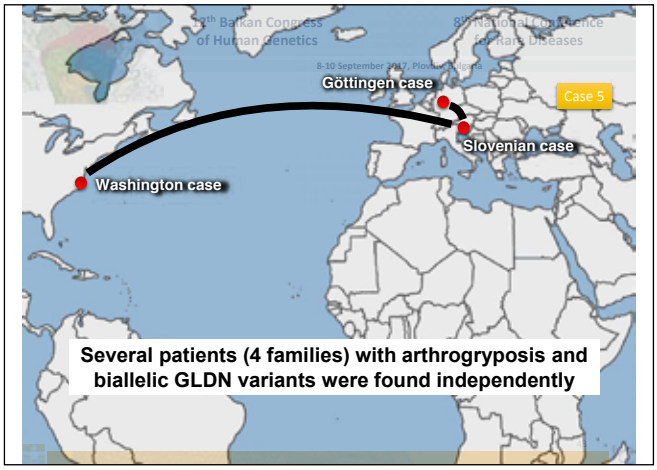
Conclusions

Phenotyping
Pre- an post-testing

Genetic variants
The effect and clinical relevance

Data sharing



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Lili Simeunović
Sabina Žitko

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Luca Lovrečić
Iryna Nikolayeva
Irena Anžel

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Marco Castori (Rome)
Laurence Faivre (Dijon)
Pablo Lapunzina (Madrid)
Andre van Kuilenburg (Amsterdam)

Lidija Kovacic (Dublin)

Jorgina Satrústegui (Madrid)
Araceli del Arco (Madrid)

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Case 5

Family with congenital arthrogryposis

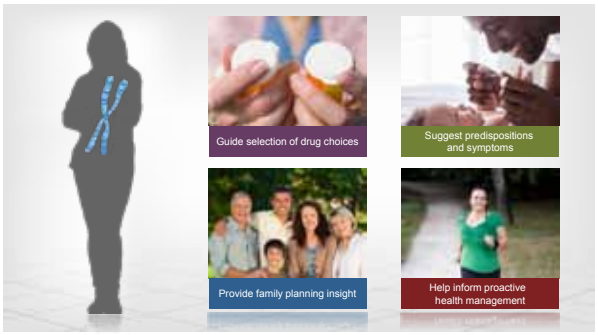
REPORT

Mutations in *GLDN*, Encoding Gliomedin, a Critical Component of the Nodes of Ranvier, Are Responsible for Lethal Arthrogryposis

NEXT GENERATION SEQUENCING INTRODUCTION BY ILLUMINA

Theodor Zamfirov

What Can We Do by Knowing Your Genome?



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Our Background

COMPANY

- Fall, 1998 — FOUNDED
- July 27, 2000 — IPO
- Francis deSouza — President and CEO
- ~5,500 — EMPLOYEES
- San Diego, CA — HEADQUARTERS
- 20 — OFFICES GLOBALLY

FINANCIALS

- $2.40B — 2016 Revenue
- 8% — REVENUE GROWTH YOY

AWARDS

- TOP 10 MOST INNOVATIVE COMPANIES IN BIOTECH — 2017 FAST COMPANY
- 10 BREAKTHROUGH TECHNOLOGIES — 2016 MIT TECHNOLOGY REVIEW
- TOP 3 SMARTEST COMPANIES — 2016 MIT TECHNOLOGY REVIEW
- TOP 10 INNOVATIONS — 2015 THE SCIENTIST MAGAZINE
- 12 MOST DISRUPTIVE NAMES IN BUSINESS — 2013 FORBES
- TOP 10 INNOVATIONS — 2012 THE SCIENTIST MAGAZINE
- FASTEST GROWING TECH COMPANIES — 2010 FORBES

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PARTNERSHIP AROUND THE GLOBE

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Cost Per Genome

$3,000,000,000	Human Genome Project	2003
$20,000,000	1st individual genome	2006
$2,000,000	1st NGS Genome	2007
$200,000	1st 30x genome	2008
$10,000	1st sub-10K genome	2010
$1,000	1st $1,000 genome	2014

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Gaining a Global Sequencing Presence

Breadth of technology enabling markets worldwide

>90% of all the sequencing performed on earth has been with Illumina systems and chemistry[1]

>9K+ Installs Worldwide[2]

Sequencing Power for Every Scale

1. Data calculations on file. Illumina, Inc., 2015.
2. Based on end of fiscal year 2016. Does not include CBOT Systems.

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Who We Serve

Innovation drives expanding market opportunities

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Reproductive Health | Oncology | Population Sequencing

Research | Complex Disease | Consumer | Infectious Disease

Forensics | Agriculture | Genetic Health | BioPharm

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TruSight® Tumor 170

Combining multiple workflows into one

Amplification Detection

Yesterday | Today

Arrays | TruSight Tumor 170

Chr	Position Start	Position End	INFO	Gene	Fold Change	Variant type
chr7	55086971	55273310	PASS	EGFR	8.7	Amplification

Identified EGFR Amplification

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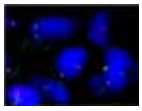
TruSight® Tumor 170

Combining multiple workflows into one

Gene Fusion Detection

Yesterday | Today



FISH | TruSight Tumor 170

Fusion Breakpoints						Wildtype Read Count		Fusion Read Count			
ChrA	PosA	StrandA	ChrB	PosB	StrandB	Supporting pairs	Split reads	Supporting pairs	Split reads	GeneA	GeneB
chr21	39817543	-	chr21	42880007	-	1264	285	3	18	ERG	TMPRSS2

Identified TPRSS2-ERG fusion

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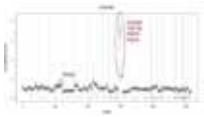
TruSight® Tumor 170

Combining multiple workflows into one

Chromosomal Aberrations

Yesterday | Today



Immunohistochemistry | TruSight Tumor 170

Identified Chromosome 11q loss

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TruSight® Tumor 170

Combining multiple workflows into one

Splice Variant Detection

Yesterday | Today

Immunohistochemistry | TruSight Tumor 170

Chr	Location	Ref	Observed	Annotation
chr7	116412014	CTGTAGACTACCGAG CTACTTTTCCAGAAGG	C	MET|NM_001127500.1 splice_donor_variant:coding_sequence_variant:feature_truncation

Identified MET Exon 14 Skipping

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Introducing NovaSeq™ Series of Systems

Theodor Zamfirov MD
CEO at ELTA 90 Group

Scalable Throughput

Complete studies faster and more economically

Run times: <1 to ~2.5d based on system, FC and read length

Configure output to match your application and study size

Single flow cell output (1 or 2 can run simultaneously)

Flow Cell Type	NovaSeq 5000	NovaSeq 6000	Reads per Flow Cell	Output (Gb) per Flow Cell: 100 cycles	200 cycles	300 cycles
S4*		✓	10B			3000
S3*		✓	6.6B			2000
S2	✓	✓	3.3 B	333	666	1000
S1*	✓	✓	1.6 B	167	333	500

*S1, S2 and S4 flow cells not currently released

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Introducing the NovaSeq Series

Any genome. Any method. Any scale.

Scalable Platform
New architecture built for scalability with better per sample economics to enable highly powered studies

Flexible Performance
Configurable to support the broadest range of applications at any scale – open to all methods

Streamlined Operation
Designed to increase lab efficiency with a simplified workflow and user interaction

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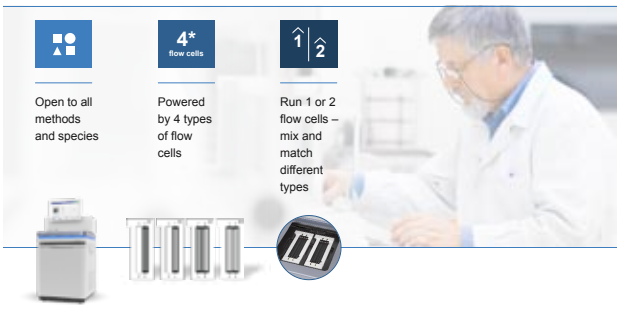
Highly Flexible

Configurable to support the broadest range of applications

Open to all methods and species

Powered by 4 types of flow cells

Run 1 or 2 flow cells – mix and match different types



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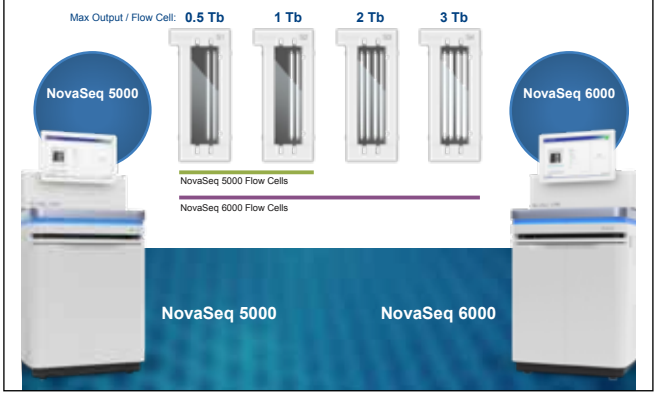
*Based on flow cells not currently released

NovaSeq System Configurations



Streamlined Operation

Increase lab efficiency with a simplified workflow

Cartridge based reagents reduce hands on time and prevent misloading

RFID encoded consumables provide traceability and ensure compatibility

Onboard cluster gen reduces hands on time and run variability

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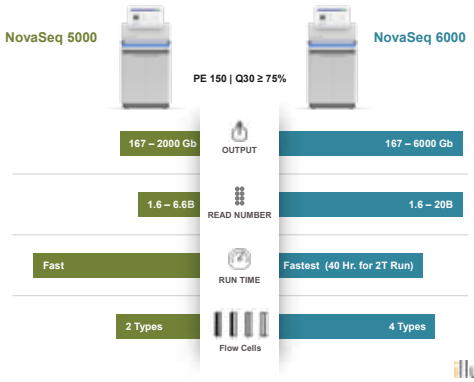
NovaSeq Series | Any Genome. Any Method. Any Scale.



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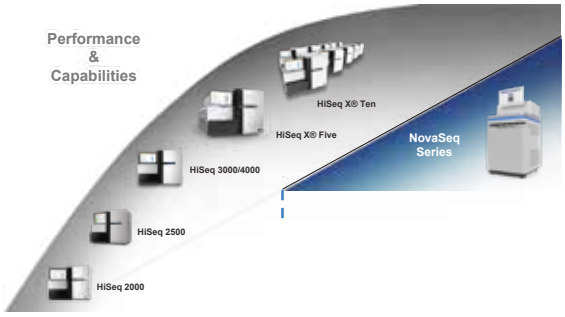
Output range shown based on currently unreleased flow cells

Illumina High Throughput Sequencing



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NovaSeq Series

Compelling price per data point enables highly-powered studies

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Next Generation Optics

Higher Density (624 nm pitch)

Custom Camera and Laser

NovaSeq Series

Compelling price points can enable highly-powered studies

NovaSeq reduction in price per Gb

	NovaSeq 6000		NovaSeq 5000 & 6000
	S3 Flow Cell	S2 Flow Cell	S1 Flow Cell
HiSeq 3000/4000	47%	23%	12%
HiSeq 2500 (v4)	66%	50%	43%
HiSeq 2000 (v3)	78%	67%	63%
HiSeq 2500 Rapid	80%	71%	67%

HiSeq 2500 based on 200 cycle RR kit and 250 cycle HO kit, all others based on 300 cycle kits

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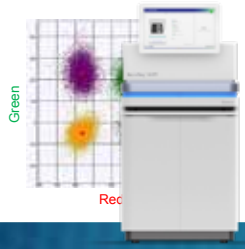
Next Generation Two-Channel Chemistry



Higher Density (624 nm pitch)

Custom Camera and Laser

New Surface Chemistry

New Dyes

Key Technology Enablers

Redesigned from the ground up

High Density Flow Cell

Higher density flow cell format (624nm pitch)

New surface chemistry

Increased signal:noise via smaller, brighter clusters

New superior imaging

4x faster scanning

Diffraction-limited performance

Data management

8x increase in primary analysis speed

Data footprint reduced by 25%

Reformulated chemistry

Reengineered nucleotide dyes

Optimization of 8 different sub-formulations

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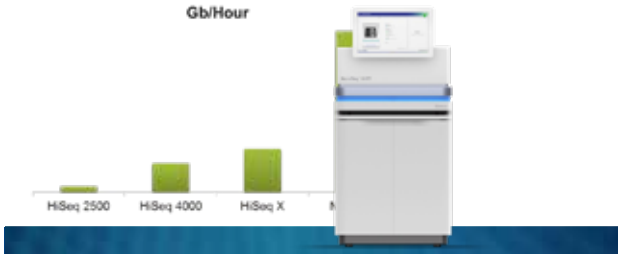
Extraordinary Data Rates



Higher Density (624 nm pitch)

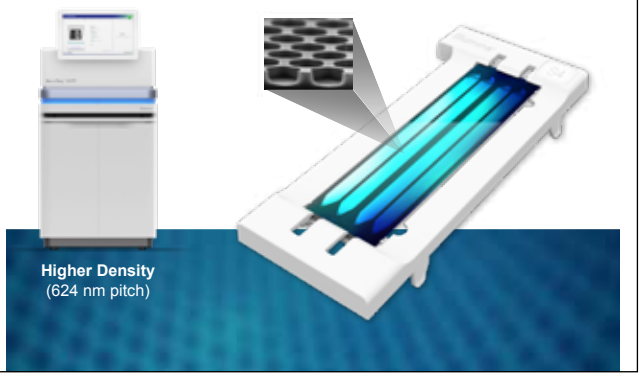
Custom Camera and Laser

New Surface Chemistry

New Dyes

New Compute Architecture

Nanofabricated Flow Cells



Higher Density (624 nm pitch)

NovaSeq Performance and Data Quality

- Data quality as good as HiSeq with initial release – significant opportunity for further improvements
  - Major R&D focus on further optimizing 2-channel chemistry with patterned flow cells
- High data quality enabled by superior optics and reformulated chemistry
  - Diffraction-limited performance optics
  - New surface chemistry, dye-sets and enzymes

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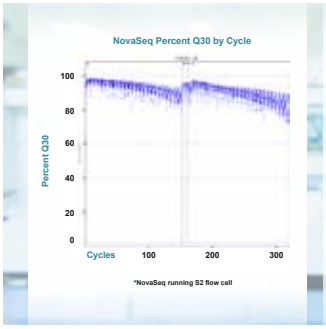
NovaSeq Performance



Platform	Read Length	Output	Percent Q30
NovaSeq*	2 x 151	>2000G	92.3
NovaSeq*	2 x 151	>2000G	88.5

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NovaSeq Ecosystem

1 MANAGE WORKFLOW
Seamless integration with BaseSpace® Clarity LIMS

2 PREPARE LIBRARIES
Access Illumina's suite of library prep methods

3 SEQUENCE
Proven data you can trust

4 ANALYZE DATA
Seamless integration into BaseSpace Sequence Hub

5 INTERPRET RESULTS
Drive discovery with BaseSpace Variant Interpreter (Beta) and BaseSpace Cohort Analyzer

Human Genome Performance on NovaSeq

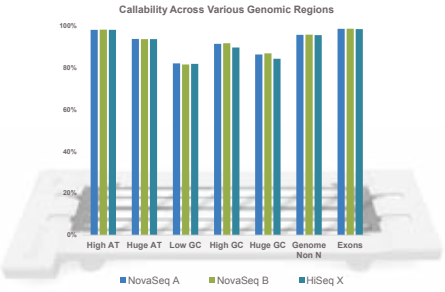
Genome build quality highly concordant with HiSeq

	NovaSeq (n4)	HiSeq X (n2)	HiSeq v4 (n2)
Genome Coverage (x)	**30.6**	**30.5**	**29.8**
Autosome Coverage	**95%**	**95%**	**91%**
Autosome Callability	**95%**	**95%**	**93%**
Autosome Exon Callability	**98%**	**98%**	**91%**
SNP Precision	**100%**	**100%**	**100%**
SNP Recall	**97%**	**97%**	**96%**
Indel Precision	**97%**	**98%**	**97%**
Indel Recall	**95%**	**95%**	**88%**

NovaSeq Prototype Instruments running S2 flow cell

Human Genome Performance on NovaSeq

Genome build quality meets or exceeds HiSeq



Streamlined Informatics with BaseSpace

Acquire | Analyze and Share | Interpret | Aggregate

BaseSpace Clarity LIMS
Automate wet lab workflows and track samples through sample prep, library prep and sequencing

Run monitoring, call variants, perform QC, store and share

BaseSpace Sequence Hub
Interpret variants, annotate for pathological significance and create reports

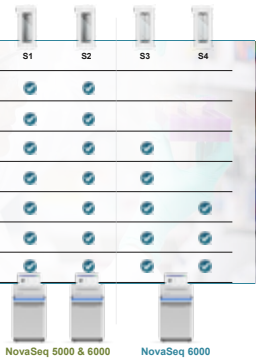
BaseSpace Variant Interpreter (Beta)
BaseSpace Cohort Analyzer
Analyze subject cohorts, generate translational insights, spur biomarker discovery

BaseSpace Correlation Engine
Correlate genes, phenotypes, compounds, tissues, and more

NovaSeq Configurations and Key Methods

Configurable to support broad range of methods and accommodate rapid turn around

Flow Cell Type	S1	S2	S3	S4
Expression profiling	✓	✓		
Whole transcriptome analysis	✓	✓		
Exome	✓	✓	✓	
Low pass WGS	✓	✓	✓	
Liquid biopsy	✓	✓	✓	✓
WGS	✓	✓	✓	✓
T/N profiling	✓	✓	✓	✓



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Streamlined Informatics with BaseSpace

Increase lab efficiency with a simplified workflow.

Simple tracking of any sample type from start to finish with integrated LIMS

No manual uploading of data — automatically stream your data to hosted storage

Fully automated aggregation, secondary analysis, and QC of data

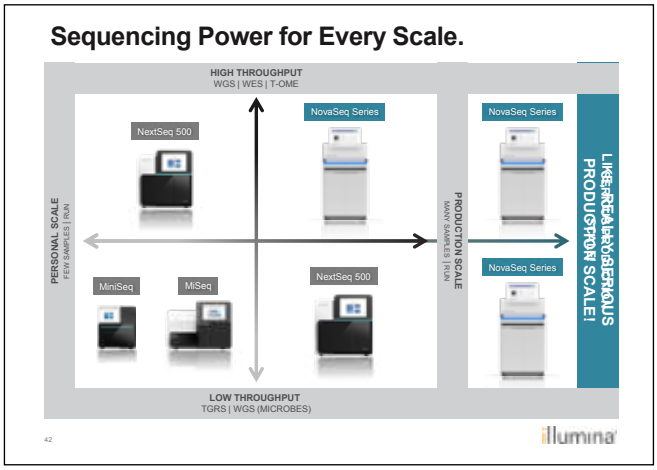
Integrated filtering and reporting tools to quickly extract information from the data

Complete studies faster and more economically.

Multiple configurations from basic to enterprise scale

Easily ramp up without investing in expensive servers and IT personnel

Pay-as-you-go for hosted storage and analysis reducing data processing costs by as much as 50%


Sequencing Power for Every Scale.
HIGH THROUGHPUT
WGS | WES | T-OME
NextSeq 500
NovaSeq Series
NovaSeq Series
NovaSeq Series
MiniSeq
MiSeq
NextSeq 500
PERSONAL SCALE
FEW SAMPLES | RUN
PRODUCTION SCALE
MANY SAMPLES | RUN
LOW THROUGHPUT
TGRS | WGS (MICROBES)
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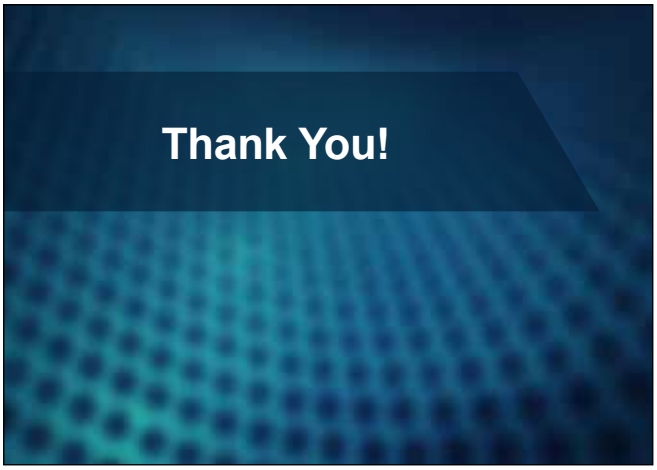

Thank You!

PARALLEL SYMPOSIUM 1
Supported by GSK

► **Mixed diseases of the connective tissue – systemic lupus erythematosus (in Bulgarian)**
V. Popova

СМЕСЕНИ ЗАБОЛЯВАНИЯ НА СЪЕДИНИТЕЛНАТА ТЪКАН (MCTD) – СИСТЕМЕН ЛУПУС ЕРИТЕМАТОДЕС

Величка Попова

Патогенеза на СЛЕ

Адаптирано по Mok CC et al. J Clin Pathol. 2003;56:481-90.

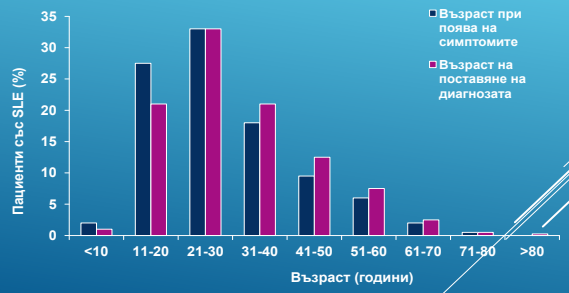
СЛЕ - ОПРЕДЕЛЕНИЕ

- Системният лупус еритематодес (СЛЕ) е хронично, многосистемно автоимунно заболяване, което може да бъде животозастрашаващо, когато са засегнати основни органи[1,2]
  - По-характерно за СЛЕ обаче е че е, свързано с инвалидизиращи клинични прояви, в резултат на възпаление на множество органни системи
  - Етиологията е неизвестна, но генетичните и хормонални фактори, както и влиянието на околната среда играят основна роля
- Естественият ход включва спорадични пикове на активност ("внезапни обостряния" на болестта), които кумулативно могат да причинят необратимо увреждане на органите[3]
- СЛЕ се характеризира с патологично продукция на антитела, насочени към собствените антигени[4,5]
  - Антинуклеарните антитела са отличителен признак на заболяването
  - Абнормална активация на B- и T-клетъчните лимфоцити

1. D'Cruz DP et al. Lancet. 2007;369:587-96. 2. ACR Ad Hoc Committee. Arthritis Rheum. 1999;42:1785-96.
3. Manzi S. Am J Manag Care. 2001;7(suppl):S474-9. 4. Muñoz LE et al. Rheumatology. 2005;44:1101-7.
5. Arbuckle MR et al. N Engl J Med. 2003;349:1526-33.

СЛЕ засяга предимно жени в детеродна възраст

Проектът "Euro-Lupus" анализира 1000 пациенти със СЛЕ:
91% са жени; Средна възраст на поява на симптомите, 29 г.



Cervera R et al. Medicine. 1993;72:113-24.

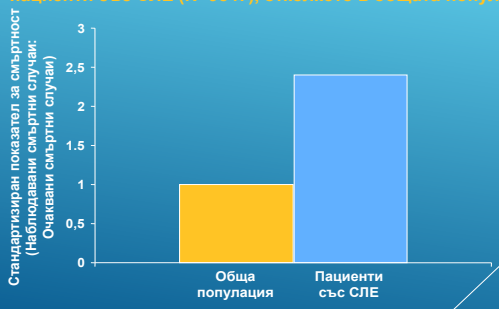
Фактори на околната среда и други фактори, асоциирани с развитието на СЛЕ

- Слънчева светлина
- EBV
- Професионално излагане: силиций, пестициди, живак
- Хормони
- Аномалии в апоптозата
- Аномална трансдукция на сигналите: toll-подобни рецептори
- Цитокинни модели: интерферонен подпис; намален интерлевкин 2 от Т-клетките

Адаптирано по D'Cruz DP et al. Lancet. 2007;369:587-96.

При пациентите със СЛЕ е налице по-висок риск за смъртност в сравнение с общата популация

Рискът от смърт по всякакви причини е 2,4 пъти по-висок при пациенти със СЛЕ (N=9547), отколкото в общата популация



Bernatsky S et al. Arthritis Rheum. 2006;54:2550-7.

Патофизиология

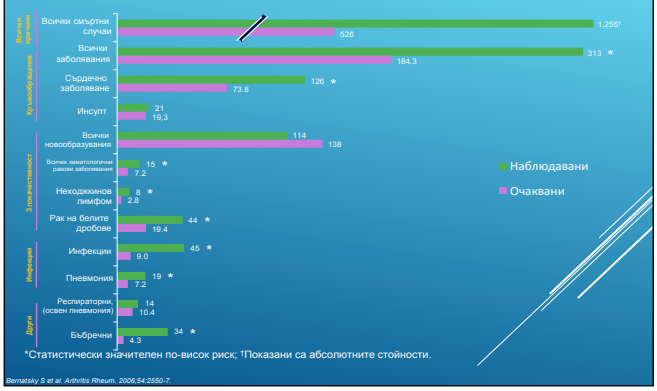
Клинични прояви

Засегнати от СЛЕ органи и системи

Указания на експертната комисия към Американския колеж по радиология (ACR) за системен лупус еритематодес. 1999;42:1785-96.

1. Rahman A, Isenberg DA. Systemic lupus erythematosus. N Engl J Med. 2008;358:929-39. 2. Buyon J. In: Klippel JH et al, eds. Primer on the Rheumatic Diseases. 13th ed. New York, NY:Springer-Verlag;2007:303-18. 3. D'Cruz DP et al. Systemic lupus erythematosus. Lancet. 2007;369:587-96. 4. Roman MJ et al. Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. N Engl J Med. 2003;349:2399-406. 5. Asanuma Y et al. Premature coronary-artery atherosclerosis in systemic lupus erythematosus. N Engl J Med. 2003;349:2407-15. 6. El-Magadmi M et al. Systemic lupus erythematosus: an independent risk factor for endothelial dysfunction in women. Circulation. 2004;110:399-404.

Сърдечно-съдови, онкологични, инфекциозни и бъбречни заболявания допринасят за повишената смъртност при СЛЕ



*Статистически значителен по-висок риск; †Показани са абсолютните стойности.

Bernatsky S et al. Arthritis Rheum. 2006;54:2550-7.

Клинични прояви на СЛЕ

- При пациентите със СЛЕ умората, болката и повишената температура може да са преобладаващи общи симптоми
- Протичането на болестта е различно при всеки пациент.
- Болестната активност флуктуира, като включва периоди на обостряне и относителен покой.
- Могат да бъдат засегнати редица органи, като редът на засягане е непредвидим. Една от най-често засяганите системи е **мускуло-скелетната**.
- При засягане на жизнено- важни органи и системи може да настъпи много бързо клинично влошаване.

Указания на експертната комисия към Американския колеж по радиология (ACR) за системен лупус еритематодес. Arthritis Rheum. 1999;42:1785-96.

Диагноза и оценка

Клинични характеристики на СЛЕ

Система	Характеристики
Общи	Умора, температура (при липса на инфекция), загуба на тегло
Скелетно- мускулна	Артралгия, артрит, миозит
Кожа	Пеперуден обрив, фоточувствителност, лезии на лигавиците, алопеция, феномен на Рейно, пурпура, уртикария, васкулит
ПОС	Хематурия, протеинурия, цилиндриурия, гломерулонефрит, нефрозен синдром
ГИТ	Гадене, повръщане, коремни болки
ДС	Плеврит, белодробна хипертония
Сърдечна	Перикардит, ендокардит, миокардит
Моноцито-макрофагеална система	Лимфаденопатия, спленомегалия, хепатомегалия
Хематологични прояви	Анемия, тромбоцитопения, левкопения
Нервно-психични прояви	Психоза, припадъци, органичен мозъчен синдром, напречен миелит, черепни невропатии, периферни невропатии

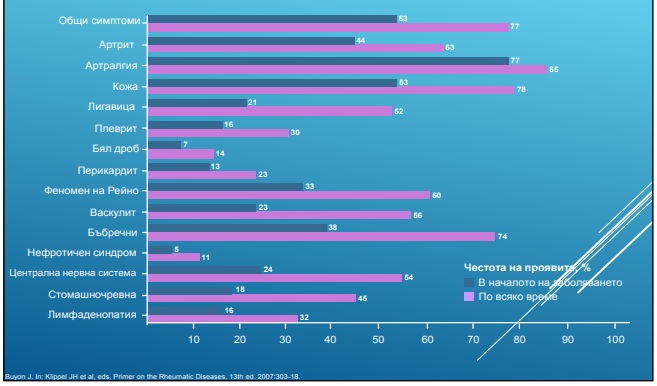
Указания на експертната комисия към Американския колеж по радиология (ACR) за системен лупус еритематодес. Arthritis Rheum. 1999;42:1785-96.

Диагноза на СЛЕ

- СЛЕ представлява предизвикателство за диагностициране, тъй като се засягат множество органи и системи и ходът на заболяването е променлив
- Указанията на ACR 1999 сочат, че СЛЕ трябва да се подозира при всеки с характеристики, засягащи ≥2 органни системи
- Диагностицирането на СЛЕ може да бъде направено с по-голяма достоверност, ако са налице 4 от 11^те^ диагностичните критерии
  - В някои случаи диагностицирането все пак е възможно с по-малко от 4 критерия

Указания на експертната комисия към ACR за СЛЕ. Arthritis Rheum. 1999;42:1785-96.

Честота на проявите на СЛЕ



Buyon J. In: Klippel JH et al, eds. Primer on the Rheumatic Diseases. 13th ed. 2007:303-18.

Натрупване на видовете автоантитела при СЛЕ

Arbuckle MR et al. N Engl J Med. 2003;349:1526-33.

Патогенни автоантитела при СЛЕ

Специфичност на антигена	Разпространение, %	Основни клинични ефекти
dsDNA	70–80	Бъбречно заболяване, кожно заболяване
Нуклеозоми	60–90	Бъбречно заболяване, кожно заболяване
Ro/SSA	30–40	Бъбречно заболяване, кожно заболяване, сърдечни проблеми на плода
La/SSB	15–20	Сърдечни проблеми на плода
Sm	10–30	Бъбречно заболяване
NMDA рецептор	33–50	Мозъчно заболяване
Фосфолипиди	20–30	Тромбоза, смърт на плода по време на бременност
α-Actinin	20	Бъбречно заболяване
C1q	40–50	Бъбречно заболяване

Адаптирано по Rahman A et al. N Engl J Med. 2008;358:929-39.

Показатели за активност на заболяването при СЛЕ

- За СЛЕ няма унифициран световен стандарт за оценка на активността на заболяването[1]
- Следните индекси са възприети за оценка на степента на активност на заболяването[2]
  - BILAG
  - SELENA - SLEDAI
  - SLAM

1. Wollaston SJ. J Rheumatol. 2004;31:2390-4. 2. Griffiths B et al. Best Pract Res Clin Rheumatol. 2005;19:685-708.

Диагностична приложимост на серологичните изследвания

- Малка вероятност за СЛЕ, показана от:
  - Липса на **антинуклеарни антитела (ANA)**
  - Наличие на антитела без засягане на органи или нормални лабораторни находки
- Други серологични изследвания може да спомогнат за диагностициране:
  - Високотитърните IgG антитела към dsДНК и антиген на Sm имат по-голяма специфичност за СЛЕ
  - Антитела към РНП протеини в РНК, Ro/SSA и La/SSB са налични при СЛЕ и други заболявания на съединителната тъкан
  - При СЛЕ се наблюдава също така ниско съдържание на комплемент (хипокомплементемия)

Указания на експертната комисия към ACR за СЛЕ. Arthritis Rheum. 1999;42:1785-96.

Показатели за активност на заболяването за СЛЕ

	BILAG	SLEDAI	SLAM
Брой описания	86	24	30
Брой органни системи	8	9	9
Интервали	28 д	10 д	28 д
Обективно/субективно	И двете	Обективно	Субективно
Променливи	Не	Да	Да
Оценка на органните прояви	Да	Не	Да
Имунологични променливи	Не	Да	Не
Актуализации	BILAG 2004	SELENA SLEDAI SLEDAI-2K*	SLAM-R
Влошавания	Да	Да (SELENA)	Не
Приложимост за клиничните изпитвания	Индивидуалните резултати за тежест ще варират, ако органната система се подобрява или влошава	Глобалният резултат за тежест не се записва, ако дадена органова система се подобрява или влошава	Трудно е да се разграничат множество леки прояви спрямо една тежка характеристика

*SLEDAI-2K не може да се използва за описание на дейността на заболяването през предходните 30 дни.

1. Griffiths B et al. Best Pract Res Clin Rheumatol. 2005;19:685-708. 2. Ramsey-Goldman R et al. Arthritis Rheum. 2003;49:S225-33. 3. Isenberg DA et al. Rheumatology. 2005;44:902-6. 4. Touma Z et al. Lupus. 2010;19:49-51.

Препоръки на EULAR за поведение пре пациенти със СЛЕ

Item	Препоръки
Прогноза	Нови клинични признаци (обриви, артрит, серозит, неврологични прояви и припадъци/психоза) и рутинни лабораторни и имунологични изследвания може да предоставят прогнозна информация и следва да бъдат взети предвид При определени пациенти трябва да се обмисли потвърждение чрез образна диагностика (ЯМР на мозъка) и патологични изследвания (бъбречна биопсия)
Мониторинг	Нови клинични прояви (напр. кожни лезии, артрит, серозит, неврологични прояви, лабораторни изследвания (ПКК), имунологични изследвания (серумни С3/С4, анти-С1q, анти-dsДНК) и утвърдени признаци на глобална дейност имат диагностична способност за мониторинг за активност на лупус
Съпътстващи заболявания	СЛЕ и/или лечението увеличават риска от съпътстващи заболявания (включително инфекции на пикочните пътища, атеросклероза, хипертония, дислипидемия, диабет, остеопороза, аваскуларна некроза, злокачествени заболявания) Препоръчва се минимизиране на рисковите фактори, своевременно оценяване и прилежно проследяване
Лечение	СЛЕ без сериозни органни прояви: • Противомаларийни средства и/или глюкокортикоиди • Целесъобразна употреба на НСПВС Пациенти, нереагиращи на лечението, или такива, които не могат да намалят стероидите до приемливи нива: • да се обмисли използването на имунопотискащи агенти като азатиоприн, ММФ, метотрексат
Спомагателна терапия	Могат да се обмислят фотозащита, промени в начина на живот и други лечения (напр. ниски дози аспирин, витамин D/калций, антихипертонични лекарства, статини)

Bertsias G et al. Ann Rheum Dis. 2008;67:195-205.

Тежест на СЛЕ

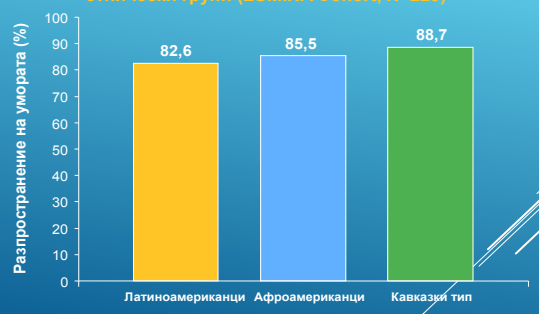
Диференциална диагноза на СЛЕ

- Недиференцирано заболяване на съединителната тъкан
- Синдром на Сьогрен
- Изолиран антифосфолипиден синдром /понякога е съпътстващ/
- Идиопатична тромбоцитопенична пурпура
- Медикаментозно индуциран лупус
- Ревматоиден артрит
- Васкулит /понякога съпътстващ/
- и др.

Адаптирано по указанията на експертната комисия към ACR за СЛЕ. Arthritis Rheum. 1999;42:1785-96.

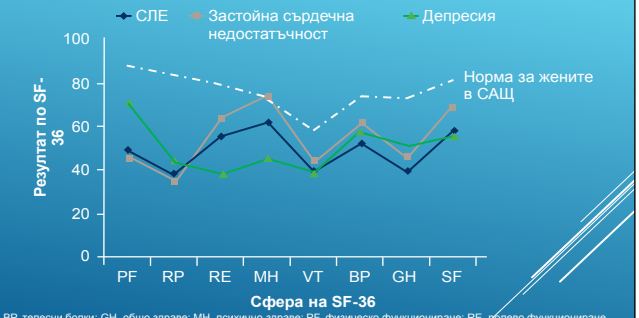
Умората е една от най-разпространените клинични прояви на СЛЕ

Умората е широко разпространена и сходна сред основните етнически групи (LUMINA Cohort, N=223)



Zonana-Nacach A et al. Lupus. 2000;9:101-9.

Свързаното със здравето качество на живот при СЛЕ е подобно на това при застойна сърдечна недостатъчност и депресия



BP, телесни болки; GH, общо здраве; MH, психично здраве; PF, физическо функциониране; RE, ролево функциониране въз основа на емоционалното състояние; RP, ролево функциониране въз основа на физическото състояние; SF, социално функциониране; VT, жизненост.

Jolly M. J Rheumatol. 2005;32:1706-8.

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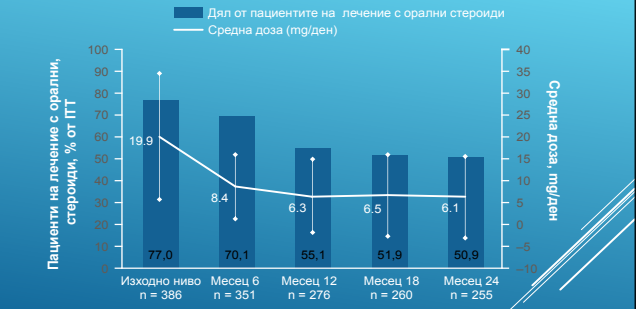
IT'S TIME FOR BENLYSTA

Benlysta е показан за допълваща терапия на възрастни пациенти с активен, автоантитяло-позитивен системен лупус еритематодес (СЛЕ) с висока степен на активност на болестта (напр. положителни анти-двойно верижни ДНК (dsDNA) антитела и нисък комплемент), въпреки приложението на стандартна терапия

Benlysta КХП **15.09.2016 г.**

Настоящи и бъдещи възможности за лечение

Проучване OBSERVE В САЩ: намаляване на средната стероидата доза за период от 24 месеца



ITT = намерение за лечение.

Collins CE, et al. Lupus Sci Med 2016; 3:e000118.

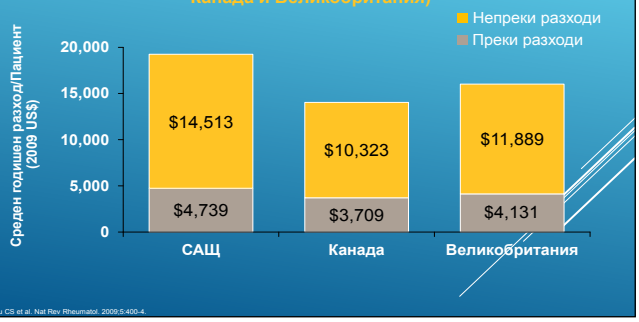
Препоръки за лечение до постигане на целта при СЛЕ

1. Целта на лечението на СЛЕ трябва да е постигане на ремисия на системните симптоми и органни прояви или, в случай че не може да се постигне ремисия, осигуряване на възможно най-ниската степен на активност на болестта, измерена чрез валидиран индекс за лупусна активност и/или органно-специфични маркери.
2. Профилактиката на изостренията на заболяването (особено на тежките тласъци) е реално постижима при СЛЕ и трябва да бъде терапевтична цел.
3. Не се препоръчва ескалиране на терапията при клинично асимптоматични пациенти само въз основа на данни за постоянна или персистираща серологична активност.
4. Тъй като увреждания са прогноза за последващи увреж дания и смърт, профилактика на тяхното натрупване трябва да бъде основна терапевтична цел при СЛЕ.
5. Факторите, които имат отрицателен ефект върху качеството на живот, свързано със здравето, като умора, болка и депресия, трябва да се допълнят към целите за контрол на активността на заболяването и профилактиката на увреждания.
6. Силно се препоръчва ранното диагностициране и лечение на бъбречното засягане при пациенти със СЛЕ.
7. При лупусен нефрит, след индукционната терапия се препоръчва имуносупресивно поддържащо лечение за най-малко три години с цел оптимизиране на резултатите.
8. Стремежът при поддържащата терапия не трябва да е към достигане на най-ниската доза глюкокортикоиди, необходима за контрол на заболяването, а вед е възможно, глюкокортикоидите трябва да бъдат напълно спрени.
9. Профилактиката и лечението на заболеваемостта, свързана с антифосфолипиден синдром (АФС), трябва да са терапевтична цел при СЛЕ, като препоръките за лечението не се различават от стандартните за АФС.
10. Независимо от другите прилагани лекарства, сериозно трябва да се обмисли лечение с антималарийни средства.
11. Заедно с имуномодулиращото лечение трябва да се обмислят адекватни адювантни терапии за контрол на придружаващите заболявания при пациенти със СЛЕ.

van Vollenhoven R, *et al. Ann Rheum Dis* 2014; **73**:958–967.

Социално-икономическите разходи за СЛЕ са значителни

Непреките разходи поради СЛЕ са 2,4-2,8 пъти по-високи, отколкото преките разходи (Три национални проучвания, включващо САЩ, Канада и Великобритания)



Lau CS et al. Nat Rev Rheumatol. 2009;5:400-4.

Употреба на глюкокортикоиди, асоциирани с тежка органова токсичност

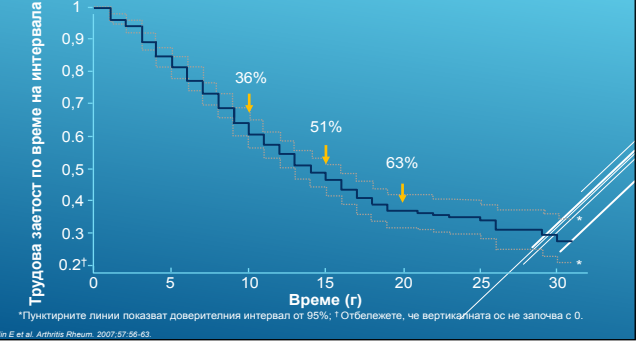
Проучвания върху увреждането на органите при пациенти със СЛЕ, провеждащи лечение с глюкокортикоиди[1]

Пациенти	N	Употреба на кортикостероиди	Находки
Hopkins	539	24% с високодозово излагане; 30% с и.в. метилпреднизолон; 11% без излагане на преднизон	Кумулативно излагане на преднизон, асоциирано с остеопорозна фрактура, симптоматично заболяване на коронарната артерия и катаракти; високи дози, свързани с аваскуларна некроза, удар
Торонто[3]	73	Употреба на преднизон от 87,7%; средна максимална доза 37,7 mg/d; средна продължителност 117,1 месеца	Средният SDI се увеличи от 0,33 до 1,9 при 15 г; скелетномускулно увреждане при >50%; 80% от смъртните случаи са свързани с кортикостероиди при 15 г.
Жени след менопауза[4]	34	Средна дневна поддържаща доза преднизон 4,0 ± 2,5 mg/d	33% остеопения и 48% остеопороза в лумбалната част на гръбнака; 74% остеопения при недоминантна бедрена шийка

1. Kalunian K, Merrill JT. Curr Med Res Opin. 2009;25:1501-14. 2. Zonana-Nacach A et al. Lupus. 2000;9:101-9. 3. Gladman DD et al. J Rheumatol. 2003;30:1955-9. 4. Mok CC et al. Lupus. 2008;14:106-12.

СЛЕ оказва съществено въздействие върху трудовата заетост

От пациентите в САЩ, които към момента на диагностициране са били трудово заети, 36%, 51% и 63% са спрели да работят 10, 15, и 20 години след диагностициране



*Пунктирните линии показват доверителния интервал от 95%; † Отбележете, че вертикалната ос не започва с 0.

Yelin E et al. Arthritis Rheum. 2007;57:56-63.

Смесени заболявания на съединителната тъкан (MCTD) – Системна склеродермия и Синдроми на припокриване

Синдроми на припокриване: класификация

Associated with specific autoantibody profile
Mixed connective tissue disease (anti-U1 snRNP)
Anti-synthetase syndrome (anti-tRNA synthetase)
Polymyositis and scleroderma (anti-PM/Scl)
Systemic lupus erythematosus and Sjögren syndrome (anti-La/SSB)
Not associated with specific autoantibody profile
Rhupus syndrome
Systemic sclerosis and Sjögren syndrome
Systemic sclerosis and rheumatoid arthritis
Systemic lupus erythematosus and systemic sclerosis
Rheumatoid arthritis and Sjögren syndrome
Polymyositis and Sjögren syndrome

Anti-U1 snRNP: anti-(U1) small nuclear RNA antibodies.

Laccarino L et al. Autoimmunity Rev 2013

• Системната склероза (SSc) се дефинира като хетерогенно заболяване, характеризиращо се с ендотелна дисфункция, нарушена функция на фибробластите с последващо свръхпроизводство на колаген и промяна във неговата структура.

• Белодробното ангажиране при SSc включва: интерстициална белодробна болест (ИББ) и ПАХ, които са най-честите белодробни прояви в наши дни и сега са водещите причини за смърт при SSc. Обикновено асоциираната с SSc - ПАХ (SSc - ПАХ) ще се развие при пациенти с ограничена форма на SSc след 10-15 години еволюция на заболяването

• Честотата на SSc - ПАХ е около 8%-15% в зависимост от използвания метод на диагностика. Следните методи се препоръчват за диагностика, проследяване на лечението и прогноза: Доплерова трансторакална ехокардиограма (TTE), функционално изследване на дишането (PFTs) , (DLCO), 6-минутен тест (6MWT) и биологични маркери: N-терминален натриуретичен пептид (NT-pro BNP)
• Инвазивна оценка- дясната сърдечна катетеризация (RHC) остава златен стандарт за стадиране и диагноза на ПАХ.

World J Rheumatol. 03.10.2012 г.; 2(2): 12-20

ОПРЕДЕЛЕНИЕ НА ПАХ

Пулмоналната Артериална Хипертония (ПАХ), хемодинамично дефинирана като средно налягане в белодробната артерия (mPAP) над 25 mmHg, средно вклинено налягане в белодробните капиляри < 15 mmHg, и белодробно съдово съпротивление над 3 Wood единици представлява прогресивен синдром на белодробната съдова мрежа, който води до прогресивна дясна камерна недостатъчност, дългосрочна инвалидност и често пъти смърт, ако бъде оставена нелекувана в рамките на 2-2,5 години

PH Mean PAP ≥25 mm Hg

PAH Mean PAP ≥25 mm Hg *plus* PCWP/LVEDP ≤15 mm Hg

ACCF/AHA includes PVR >3 Wood Units

ACCF = American College of Cardiology Foundation
AHA = American Heart Association
LVEDP = left ventricular end-diastolic pressure
PAP = pulmonary arterial pressure
PCWP = pulmonary capillary wedge pressure
PVR = pulmonary vascular resistance

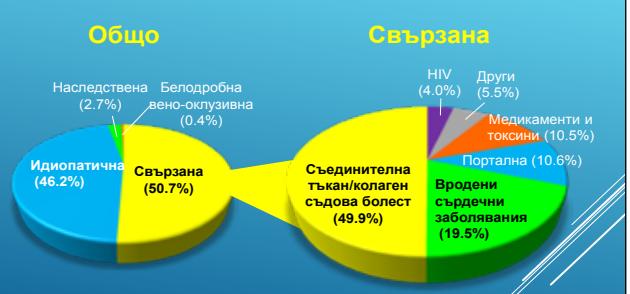
Badesch D et al. *J Am Coll Cardiol.* 2009;54:S55-S66.
McLaughlin VV et al. *J Am Coll Cardiol.* 2009;53:1573-1619.
World J Rheumatol. 03.10.2012 г.; 2(2): 12-20

Белодробна артериална хипертония (ПАХ), асоциирана със заболяване на съединителната тъкан (ЗСТ)

- Защо да говорим за ЗСТ?
- При до 30% от пациентите с ПАХ тя е асоциирана със заболяване на съединителната тъкан[1]
- Видовете ЗСТ, свързани с ПАХ, включват системна склероза (ССз), системен лупус еритематозус (СЛЕ), застъпващи се/смесени ЗСТ, дерматомиозит, полимиозит, синдром на Сьогрен и евентуално ревматоиден артрит[1,2]
- При пациенти с ПАХ-ЗСТ често се среща широк спектър значителни съпътстващи заболявания[1]

1. Coglan JG *et al. Curr Opin Pulm Med* 2010; 16 (suppl 1):S27–S34
2. Condliffe R *et al. Am J Respir Crit Care Med* 2009; 179:151–157

Почти 25% от ПАХ е свързана с ССТБ: REVEAL регистър



Based on Venice Clinical Classification (2003); 2967 patients.
Adapted from Badesch DB et al. *Chest.* 2010;137:376-387.

Пулмоналната артериална хипертония (ПАХ), асоциирана със заболяване на съединителната тъкан (ССТБ)

- Пациентите с ПАХ-ССТБ имат по-лоша прогноза, отколкото тези с идиопатична ПАХ и при тях заболяването е по-тежко и прогресивно[1]
- ПАХ е една от най-честите причини за свързана със заболяването смърт при ССТБ[1]
- Едногодишният коефициент на преживяване при нелекувани пациенти с ПАХ-ССТБ е ~45%[4]
- Честота в UK на ПАХ-ССТБ е 4,23 на милион[3]
- ПАХ-ССТБ е призната като по-трудно за справяне състояние в сравнение с идиопатичната ПАХ[2]
- Важна е ранната диагноза и адекватно лечение с оглед - удължаване на живота и подобряване на функционалния клас на тъканна увреда [1]

1. Coglan JG *et al. Curr Opin Pulm Med* 2010; 16 (suppl 1):S27–S34; 2. Badesch D. American College of Rheumatology Annual Meeting 2007. Abstract 2166; 3. Condliffe et al Am J Respir Crit Care Med 179: 151-157; 4. Denton *et al.*, 2006 Ann Rheum Dis 2006; 65: 1336-1340

Клинична класификация на пулмоналната хипертония (Дана Пойнт)

1. ПАХ
- Идиопатична
- Наследствена
- Индуцирана от медикаменти и токсини
- Персистираща ПХ на новороденото
- Асоциирана с:
  - Съединително-тъканна болест
  - HIV инфекция
  - Портална хипертония
  - Конгенитални сърдечни заболявания
  - Шистозомиаза
  - Хронична хемолитична анемия

1'. Белодробна венооклузивна болест (БВОБ) и/или белодробна капилярна хемангиоматоза (БКХ)

2. ПХ с левостранно сърдечно заболяване
- Систолна дисфункция
- Диастолна дисфункция
- Клапни заболявания

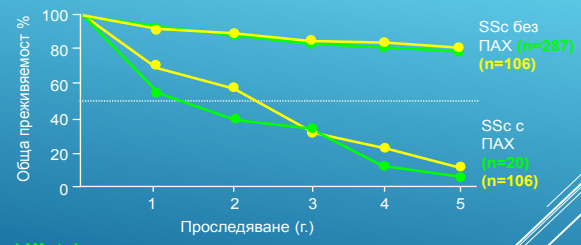
3. ПХ асоциирана с белодробно заболяване и/или хипоксемия
- ХОББ
- Интерстициална белодробна болест
- Други белодробни заболявания със смесен рестриктивно-обструктивен механизъм
- Сънни нарушения на дишането
- Алвеоларна хиповентилация
- Хронична експозиция на висока надморска височина
- Нарушения на развитието

4. Хронична тромбоемболична белодробна хипертония

5. ПХ с неясни и мултифакторни механизми
- Хематологични заболявания
- Системни заболявания
- Метаболинти заболявания
- Други

Simonneau G et al. *J Am Coll Cardiol.* 2009;54:S43-S54.

ВЛИЯНИЕ НА ПАХ ВЪРХУ ПРЕЖИВЯЕМОСТТА ПРИ СИСТЕМНА СКЛЕРОЗА

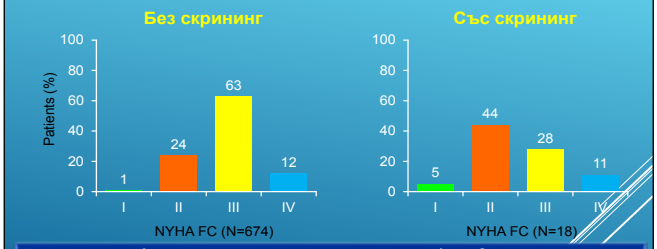


Stupi AM et al:
Средно налягане в БА: 82/35 (50) mm Hg
Среден резистанс в БА: 16 Wood units
Среден сърдечен индекс: 2.1 L/min/m²

Steen V et al:
Средно налягане на пулмоналната артерия: 76 mm Hg

Адаптирано по Stupi AM et al. *Arthritis Rheum* 1986;29:515-524. и Steen VD, Medsger TA Jr. *Arthritis Rheum*. 2003;48:516-522.

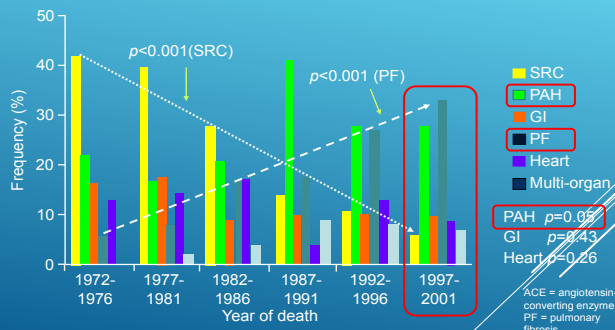
СКРИНИГЪТ МОЖЕ ДА ПОМОГНЕ ЗА ДИАГНОСТИЦИРАНЕ НА БОЛЕСТА В ПО-РАНЕН ЕТАП



Без скрининг болшинството от пациентите са били диагностицирани с функционален клас III или IV по NYHA, и само 24% от пациентите са били с ФК II по NYHA при поставяне на диагнозата.

Humbert M et al. *Am J Respir Crit Care Med*. 2006;173:1023-1030.
Hachulla E *et al. Arthritis Rheum* 2005: 52:3792-3800.

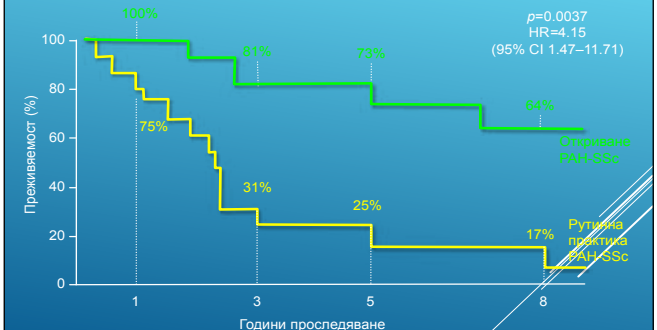
ПРОМЯНА НА ТЕНДЕНЦИЯТА НА СМЪРТНОСТ ПРИ СКЛЕРОДЕРМИЯ



ACE = angiotensin-converting enzyme
PF = pulmonary fibrosis
SRC = scleroderma renal crisis

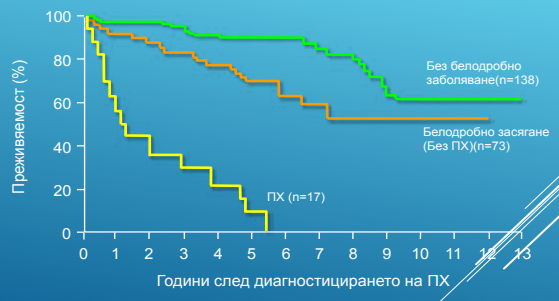
Steen VD. *Ann Rheum Dis*. 2007;66:940-944.

ПРОГНОЗА ПРИ ПАЦИЕНТИТЕ С ПАХ- SSc ПРИ "ОБИЧАЙНА ПРАКТИКА" И "ОТКРИВАНЕ"



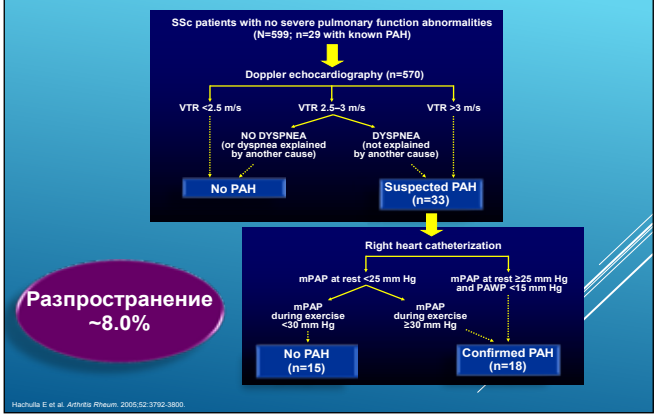
Adapted from Humbert M et al. *Arthritis Rheum*. 2011;63:3522-3530.

ВЛИЯНИЕ НА НЕЛЕКУВАНАТА БЕЛОДРОБНА БОЛЕСТ ВЪРХУ ПРЕЖИВЯЕМОСТТА ПРИ СИСТЕМНА СКЛЕРОЗА



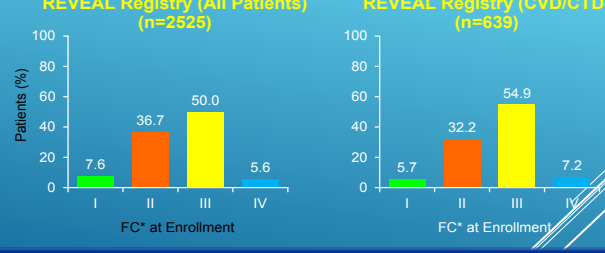
Koh ET. *Br J Rheumatol*. 1996;35:989-993.

СКРИНИНГ ЗА ПАХ ПРИ СКЛЕРОДЕРМИЯ



Hachulla E et al. *Arthritis Rheum*. 2005;52:3792-3800.

ПАЦИЕНТИТЕ СЪС ССТБ СЕ ДИАГНОСТИЦИРАТ С ПО-НАПРЕДНАЛИ СИМПТОМИ



Пациентите със ЗСТ са известни като високорискова пациентска популация. Защо не са идентифицират по-рано от пациентите с ПАХ?

*Modified NYHA/WHO functional class.

Badesch DB et al. *Chest*. 2010;137:376-387

ПРЕПОРЪКИ ЗА СКРИНИНГ И ОТКРИВАНЕ НА СВЪРЗАНИ СЪС ССТБ ПАХ

- Всички пациенти с ССТБ трябва да се изследват за ПАХ
- Всички пациенти с положителен неинвазивен скрининг трябва да бъдат насочени за ДСК
- ДСК е задължително за диагностициране на ПАХ

Първоначален скрининг за ССТБ

- Белодробен функционален тест на DLCO (надежден)
- Трансторакална ехокардиограма (ТТЕ) (надежден)
- N-краен про В-тип натриуретичен пептид NT-Pro BNP (умерен)
- DETECT алгоритъм ако DLCO% < 60% и >3 год. Продължителност на заболяването (умерен)

Честота на неинвазивните методи

- ТТЕ ежегодно за скрининг(**нисък**); ако се развият нови признаци или симптоми (висок)
- Белодробен функционален тест на DLCO ежегодно като скрининг (ниско качество); ако се развият нови признаци или симптоми (ниско)
- N-терминален про В-тип натриуретичен пептид NT-Pro BNP ако се развият нови признаци или симптоми (ниско)

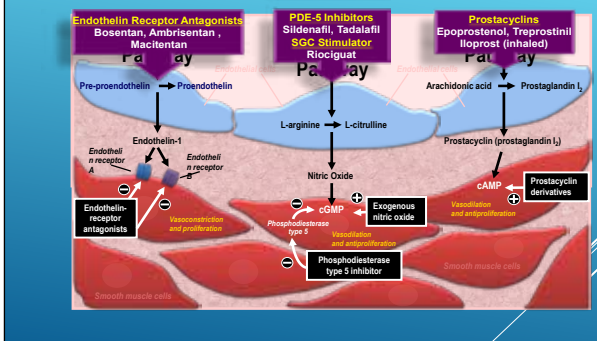
Khanna D et al. *Arthritis Rheum*. 2013 Sep 10. doi: 10.1002/art.38172.

ЛЕЧЕНИЕ НА СВЪРЗАНАТА СЪС СКЛЕРОДЕРМИЯ ПАХ

ЗАКЛЮЧЕНИЕ

- Приблизително 1 от 8 пациента със SSc развива ПАХ
- Ранното диагностициране и лечение на ПАХ е критично за забавяне на прогресията на заболяването
- Налични са много терапевтични възможности на свързаните с ПАХ ССТБ. Те могат да бъдат предизвикателство за лечение поради различният терапевтичен отговор
  - Комбинираната терапия се превръща в основна
- Продължават да се изучават нови таргетни механизми, които се очаква да обърнат прогресивният характер на заболяването

ЛЕЧЕНИЕ НА ПАХ: НАСОЧЕНО КЪМ ИЗВЕСТНИТЕ НИ ПАТОФИЗИОЛОГИЧНИ ПЪТИЩА



Adapted from Humbert M et al. *N Engl J Med.* 2004;351:1425-1436.

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Съкратена лекарствена информация

▼ Този лекарствен продукт подлежи на допълнително наблюдение. Това ще позволи бързото установяване на нова информация относно безопасността. От медицинските специалисти се изисква да съобщават всяка подозирана нежелана реакция.

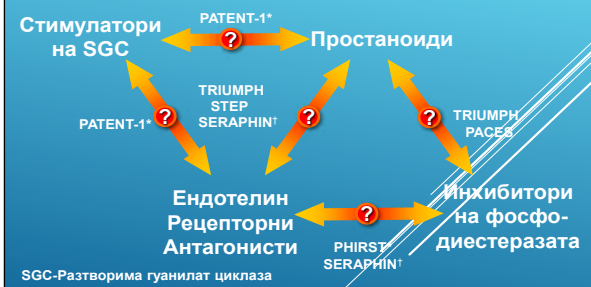
Benlysta 120 mg **прах за концентрат за инфузионен разтвор**. Всеки флакон съдържа 120 mg belimumab.

Benlysta 400 mg **прах за концентрат за инфузионен разтвор**. Всеки флакон съдържа 400 mg belimumab.

След приложение, разтворът съдържа 80 mg belimumab на ml. Benlysta е показан за допълваща терапия на възрастни пациенти с активен, автоантитяло-позитивен системен лупус еритематодес (СЛЕ) с висока степен на активност на болестта (напр. пациентите анти-двойно верижна ДНК (dsDNA) антитела и ниски комплемент), въпреки приложението на стандартна терапия. Лечението с Benlysta трябва да се започне и да се наблюдава от квалифициран лекар с опит в диагностицирането и лечението на СЛЕ. Инфузията Benlysta трябва да се прилага от квалифициран медицински специалист, обучен за приложение на инфузионна терапия. Приложението на Benlysta може да причини тежки или животозастрашаващи реакции на свръхчувствителност и инфузионни реакции. Съобщавано е за развитие на симптоми на остра реакция на свръхчувствителност няколко часа след приложение на инфузията. Също така са наблюдавани и рецидиви на клинично значими реакции след начално правилно лечение на симптомите. Поради това, Benlysta трябва да се прилага само при наличие възможности за незабавно овладяване на такива реакции. Пациентите трябва да останат под медицинско наблюдение за продължителен период от време (за няколко часа) поне след всяка инфузия, като се има предвид възможността за реакции от забавен тип. Пациентите на лечение с Benlysta трябва да бъдат запознати с потенциалния риск от тежки или животозастрашаващи реакции на свръхчувствителност и с възможността от забавено начало или рецидив на симптомите. На пациента трябва да се предоставя листовка при всяко приложение на Benlysta. **Дозировка** Премедикация, включително антихистаминик, съл или без антипиретик, може да се приложи преди инфузията на Benlysta. Препоръчителният режим на дозиране е 10 mg/kg Benlysta на 0, 14-ия и 28-ия ден, а след това през интервали от по 4 седмици. Състоянието на пациента трябва да се оценява непрекъснато. Спиране на лечението с Benlysta трябва да се обмисли, ако няма подобрение в степента на контрол на заболяването след 6 месеца лечение. **Начин на приложение** Benlysta се прилага интравенозно чрез инфузия и преди приложение трябва да се разтвори и разреди. Инфузията на Benlysta трябва да се прави в продължение на един час. Benlysta не трябва да се прилага като интравенозен болус. Скоростта на инфузия може да се забави или инфузията да се прекъсне, ако пациентът развие инфузионна реакция. Инфузията трябва да се спре незабавно, ако при пациента се проявят потенциално животозастрашаваща нежелана реакция. **Противопоказания** Свръхчувствителност към активното вещество или към някое от помощните вещества. **Специални предупреждения и предпазни мерки при употреба** Benlysta не е проучвана при посочените по-долу групи пациенти и не се препоръчва при: тежък активен лупус на централната нервна система; тежък активен лупусен нефрит; HIV; знаменателни данни за минала или настояща инфекция с хепатит B или C, хипогамаглобулинемия (IgG <400 mg/dl) или IgA дефицит (IgA <10 mg/dl), знаменателни данни за голяма органна трансплантация или трансплантация на хемопоетични стволови клетки или костен мозък, както и бъдеща трансплантация. Benlysta не е проучвана в комбинация с други терапии, насочени срещу B клетките, или с интравенозно приложен циклофосфамид. Едновременното приложение на Benlysta с други терапии, насочени срещу B клетките, или с циклофосфамид, трябва да се е повишено внимание. Механизмът на действие на Benlysta може да повиши риска за развитие на инфекции, включително опортюнистични инфекции. Тежки инфекции, включително случаи с летален изход, са съобщени при пациенти със СЛЕ, приемащи имуносупресивна терапия, включително белимумаб. Лекарите трябва да са с повишено внимание, когато обмислят приложение на Benlysta при пациенти с тежки или хронични инфекции, или с анамнеза за рецидивираща инфекция. Пациенти, които развиват инфекция по време на лечение с Benlysta, трябва да се проследяват непосредствено и внимателно да се обмисли прекъсване на имуносупресивната терапия, включително белимумаб, до овладяване на инфекцията. Не е известен риска от приложение на Benlysta при пациенти с активна или латентна туберкулоза. Прогресивна мултифокална левкоенцефалопатия (ПМП) е докладвана при лечение на СЛЕ с Benlysta. Специалистите трябва да бъдат особено внимателни относно симптоми, насочващи към ПМП, които пациентите могат да не забележат (напр. когнитивни, неврологични или психиатрични прояви или признаци). Пациентите трябва да се проследяват за някои от тези нови или влошаващи се симптоми или признаци и ако такива симптоми/признаци се появят, трябва да се обмисли консултация с невролог и подходяща диагностична оценка за ПМП. Ако има съмнения за ПМП, приемът трябва да се прекратяват до изключване на ПМП. Не трябва да се приемат живи ваксини 30 дни преди или едновременно с Benlysta. Тъй като не е установена клиничната безопасност, необходимо е повишено внимание при обмисляне на лечение с белимумаб при пациенти с анамнестични данни за злокачествено заболяване или при обмисляне на продължаване на лечението при пациенти, които междувременно развият злокачествено заболяване. **Бременност и кърмене** Жените с детероден потенциал трябва да използват ефективни контрацептиви по време на лечението с Benlysta и най-малко 4 месеца след последното приложение. Benlysta не трябва да се приема по време на бременност, освен ако потенциалната полза оправдава потенциалния риск за плода. Тъй като майчините антитела (IgG) се екскретират в кърмата, се препоръчва решението за спиране на кърменето или спиране на терапията с Benlysta да се направи, като се вземат предвид ползата от кърменето за детето и ползата от лечението за майката. Не са провеждани проучвания на **способността за шофиране и работа с машини.** От фармакологичните характеристики на Benlysta не се предвиждат нежелани ефекти върху тази дейност. **Нежелани лекарствени реакции** Безопасността на Benlysta при пациенти със СЛЕ е оценена в 3 плацебо-контролирани проучвания. Данните отразяват експозицията на Benlysta 10 mg/kg при 674 пациенти със СЛЕ, включително 472 пациенти с експозиция най-малко 52 седмици. Включени са и данни от следрегистрационни доклади. Най-често съобщаваните нежелани реакции (≥10% от пациентите със СЛЕ на лечение с Benlysta плюс стандартни грижи и с честота с ≥1% по-голяма от плацебо) са гадене, диария, пирексия и бактериални инфекции, напр. бронхит, цистит. При пациенти на лечение с Benlysta са съобщавани подтипични инфекции. Някои инфекции са тежки или летални. Пациентите, които развиват лечението поради нежелани лекарствени реакции, са 7%, както за пациентите на лечение с Benlysta, така и за тези на лечение с плацебо. **Предозиране** Има ограничен клиничен опит с предозиране на Benlysta. Съобщените нежелани реакции, свързани със случаи на предозиране, са в съответствие с тези, очаквани за белимумаб. При случайно предозиране пациентите трябва да се наблюдават внимателно и според случая да се прилага поддържаща терапия. **Притежател на разрешението за употреба** Glaxo Group Limited, 980 Great West Road, Brentford, Middlesex TW8 9GS, Великобритания. **От медицинските специалисти се изисква да съобщават всяка подозирана нежелана реакция чрез националната система за съобщаване. Изпълнителна агенция по лекарствата ул. „Дамян Груев" № 8 1303 София тел.: +359 2 890 34 17 уебсайт: www.bda.bg**

Дата на последно одобрение на КХП от регулаторния орган към датата на издаване на материала: 15.09. 2016 г.

КОМБИНИРАНА ТЕРАПИЯ



SGC-Разтворима гуанилат циклаза

*Half of patients on combination therapy

†SERAPHIN, 64% on combination therapy, 5% of patients on prostanoid; PATENT 1, 6%.

PACES: Simonneau G et al. *Ann Intern Med.* 2008;149:521-530; PHIRST: Galiè N et al. *Circulation.* 2009;119:2894-2903; STEP: McLaughlin VV et al. *Am J Respir Crit Care Med.* 2006;174:1257-1263. TRIUMPH: McLaughlin VV et al. *J Am Coll Cardiol.* 2010;55:1915-1922; SERAPHIN: Pulido T et al. *N Engl J Med.* 2013;369:809-18; PATENT-1: Ghofrani AH et al. N Engl J Med 2013; 369:330-340.

BENLYSTA е запазена марка на ГлаксоСмитКлайн. Лекарствен продукт по лекарско предписание. Прилага се при възрастни. Безопасността на пациентите е от първостепенно значение за ГлаксоСмитКлайн. Ако считате, че сте наблюдавали нежелана лекарствена реакция, предозиране или неправилна употреба, ако междувременно е настъпила бременност, ако сте наблюдавали неочаквана полза или липса на ефект, моля да се свържете с нас на телефон: (02) 953 10 34/ факс: (02) 950 56 05. Моля съблюдавайте също изискванията за докладване на нежелани лекарствени реакции към Изпълнителната агенция по лекарствата. За информация за медикаменти на ГлаксоСмитКлайн може да се свързвате с нас на горепосочените телефони. Материалът се придружава от актуална Кратка характеристика на продукта. Актуална Кратка характеристика на продукта можете да намерите на: http://bg.gsk.com/media/784401/benlysta_spc.pdf

VOLIBRIS е запазена марка на ГлаксоСмитКлайн. Лекарствен продукт по лекарско предписание. Прилага се при възрастни. Безопасността на пациентите е от първостепенно значение за ГлаксоСмитКлайн. Ако считате, че сте наблюдавали нежелана лекарствена реакция, предозиране или неправилна употреба, ако междувременно е настъпила бременност, ако сте наблюдавали неочаквана полза или липса на ефект, моля да се свържете с нас на телефон: (02) 953 10 34/ факс: (02) 950 56 05. Моля съблюдавайте също изискванията за докладване на нежелани лекарствени реакции към Изпълнителната агенция по лекарствата. За информация за медикаменти на ГлаксоСмитКлайн може да се свързвате с нас на горепосочените телефони. Материалът се придружава от актуална Кратка характеристика на продукта. Актуална Кратка характеристика на продукта можете да намерите на: http://bg.gsk.com/media/738392/volibris_spc.pdf

Уведомление до ИАЛ по чл. 254а(2) от ЗЛПХМ IAL-35967/ 24.08.2017г. Номер BG/BEL/0019/17 Дата на издаване: Август 2017 г.

AMBRISENTAN Е ET_A РЕЦЕПТОРЕН АНТАГОНИСТ С ВИСОК АФИНИТЕТ И ВИСОКА СЕЛЕКТИВНОСТ КЪМ ET_A СПРЯМО ET_B

- Ambrisentan е перорално активен селективен към ET_A рецептора антагонист от класа на пропановата киселина
- Ambrisentan блокира субтип ET_A рецепторите, локализирани предимно в съдовите гладкомускулни клетки и кардиомиоцитите
- Това предотвратява ендотелин-медиираната активация на системи от вторични медиатори, което води до вазоконстрикция и пролиферация на на гладкомускулните клетки
- Би могло да се очаква, че селективността на ambrisentan към ET_A рецептора ще запази ET_B рецептор - медиираната продукция на вазодилататори – NO и простациклин.

"Ambrisentan е показан за лечение на възрастни пациенти с БАХ, II и III функционален клас по СЗО за подобряване на физическия капацитет.
- Показал е ефикасност при иБАХ и БАХ, асоциирана с болест на съединителната тъкан"

ET_A, endothelin A; ET_B, endothelin B

Volibris КХП 26.04.2017

VOLIBRIS®

Съкратена лекарствена информация

Volibris 5 mg филмирани таблетки. Всяка таблетка съдържа 5 mg амбрисентан (ambrisentan). **Терапевтични показания** Volibris е показан за лечение на възрастни пациенти с белодробна артериална хипертония (БАХ), класифицирана като функционален клас II и III по СЗО, включително и за употреба в комбинирана терапия. Доказана е ефикасността му при идиопатична БАХ (иБАХ) и при БАХ, свързана със заболяване на съединителната тъкан. **Дозировка и начин на приложение** Лечение трябва да се започне само от лекар с опит в лечението на БАХ. Volibris трябва да се приема перорално в доза 5 mg веднъж дневно за започване на лечението и в зависимост от клиничния отговор и поносимостта, дозата може да се повиши до 10 mg дневно. Когато се използва в комбинация с тадалафил, Volibris трябва да се титрира до 10 mg веднъж дневно.
При едновременно прилагане с циклоспорин А, дозата на амбрисентан трябва да се ограничи до 5 mg веднъж дневно. Препоръчва се таблетката да се гълта цяла с или без храна. Препоръчва се таблетката да не се разделя, разтрошава или дъвче. **Противопоказания** Свръхчувствителност към активното вещество, към соя или към някое от помощните вещества. Бременност: Жени от детероден потенциал, които не използват надеждан метод за контрацепция. Кърмене: Тежко чернодробно увреждане (със или без цироза); Начални стойности на чернодробните аминотрансферази (аспартатаминотрансферази (AST) и/или аланинаминотрансферази (ALT)) >3xULN. Идиопатична белодробна фиброза (ИБФ), със или без вторична белодробна хипертония. **Специални предупреждения и предпазни мерки при употреба:** Амбрисентан не е проучен при достатъчен брой пациенти с цел да се установи съотношението полза/риск при БАХ, функционален клас I по СЗО. Ефикасността на амбрисентан като монотерапия не е установена при пациенти с БАХ функционален клас IV по СЗО. Пациентите трябва да се проследяват за чернодробно увреждане, вкл. ежемесечно ALT и AST. Ако пациентите развият необяснимо, значимо повишение на трансаминазите, или ако повишаването на трансаминазите е придружено от признаци или симптоми на чернодробно увреждане, лечението с амбрисентан трябва да се преустанови. Не се препоръчва започване на лечение с амбрисентан при пациенти с клинично значима анемия. По време на лечение с амбрисентан периодично се проследяват нивата на хемоглобина. Ако по време на лечение с амбрисентан се развие клинично значимо задържане на течности трябва да се определи причината, и възможната нужда от специфично лечение или прекъсване на терапията с амбрисентан. Ако пациентите с БАХ развият остър белодробен оток по време на лечение с амбрисентан, трябва да се обмисли възможността за белодробна венооклузивна болест. Пациенти, лекувани с амбрисентан, трябва внимателно да бъдат наблюдавани при започване на лечение с рифампицин. Volibris таблетки съдържа лактоза. Пациентите с редки наследствени проблеми на галактозна непоносимост, Lapp лактазен дефицит или глюкозо-галактозна малабсорбция не трябва да приемат това лекарство. Volibris таблетки съдържа лецитин, получен от соя. Амбрисентан не трябва да се приема, ако пациентът е свръхчувствителен към соя. **Фертилитет, бременност и кърмене** Не трябва да се започне лечение с амбрисентан при жени с детероден потенциал, освен при отрицателен тест за бременност и надеждна контрацепция. Амбрисентан е противопоказан по време на бременност и кърмене. Жени, приемащи амбрисентан, трябва да бъдат предупредени за риска от увреждане на фетуса, а ако настъпи бременност трябва да се започне алтернативно лечение. Ефектът върху фертилитета при мъже не е установен, но не може да се изключи влошаване на сперматогенезата. **Ефекти върху способността за шофиране и работа с машини** Амбрисентан има лек или умерено влияние върху способността за шофиране и работа с машини. **Нежелани лекарствени реакции** Най-честите нежелани реакции, наблюдавани при лечение с амбрисентан са периферен оток, задържане на течности и главоболие. По-високата доза (10 mg) е свързана с по-висока честота на тези нежелани реакции, а периферният оток е по-тежък при пациенти с 65 години и повече. НПР – анемия, намаление на хемоглобина, намаление на хематокрита, палпитации, хипотония, зачервяване, епистаксис, диспнея, конгестия в горните части на дихателната система, синузит, назофарингит, ринит, коремна болка, запек, гадене, повръщане, диария, повишаване на чернодробните трансаминази, грайпоподобна болка/дискомфорт, астения и умора. С неизвестна честота - замъглено зрение, зрително увреждане. **Срок на годност:** 5 години. **Специални условия на съхранение:** Този лекарствен продукт не изисква специални условия за съхранение. **Притежател на разрешението за употреба:** ViiV Healthcare UK Limited, 980 Great West Road, Brentford, TW8 9GS, Великобритания. **Местен представител на притежателя на разрешението за употреба:** ГлаксоСмитКлайн ЕООД, бул. Цариградско шосе № 115Г, 1784 София, България. **Съобщаване на подозирани нежелани реакции.** Съобщаването на подозирани нежелани реакции след разрешаване за употреба на лекарствения продукт е важно. Това позволява да продължи наблюдението на съотношението полза/риск за лекарствения продукт. От медицинските специалисти се изисква да съобщават всяка подозирана нежелана реакция чрез националната система за съобщаване: Изпълнителна агенция по лекарствата ул. „Дамян Груев" № 8 1303 София тел.: +359 2 890 34 17 уебсайт: www.bda.bg.

Дата на последно одобрение на КХП от регулаторния орган към датата на издаване на материала: 26.04.2017 г.

SESSION 2

Moderators: Rada Staneva, Ralitsa Yordanova

- **RASopathies: clinical and molecular correlations**
  G. Neri

- **Cherubism – a rare genetic disorder**
  E. Severin

Oral presentations:

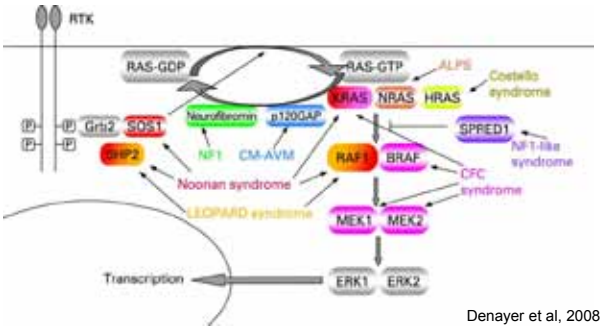
- **A rare disease of the mitochondrial respiratory chain – 3-methylglutaconic aciduria. Approach to diagnosis and rehabilitation**
  O. Grechanina

- **Selective screening of mitochondrial dysfunction in a region with a high level of neurological diseases**
  Y. Grechanina

RASOPATHIES: CLINICAL AND MOLECULAR CORRELATIONS

Giovanni Neri

THE RAS-ERK SIGNALING PATHWAY

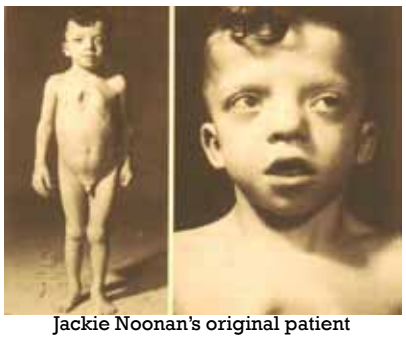


Denayer et al, 2008

OUTLINE OF PRESENTATION

- Definition and nosology
- Genes and pathways
- Clinical description
- Treatment

NOONAN SYNDROME



Jackie Noonan's original patient

RASOPATHIES

A group of developmental disorders characterized by:

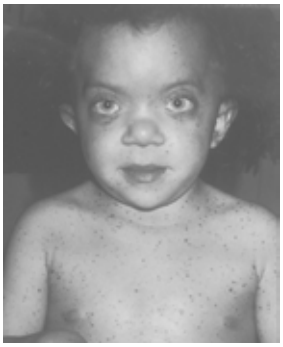
- peculiar facies
- cardiac defects
- growth failure
- ectodermal anomalies
- musculoskeletal anomalies
- intellectual disability
- autosomal dominant inheritance

MAIN CLINICAL CHARACTERISTICS OF NOONAN SYNDROME

Short stature
Relative macrocephaly
Peculiar face
Pectus excavatum
Congenital heart defect (PS)
Cryptorchidism
Hemorrhagic diathesis
Mild intellectual disability (in some cases)

THE RASOPATIES: SYNDROMES AND GENES

SYNDROMES	OMIM	GENES
Noonan	163950	*PTPN11, KRAS, RAF1, SOS1, SOS2, KRAS, NRAS, BRAF, RIT1*
Noonan-multiple lentigines	151100	*PTPN11, BRAF, RAF1*
Costello	218040	*HRAS*
Cardio-facio-cutaneous (CFC)	115150	*BRAF, MEK1, MEK2, KRAS*
Noonan-like with anagen hair	607721	*SHOC2, PPP1CB*
Noonan-like, with or without juvenile myelomonocytic leukemia	613563	*CBL*
Neurofibromatosis 1	162200	*NF1*
Legius (Neurofibromatosis type1-like syndrome)	611431	*SPRED1*



NOONAN SYNDROME WITH LENTIGINES

CFC SYNDROME

New Multiple Congenital Anomalies/Mental Retardation Syndrome With Cardio-Facio-Cutaneous Involvement—The CFC Syndrome

James F. Reynolds, Giovanni Neri, Jurgen P. Herrmann, Bruce Blumberg, James G. Caldwell, Paul V. Miles, and John M. Opitz

Shodair Children's Hospital, Helena, Montana (J.F.R., J.M.O.), Instituto di Genetica Umana, Facolta di Medicina "A Gemelli" UCSC, Rome, Italy (G.N.), Great Lakes Genetics SC, Wauwatosa, Wisconsin (J.P.H.), Permanente Medical Group, San Francisco, California (B.B.), Children's Medical Center, Tulsa, Oklahoma (J.G.C.), and Pediatric Center, Twin Falls, Idaho (P.V.M.)

A SCIENTIFIC CONTROVERSY

American Journal of Medical Genetics 39:367–370 (1991)

Editorial Comment

The Noonan-CFC Controversy

Giovanni Neri, Marcella Zollino, and James F. Reynolds

Istituto di Genetica Umana, Facoltàdi Medicina "A. Gemelli", U.C.S.C., Roma, Italy (G.N., M.Z.) and Department of Medical Genetics, Shodair Children's Hospital, Helena, Montana

Reynolds et al. (1986) described 8 patients presenting with

- Cardiac defects
- Distinct facial appearance
- Ectodermal abnormalities
- Growth retardation
- Intellectual disability

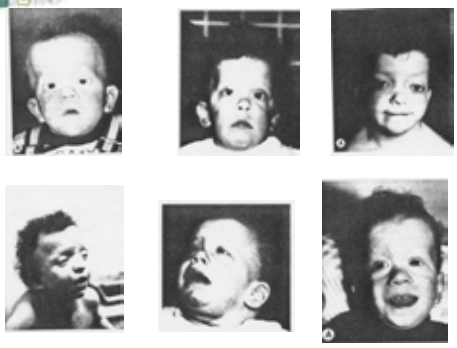
European Journal of Human Genetics (2003) 11, 64–68

ARTICLE

PTPN11 mutations are not responsible for the Cardiofaciocutaneous (CFC) syndrome

MI Kavamura*[1,2,3], MG Pomponi[1], M Zollino[1], R Lecce[1], M Murdolo[1], D Brunoni[2], MMA Alchorne[2], JM Opitz[1,4] and G Neri[1]

[1]Istituto di Genetica Medica, Università Cattolica del Sacro Cuore, Rome, Italy; [2]Centro de Genética Médica, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brazil; [3]Departamento de Dermatologia, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brazil; [4]Pediatrics (Medical Genetics), Human Genetics, Obstetrics and Gynecology, University of Utah, Salt Lake City, USA



Germline Mutations in Genes Within the MAPK Pathway Cause Cardio-facio-cutaneous Syndrome

Pablo Rodriguez-Viciana,[1]* Osamu Tetsu,[2,3]* William C. Tidyman,[2] Anne L. Estep,[2] Brenda A. Conger,[2] Molly Sands Cruz,[2] Frank McCormick,[1,2]† Katherine A. Rauen[2,3]†

***Science*, March 2006**

Nature Genetics 38, 294 - 296 (2006)

Germline *KRAS* and *BRAF* mutations in cardio-facio-cutaneous syndrome

Tetsuya Niihori[1], Yoko Aoki[1], Yoko Narumi[1], Giovanni Neri[2], Hélène Cavé[3], Alain Verloes[3], Nobuhiko Okamoto[4], Raoul C M Hennekam[5], Gabriele Gillessen-Kaesbach[6], Dagmar Wieczorek[6], Maria Ines Kavamura[7], Kenji Kurosawa[8], Hirofumi Ohashi[9], Louise Wilson[10], Delphine Heron[11], Dominique Bonneau[12], Giuseppina Corona[13], Tadashi Kaname[14], Kenji Naritomi[14], Clarisse Baumann[3], Naomichi Matsumoto[15], Kumi Kato[1, 16], Shigeo Kure[1] & Yoichi Matsubara[1, 16]

Clinical manifestations of the CFC syndrome

- Short stature
- Characteristic face
- Congenital heart defect (HC, PS)
- Skin and hair abnormalities
- Feeding difficulties
- Seizures
- Intellectual disability

CFC (BRAF p.Q257R missense mutation)

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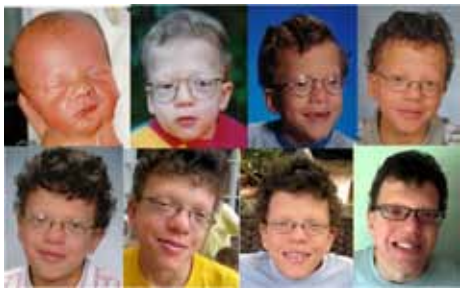

CFC (MEK2 p.F57C missense mutation)

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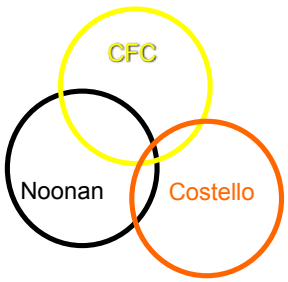
Differential diagnosis



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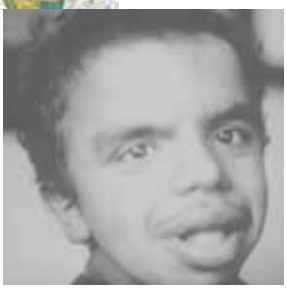

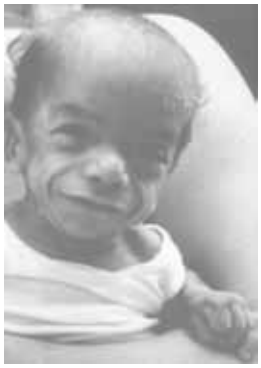

COSTELLO SYNDROME

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THERAPEUTIC PERSPECTIVES

- ✓ Tyrosine kinase inhibitors(sorafenib)
- ✓ Farnesylation inhibitors(lonafarnib, statins)
- ✓ **Need for clinical trials**

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PEDIATRICS
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Cardio-Facio-Cutaneous Syndrome: Clinical Features, Diagnosis, and Management Guidelines

Mary Ella M. Pierpont, Pilar L.Magoulas, Saleh Adi, Maria Ines Kavamura, Giovanni Neri, Jacqueline Noonan, Elizabeth I. Pierpont, Kent Reinker, Amy E.Roberts, Suma Shankar, Joseph Sullivan, Melinda Wolford, Brenda Conger, Molly Santa Cruz, and Katherine A. Rauen

Pediatrics Vol. 134 No. 4 October 1, 2014 pp. e1149 -e1162

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Costello syndrome phenotype

- ✓Polyhydramnios, macrosomia, macrocephaly
- ✓Short stature
- ✓ "Coarse" face with depressed nasal bridge, thick lips, epicanthal folds, strabismus, downslanting palpebral fissures, curly and sparse hair, low-set ears with thickened lobes
- ✓ Macroglossia and high-arched palate
- ✓ Short neck with loose, redundant skin
- ✓Congenital heart defect (hypertrophic cardiomyopathy, pulmonic stenosis, mitral valve prolapse, ventricular septal defect, dysrhythmias)
- ✓ Deep palmar creases
- ✓ Cutis laxa (especially hands and feet), dark skin pigmentation, papillomas (perioral, nasal, and anal regions), acanthosis nigricans
- ✓ Intellectual disability
- ✓ Increased incidence of tumors

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COMMON	MAY OCCUR	RARE
	Cardiovascular	
Pulmonary valve stenosis	Supravalvular pulmonary stenosis	Aortic root dilation
Hypertrophic cardiomyopathy	Bicuspid aortic valve	Restrictive cardiomyopathy
Atrial septal defect	Coarctation of the aorta	Ebstein's anomaly of the tricuspid valve
	Ventricular septal defects	Coronary artery abnormality
	Mitral valve anomalies	Aortic valve thickening
		Rhythm disturbance

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Cardiovascular	**At risk for: pulmonary stenosis, hypertrophic cardiomyopathy, septal defects.** **At diagnosis:** • **Echocardiogram, electrocardiogram, chest x-ray.** • **Refer to cardiologist.** **Ongoing Management:** • **Cardiology follow-up if cardiac disease found at diagnosis or at each of the age intervals below.** • **Infancy up to 1 year: If arrhythmias present, 24-hour Holter evaluation.** • **Childhood and Adolescence (up to 20 years): If no cardiac disease found initially, repeat echocardiogram every 2-3 years. Measurement of blood pressure at each visit.** • **Adulthood (>20 years): Echocardiogram every 3-5 years if no previous heart disease found. Measurement of blood pressure at each visit.**

CHERUBISM – A RARE GENETIC DISORDER

Emilia Severin, Octavian Dincă, Cristian Vladan, Dana Cristina Bodnar and Alexandru Bucur

CLASSIFICATION CODES

CHERUBISM

ICD-10
K 10.8
Orpha number:
ORPHA184

OMIM
118400
POSSUM
3266

DISEASE DEFINITION

CHERUBISM is a rare, self-limiting, fibro-osseous, genetic disease of childhood and adolescence characterized by varying degrees of progressive bilateral enlargement of the mandible and/or maxilla, with clinical repercussions in severe cases. (orphanet)

Synonym(s):
- Fibrous dysplasia of jaws
- Familial multi-locular cystic disease
- Jones syndrome

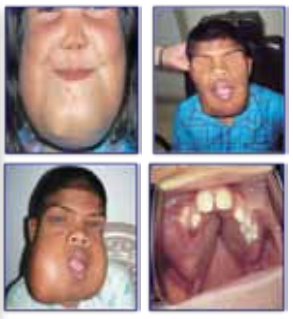
Fullness of the face with cherub-like physiognomy.

EPIDEMIOLOGY

CHERUBISM

- PREVALENCE - unknown
- SEX-RATIO – 1M:1F
- AGE of ONSET - childhood
- All racial and ethnic groups affected
- Unequal penetrance between males and females ?

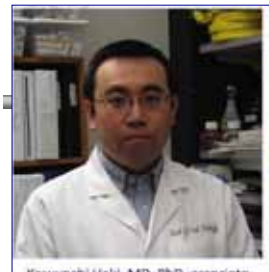
CLINICAL PHENOTYPE



I do wonder why, in the 21st century, non-disabled people still have a problem with us. Are we really so frightening? Victoria

http://www.primehealthchannel.com/cherubism.html

TIMELINE of CHERUBISM



Yasuyoshi Ueki, MD, PhD, associate professor at the University of Missouri–Kansas City School of Dentistry

Innovative studies of cherubism may aid development of therapies for common inflammatory bone diseases

WHAT CAUSES CHERUBISM ?

- Cherubism is caused by missense mutations in the SH3BP2 gene (4p16.3) in approximately 80% of cases, suggesting genetic heterogeneity.
- The exact mechanism underlying fibrous expansion has not been elucidated. Experimental data points to possible auto-inflammatory disease.

SH3-domain binding protein 2 [*Homo sapiens*]

(OMIM 602104)

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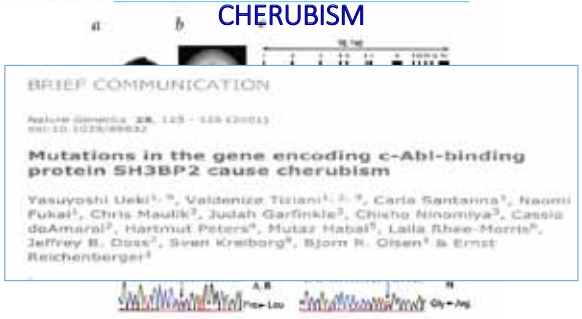
FIRST GENE MUTATION DISCOVERED IN CHERUBISM



BRIEF COMMUNICATION

Mutations in the gene encoding c-Abl-binding protein SH3BP2 cause cherubism

Yasuyoshi Ueki[1-3], Valdenize Tiziani[1,2,3], Carla Santanna[1], Naomi Fukai[1], Chris Maulik[1], Judah Garfinkle[1], Chisho Ninomiya[1], Cassio doAmaral[1], Hartmut Peters[4], Nutaz Habal[5], Laila Rhee-Morris[5], Jeffrey B. Doss[5], Sven Kreiborg[6], Bjorn R. Olsen[1] & Ernst Reichenberger[1]

CHERUBISM - a rare genetic disorder

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TWO CASE REPORTS:

CASE 1- familial

A. Presenting patient: 9-year-old Caucasian girl

B. Chief complaint: she complained of painless, progressive and disfiguring enlargement of the lower face, speech and swallowing problems.

CASE 2- non-familial

A. Presenting patient: 12-year-old Caucasian girl

B. Chief complaint: she complained of facial appearance with swollen cheeks, round and asymmetrically full lower face. There was no functional complaint.

CHERUBISM - a rare genetic disorder

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DIAGNOSTIC METHODS / INVESTIGATIONS

Diagnosis is based on a combination of:

- clinical signs,
- patient age,
- radiographic findings (panoramic x-rays, CT scan),
- biopsy,
- family history,
- and can be confirmed by molecular genetic testing.
- Histology shows spindle cells embedded in interstitial collagen fibers and osteoclastic giant-cells.

CHERUBISM - a rare genetic disorder

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8-10 September 2017, Plovdiv, Bulgaria

TWO CASE REPORTS:

CASE 1- familial

C. Social history : patient attends normal public school; lives at home with both parents and two older sisters; socio-economic status is lower middle class.

CASE 2- non-familial

C. Social history : patient attends normal public school; lives at home with both parents; no siblings; lower middle class socio-economic status.

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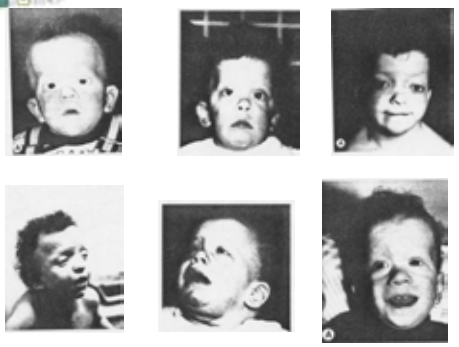

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MEDICAL CONSULT:

CASE 1- familial

D. Extra-oral exam : revealed swellings at the angles of the mandible, a little asymmetrical enlargement of the mandible and chubbiness of the face ; the patient did not exhibit any physical abnormality and showed no signs of mental retardation.

E. Intra-oral exam: Mixed dentition, bad alignment of the teeth, and poor occlusion were noticed.

CASE 2- non-familial

D. Extra-oral exam : revealed fullness of the lower face, painless enlargement at the angles of mandible, bilateral swelling but right side was more prominent then the left side, no evident enlarged lymph nodes under mandible.

E. Intra-oral exam: normal soft tissue of the mouth, normal tongue, normal palate and no delayed eruption or missing teeth. Poor occlusion was noted. No extension of the swelling in the mouth was observed.

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STEPS OF SURGICAL TREATMENT

- surgical intervention involving resection, curettage or contouring may be required in patients with functional manifestations or for esthetic reasons and to improve quality of life. Surgery is generally indicated once lesions have become quiescent and does not alter disease progression.

http://www.craniofacial.net/conditions-cherubism

CHERUBISM - a rare genetic disorder

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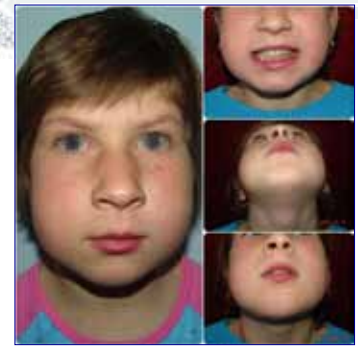

CASE 1 - Clinical examination revealed swellings at the angles of the mandible, a little asymmetrical enlargement of the mandible and chubbiness of the face.

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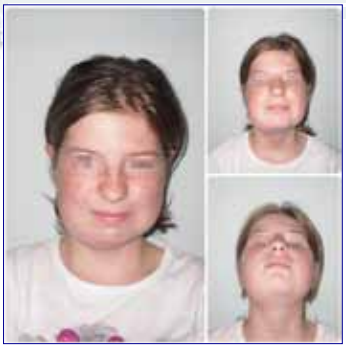

CASE 2 - Facial appearance (a, b – frontal and c – submental images) showing asymmetrical swelling of the cheeks caused by unequal mandibular enlargement (the right side is more prominent than the left one).

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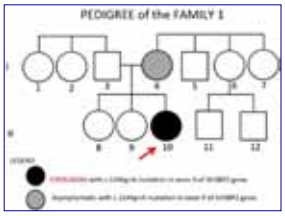
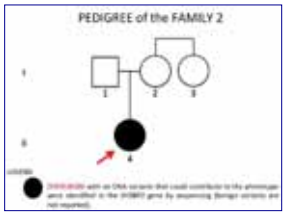
FAMILY STUDY



Familial case



Sporadic case

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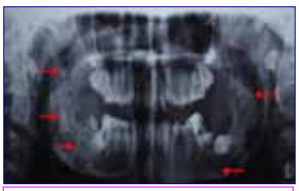
PANORAMIC RADIOGRAPHS:

F. **CASE 1:** OPG showed clearly that the facial enlargement is the result of bone changes. Radiological examination revealed bilateral multilocular areas of diminished density in the mandible and congenitally missing 2nd and 3rd lower molars. The maxillary dentition was not affected.



F. CASE 2: OPG showed clearly that the facial enlargement is the result of abnormal bone pattern in the mandible. Radiological examination revealed bilateral multilocular radiolucent areas within the bone. The lesions are restricted to the angle and body of mandible. Maxilla was not involved.

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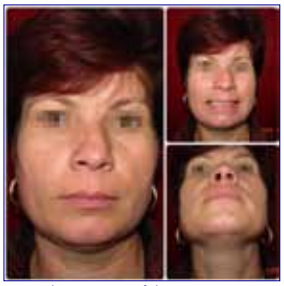
MOTHER of CASE 1



Facial appearance of the asymptomatic mother.

Recent orthopantomography of the mother shows normal radiological appearance without any remnants.

Identical Mutation in SH3BP2 Gene Causes Clinical Phenotypes with Different Severity in Mother and Daughter

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BIOPSY and HISTOLOGICAL FINDINGS:

Cherubism was suspected based on suggestive clinical, radiological and histological findings.

G. **CASE 1:** Histological examination demonstrated the replacement of the normal bony structure with proliferating fibrous tissue containing numerous multinucleated acidophilic giant cells randomly distributed in a fibro-vascular stroma of nuclear spindle-shaped cells.

G. **CASE 2:** Histological examination reported the replacement of the normal bony structure with fibrous tissue containing a reduced number of multinucleated giant cells scattered in a fibro-vascular stroma of nuclear spindle-shaped cells.

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PARENTS of CASE 2

Both parents are normal.

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GENETIC TESTING

Molecular analysis consisted of PCR amplification and direct sequencing of exons 3, 4 and 9, an intron–exon boundaries of SH3BP2 gene harboring all variants reported to date. The reference sequence and exon numbering are according to GenBank accession number NM_003023.4 with the A of the ATG start codon on position 1.

H. CASE 1: A c.1244G > A mutation was identified in exon 9 of the SH3BP2 gene for patient and her mother.

H. CASE 2 : No DNA variants that could contribute to the phenotype were identified in the SH3BP2 gene by sequencing.

Failure to identify a SH3BP2 mutation in affected individuals suggests possible genetic heterogeneity, and a genetic cause is still likely.

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TREATMENT PLAN:

I. CASE 1: No operative treatment was undertaken. The patient was scheduled for regular follow-up (including clinical, radiographic, dental, orthodontic, and ophthalmologic evaluations).

I. CASE 2: No operative treatment was undertaken. Follow-up was recommended with regular medical checkup in order to identify any change of patient's disorder evolution.

CHERUBISM - a rare genetic disorder

PARTICULARITY OF:

CASE 1 the incomplete penetrance of the disease caused by the same mutation in 2 female members of the same family and the possibility to under-diagnose the condition without molecular genetic testing.

CASE 2 failure to identify a SH3BP2 mutation in 20% of affected individuals suggests possible genetic heterogeneity.

CHERUBISM PREVENTION:

- Couples with a family history of this disease should consider genetic counseling before deciding having a child.
- Jones pointed out that lack of signs or history in either parent does not exclude the possibility of one's being affected.
- Naturally, genetic testing is the only way of prevention. However, the gene responsible for it is sometimes known to undergo spontaneous mutation.

In these cases, there is no way of preventing the disorder.


Cherubism: best clinical practice

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- https://rarediseases.info.nih.gov/diseases/6036/cherubism/cases/27480#3583.
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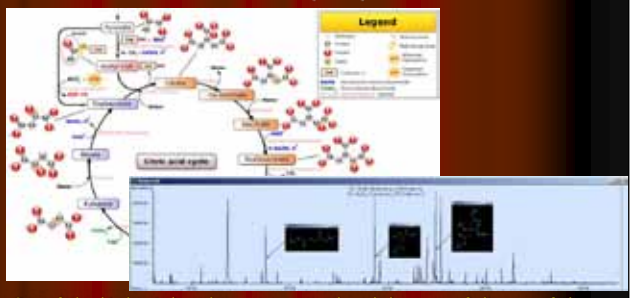
- Cherubism: a case report – Octavian Dincă, **Emilia Severin**, Cristian Vlădan, Dana Cristina Bodnar, Alexandru Bucur, Romanian Journal of Morphology and Embryology, 2014, 55 (2 Suppl): 3-6, 2014.
- Identical mutation in SH3BP2 gene causes clinical phenotypes with different severity in mother and daughter – case report – L.Preda, O.Dinca, A. Bucur, Cristina Dragomir, **Emilia Severin**, Molecular Syndromology, 2010; 1: 87-90, ISSN 1661-8769 (print). DOI:10.1159/000314268, 2010, PMID: 21045962 [PubMed]

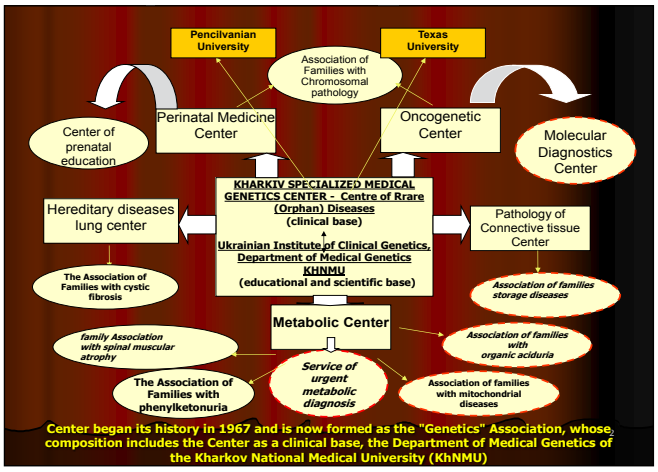
A RARE DISEASE OF THE MITOCHONDRIAL RESPIRATORY CHAIN – 3-METHYLGLUTACONIC ACIDURIA (MGA).APPROACH TO DIAGNOSIS AND REHABILITATION

Olena Grechanina

Biochemical markers common to the whole group of diseases of the mitochondrial respiratory chain



One of the biochemical markers common to the whole group of diseases of the mitochondrial respiratory chain is the metabolites of the Krebs cycle, including 3-methylglutaconic acid. For this group of diseases there is no ethiopathogenetic therapy. But at the same time, a number of researchers have developed methods for correcting oxidative phosphorylation, which, with dynamic observation of the patient with an individual assessment of his condition, improve the quality of life of the patient. 5

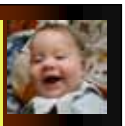


Center began its history in 1967 and is now formed as the "Genetics" Association, whose composition includes the Center as a clinical base, the Department of Medical Genetics of the Kharkov National Medical University (KhNMU)

The purpose of our investigation was to study the time of the manifestation, the nature of the clinical and biochemical phenotypes of methylglutaconic aciduria

6

Dynamics of the number of consultations of patients with suspected hereditary pathology. It looks like on this table. More than 35000 families consults every year. 4614 nosological forms stored in the register

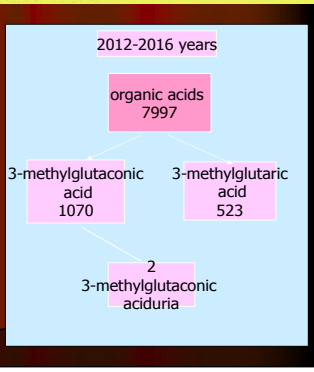


	2015	2014	2013	2012	2011	2010	2009	2008	2007	2006	Over 10 years
Total people	38612	24060	38548	38489	35464	35041	35036	34699	34683	34505	373137
Primary	12000	13018	13867	11258	10429	10837	18746	18018	17630	12641	138444
Repeated	26612	29042	21681	24240	25035	24204	16290	16681	17053	21864	222702
Refined nosological forms	533	424	430	445	387	444	591	523	432	405	4614

Hereditary diseases of the mitochondrial respiratory chain include a significant number of clinically polymorphic and genetically heterogeneous forms

7

Over the past 5 years, 7997 studies of organic acids with GC-MS in patients with suspected hereditary metabolic diseases have been carried out. Revealed only 2 patients.



4

3-methylglutaconic acid is an intermediate (as well as CoA thioester) in the pathway of leucine decay, in addition, the mevalonate metabolic pathway binds to the metabolism of isoprenoids with the participation of the mitochondrial acyl-CoA metabolism (PMID: 7603789). A significant increase in it leads to a decrease in the ability to metabolize leucine. Altered leucine values were found with methylglutaconic aciduria and mitochondrial disorders, but changes in leucine metabolites may not be associated with the MD (figure)

8

CHAIN-3-METHYLGLUTACONIC ACIDURIA IS CHARACTERIZED OF VARIETY OF CLINICAL FEATURES

3-MGA type I (hydrate-3-methylglutaconyl-CoA deficiency)

- Ammonia - ↑ (www.metagene.de), N or ↑ (N. Blau)
- Glucose - N or ↓
- Transaminases (aspartate transaminase, alanine transaminase) in serum - N or ↑
- Carnitine, free and common in plasma - ↓
- C5-hydroxyacylcarnitine - ↑ - ↑↑↑
- C6-unsaturated acylcarnitine - ↑ - ↑↑↑
- 3-methylglutaconic acid - ↑↑↑
- 3-methylglutaric acid - ↑↑ - ↑↑↑
- 3-hydroxyisovaleric acid - ↑ - ↑↑

9

Clinical phenotype:

- Cognitive impairment
- Delay in speech development
- Attention Deficit
- Fibrillation cramps
- Growth retardation
- Dysatria
- Progressive cerebral atrophy
- Cerebella ataxia
- Atrophy of the basal ganglia
- Leukoencephalopathy
- Enuresis (in adults)

The molecular basis is the mutation of the gene in the AUA-specific RNA-binding protein (AUH, 600529.001) 9q22.31.

10

3-MGA II type (Barth's syndrome, cardio myopathy, skeletal myopathy with atypical mitochondropathy)

- Uric acid in the blood - N or ↑
- Carnitine, free and common in plasma - N or ↓
- 3-methylglutacon sorbitol - ↑ - ↑↑
- 3-methylglutaric acid - ↑ - ↑↑
- 2-ethylhydrazrylic acid - N or ↑
- The ratio of Monolysocardiolipin (Monolysocardiolipin - MLCL) and tetralinoyl-cardio-lipin (tetralinoleyl-cardiolipin - CL4) - ↑

11

Clinical phenotype:

- Cognitive impairment
- Delay in speech development
- Attention Deficit
- Fibrillation cramps
- Growth retardation
- Dysatria
- Progressive cerebral atrophy
- Cerebella ataxia
- Atrophy of the basal ganglia
- Leukoencephalopathy
- Enuresis (in adults)

The molecular basis is the mutation of the gene in the AUA-specific RNA-binding protein (AUH, 600529.001) 9q22.31.

12

3-MGA III type (Kostefe's syndrome)
Atrophy of the optic nerves 3, autosomal recessive

- 3-methylglutaconic acid - ↑↑↑
- 3-methylglutaric acid - ↑↑ - ↑↑↑
- Clinical phenotype
- Cognitive disorders, dysarthria
- Visual atrophy
- Decreased visual acuity
- Ataxia
- Spasticity
- Hyperreflection
- Plantar extension reaction
- Extrapyramidal signs

There were mutations in the OPA3 gene (OPA3, 606580.0001).

13

3-MGA IV type

- 3-methylglutaconic acid - ↑↑↑
- 3-methylglutaric acid - ↑↑ - ↑↑↑
- Clinical phenotype
- Severe delay of psychomotor development
- Neonatal hypotension
- Absence of reflexes
- Subaortic stenosis
- Biventricular hypertrophy

Molecular-genetic characteristics - mutations MGCA1, MGA4.

14

3-MGA V type
(advanced cardiomyopathy with ataxia)

- 3-methylglutaconic acid - ↑ - ↑ ↑
- 3-Methylglutaric acid - ↑ - ↑ ↑
- Transaminases (aspartate transaminase, alanine transaminase) in serum - N or ↑

15

Clinical phenotype:

- violation of prenatal development;
- violation of postnatal development;
- visual atrophy (in some patients);
- dilated cardiomyopathy, early onset;
- long qt syndrome;
- heart failure;
- spongy cardiomyopathy;
- sudden death from cardiac arrest;
- microvesicular steatosis of the liver with hypospadias;
- the chord of the penis;
- small atrophied testicles (testicles);
- cryptorchidism;
- muscle weakness;
- a slight decrease in the activity of the mitochondria of the respiratory chain;
- cerebellar ataxia, non-progressive;
- mental retardation, mild, non-progressive;
- microcytic anemia.

Molecular-genetic features-induced mutations in the homologous gene of E. coli DNAJ subfamily C term 19, TIM 14, 3q25.33

16

MEGDEL, (3-MGA with deafness, encephalopathy and Leia like syndrome), 3-MGA type VI

- Ammonia - ↑ (www.metagene.de), N or ↑ (N. Blau)
- Glucose - N or ↓
- Methionine in the blood - N or ↑↑
- Creatine kinase in serum - N or ↑
- Transaminases (aspartate transaminase, alanine transaminase) in serum - N or ↑
- Carnitine, free and common in plasma - ↓
- 3-methylglutaconic acid - ↑ - ↑↑↑
- 3-methylglutaric acid - ↑ - ↑↑↑
- Lactate in urine - N or ↑
- Lactate in the blood - N or ↑
- Serum cholesterol - N or ↓
- Intracellular accumulation of unesterified cholesterol
- Abnormal phospholipid profile

17

Clinical phenotype:

- violation of growth,
- microcephaly,
- sensorineal deafness,
- malnutrition,
- hypotension,
- psychomotor regression,
- mental retardation,
- spasticity,
- dystonia,
- extrapyramidal symptoms,
- convulsions (less often),
- leigh syndrome,
- defeat of the basal ganglia,
- atrophy of the brain and cerebellum,
- lactic acidosis,
- hypoglycemia,
- recurrent infections,
- neonatal sepsis,

Molecular genetic features of SERAC1, MEGDEL 6q25.3

18

3-MGA with cataracts with neurological involvement and neutropenia (MEGCANN, 3-MGA VII type)

- 2-methyl-2,3-dihydroxybutyric acid - ↑ - ↑↑
- S- (2-carboxypropyl) -cysteine
- 3-methylglutaconic acid - ↑↑ - ↑↑↑
- 3-methylglutaric acid - ↑↑ - ↑↑↑
- A secondary decrease in the activity of pyruvic acid dehydrogenase
- Lactate in the urine in the blood - ↑

19

Clinical features:

- weak (low) growth;
- microcephaly;
- dysmorphic features (in some patients);
- cataract (in most patients);
- malnutrition;
- hypotension, neonatal;
- increased muscle tone, in newborns (in severely affected patients);
- delayed psychomotor development (in most patients);
- intellectual disabilities;
- regression development (in some patients);
- spasticity;
- pyramidal symptoms;
- extrapyramidal symptoms;
- abnormal movements;
- convulsions (in some patients);

The mutations induced in caseinolytic peptidase B of the gene (CLPB, 616254.0001)

20

3-MGA VIII type

- uric acid in the blood - N or ↑
- 3-methylglutaconic acid - ↑ - ↑↑
- 3-methylglutaric acid - ↑ - ↑↑

21

Insufficiency of hydratase 1 of short-chain enoyl-CoA

- 2-methyl-2,3-dihydroxybutyric acid - ↑ - ↑↑
- S- (2-carboxypropyl) -cysteine
- 3-methylglutaconic acid - ↑↑ - ↑↑↑
- 3-methylglutaric acid - ↑↑ - ↑↑↑
- A secondary decrease in the activity of pyruvic acid deplexide dehydrogenase
- Lactate in urine - ↑
- Lactate in the blood - ↑

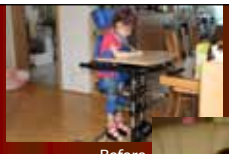
22

3-hydroxy-3-MGA (insufficiency of the lyase of 3-hydroxymethylglutaryl-CoA)

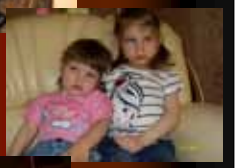
- Ammonia - ↑ (www.metagene.de), N or ↑ (N. Blau)
- Glucose - N or ↓
- Transaminases (aspartate transaminase, alanine transaminase) in serum - N or ↑
- Carnitine, free and common in plasma - ↓
- C5-hydroxyacylcarnitine - ↑ - ↑↑↑
- C6-unsaturated acylcarnitine - ↑ - ↑↑↑
- 3-methylglutaconic acid - ↑↑↑
- 3-methylglutaric acid - ↑↑ - ↑↑↑
- 3-hydroxyisovaleric acid - ↑ - ↑↑

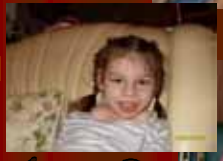
23

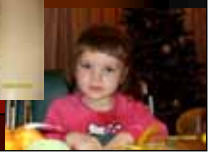
Among all the cases found, the family G., who have 2 children with MGA, deserves the most attention, and is registered with the Center since 2014



Before







After

Patient Yu. - complaints about

- the delay of development
- she does not hold objects
- salivation
- burping
- hypotrophy
- musclular hypertonicity

25

In this slide you can see results of the conducted researches:

- Karyotype - 46 XX, G-, C-staining, 1% chromosomal instability

Inspection Yu .:

- HPLC of blood amino acids from 06.11.2014: methionine ↑, tryptophan ↑, alanine ↑), threonine ↑, lysine ↑, other amino acids - N.
- HPLC of blood amino acids from 10.09.2015: methionine ↑, cystine ↓, glutamate ↑, glutamine ↑, aspartate ↑, asparagine ↑, arginine ↑, ornithine ↑, alanine ↑, lysine ↑, serine ↑.
- Blood homocysteine ↑; lactate of blood ↑; vitamins of group B - N.
- Virological examination (PCR-saliva): CMV, EBV, herpes simplex virus, type 1, type 2 - not detected.
- Herpes virus, type 6 PCR (saliva) - detected.
- Biotin - N
- Pyruvate - N
- Ammonia - ↓
- Blood electrolytes: calcium, phosphorus, iron, fasting glucose, ceruloplasmin - N, zinc ↓, copper - ↓.
- Biochemical blood test: uric acid- ↓, creatinine, urea within the reference values.
- Polymorphic variants of genes of folate cycle enzymes were studied: MTHFR 677 CT gene was detected; MTRR 66 GG.

29

The anamnesis reflects the progression of the disease from the moment of birth

- *Anamnesis of life:*

- polyhydramnios during pregnancy;
- weakness of labor;
- time of manifestation is 2 months.

- *First signs:*

- does not follow toys;
- established-minimal external hydrocephalus;

- *MRI at 6 months:*

- extended subarachnoid spaces;
- hypoplasia of the frontal lobes

26

- **Urine analysis by gas chromatography** - the dynamics revealed the following changes:

moderate increases in 3-methylglutaric (7.68 mmol/ mol crea), 3-methylglutaconic (130.18mmol/mol crea) acids and changes in metabolites: leucine; insufficiency of coenzyme Q10; connective tissue and/or vitamin C deficiency.

In dynamics the involvement of different aspects of metabolism is observed: neurotransmitters; ketosis, disturbance of BC oxidation with branched chain; insufficiency B3, B6, biotin, folic acid, B12, coenzyme Q10, Fe, Cu, Mg, Zn; increase of lactic acid, violations of the gastrointestinal microflora; the insufficiency of tryptophan and/or serotonin can not be ruled out.

30

Obvious is the progress of the current

- At 7 months:

- wince;
- muscular hypotension;
- delay of mental, pre-speech and stato-kinetic development;
- proximal hypotension with pyramidal insufficiency;
- symptomatic epilepsy;

- In 1 year 7 months:

- unmotivated laughter;
- closed;
- torsion of the trunk from side to side;
- atrophic changes in the frontal lobes.

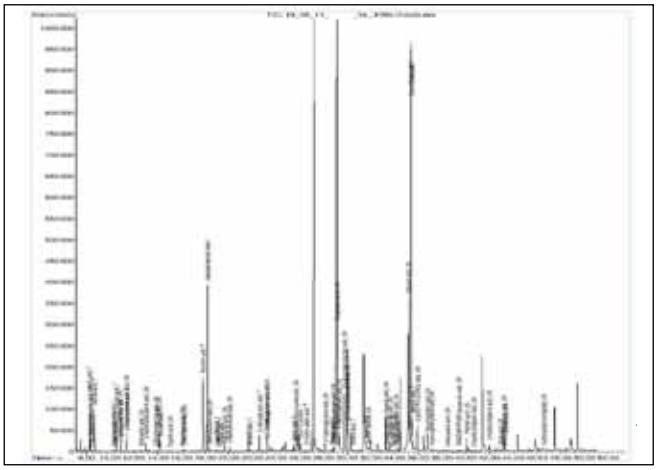
- In 4.5 years:

- myoclonic absences

- Examination at the InstitutFurKlinischeGenetikBoon Clinic:

- excluded are the Rett and Angelmann syndrome,the diagnosis is not established, cranio-sacral therapy launched

27



At the age of 5, she was inspected in KhMSMGC:

- a slight decrease in body weight with normal growth: Yu weight 13kg (N 18 kg), height 108 cm (N);
- pallor, marbling of the skin, superficial arrangement of the vessels;
- hypoplasia of subcutaneous fat;
- thinning hair;
- the face is narrow;
- nose short;
- gingival hyperplasia;
- narrow chest;
- kyphoscoliotic curvature of the spine;
- narrow brushes, hypermobility of the joints of the upper limbs, pronounced muscular hypotension.

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- These data were taken into account in determining the refining diagnosis from the development of tactics of symptomatic treatment.
- Diadnosis: Disturbance of the exchange of the respiratory chain of the mitochondria - methyl glutaconic aciduria
- Insufficiency of methionine synthase reductase reduced activity of methylenetetrahydrofolate reductase.

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You can see, that clinical and biochemical features are the same

Patient K. 3 years:
- the delay of development
- she does not hold objects
- salivation, burping
- hypotrophy, hypertonicity
- in the neonatal period, jaundice

of newborns, acute respiratory illness
- stereotype movements
- there is no eye contact
- signs of immature myelenia

The diagnosis is not established in the clinic InstitutFurKlinischeGenetikBoon. Cranio-sacral therapy was started.

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- Creatinine 2.34 ↓
- The polymorphic variants of the genes of the enzymes of the folate-methionine cycle were studied: the MTHFR 677 CT gene was detected; polymorphism MTRR 66 GG.
- HPLC of blood amino acids: valine ↓ 0.115

Because of the polymorphisms of genes (MTHRR 677CT gene, MTRR 66GG gene combined with moderately elevated homocysteine), there is reason to assume methionine-syntazide reductase deficiency (cobalamin-type E metabolism) in the study of both girls clinical significance, being the genetic background for these children.

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Phenotypically:

- hypotrophy, psychomotor development delay, attention is drawn to the pallor, the marbling of the skin, the superficial arrangement of the vessels
- hypoplasia of subcutaneous fat
- thinning hair
- the face is narrow
- nose short
- gingival hyperplasia
- narrow chest
- kyphoscoliotic curvature of the spine
- narrow brushes, hypermobility of the joints of the upper extremities

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Diagnosis was established:

- Patient K. - The disturbance of the exchange of the respiratory chain of mitochondria is methylglutaconic aciduria.
- Violation of the metabolism of leucine,
- Connective tissue dysplasia, vitamin C deficiency.
- Insufficiency of methionine synthase reductase (violation of cobalamin metabolism)
- Reduced activity of methylenetetrahydrofolate reductase

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Conclusions

- 3-Methylglutaconic aciduria is an extremely rare hereditary disease with an autosomal recessive type of inheritance
- Markers biochemical signs of MGA are stably changed indicators of the level of 3-methylglutaconic, 3-methylglutaric organic acids and leucine
- In the described observations, a manifestation from birth was noted, the signs of which were not adequately regarded by the doctors, since they were of a nonspecific character
- High diagnostic value of the AK and OK research, requires their use in early childhood in the presence of convulsive syndrome, dystonia, paresis, delay in psycho-speech and psychomotor development, atoxy, dysarthria, intellectual disorders of varying severity

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Metabolicum analyzes

- Urine analysis by gas chromatography - the dynamics revealed the following changes:

- moderate increases in 3-methylglutaric, 3-methylglutaconic acids and changes in metabolites: AK leucine; connective tissue and / or vitamin C deficiency; low levels of metabolites of the Krebs cycle, glutathione depletion, folic acid deficiency, changes in the microflora of the gastrointestinal tract. It is impossible to exclude the lack of tryptophan or serotonin.

In dynamics: excessive growth of bacteria in the gastrointestinal tract; insufficiency B3, Mg; polyphenols and flavonoids, phenol.

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SELECTIVE SCREENING OF MITOCHONDRIAL DYSFUNCTION IN A REGION WITH A HIGH LEVEL OF NEUROLOGICAL DISORDERS

Y Grechanina, O Grechanina, S Biletska

According to the UMDF (United Mitochondrial Disease Foundation), an affected individual may have

- Strokes
- Seizures
- Gastrointestinal problems
- Swallowing difficulties
- Failure to thrive
- Blindness
- Deafness
- Heart and kidney problems
- Muscle failure
- Heat/cold intolerance
- Diabetes
- Lactic acidosis
- Immune system problems
- Liver disease

Neurological disorders

- 1 billion patients in the world
- 9.5 million deaths annually
- 4.1 million patients in Ukraine (more than 50% - cerebrovascular pathology, 4-th place in the structure of the total incidence)

Challenges…

- High level of clinical polymorphism (Suhorukov V.S., 2007)
- Genetic heterogeneity
- Effect of synthropy
- Specificity of phenotypic manifestation
- Wide age range of manifestation
- Variability of biochemical parameters

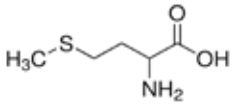
Modern medicine

- Predisposition (SNP) →
- Mediators →
- Triggers →

- Epigenetic diseases
- Heterochromatic diseases
- Mitochondrial dysfunctions
- Effect of synthropy
- Autism «epidemic»

O.Grechanina, 2011

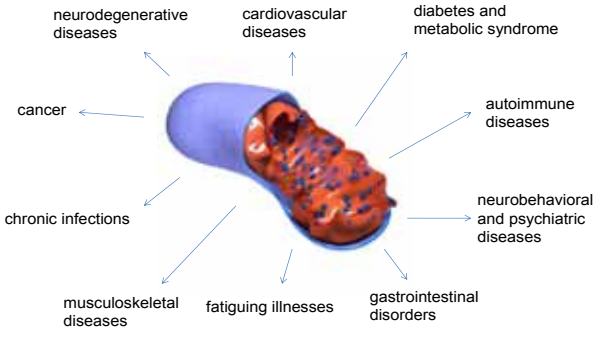
«Genetics predisposes, and epigenetics dispose» (*Piter Medawar*)



Methionine

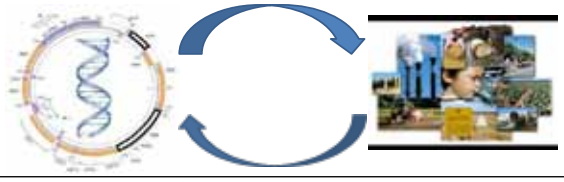
- Causes epigenetic modifications of mtDNA (*Bender A., 2008*)
- Accumulates in the proteins of the respiratory chain, acting as an antioxidant (*Sengupta S., 2007; M. A. Marchetti, 2006*)

Mitochondrial dysfunction



Aim of the study

Development of a new direction of studying the fundamental and applied problems of clinical polymorphism and genetic heterogeneity of mitochondrial dysfunction in patients with pathology of the nervous system, associated with the complex interaction of population-genetic factors that can form a predisposition to disorder of energy metabolism on the background of altered epigenetic status.



Tasks

1. To determine the genetic epidemiology, genetic specificities – haplogroups of mtDNA in the population of Ukraine.
2. To study the character of phenotypic features in carriers of mtDNA polymorphisms.
3. To determine the spectrum of systems and organs pathology associated with the tRNA-leucine and tRNA-lysine genes of mtDNA.
4. To determine the efficiency of the new approach to clarifying diagnostics of mitochondrial dysfunction.

Haplogroups mtDNA (Ukrainian population)

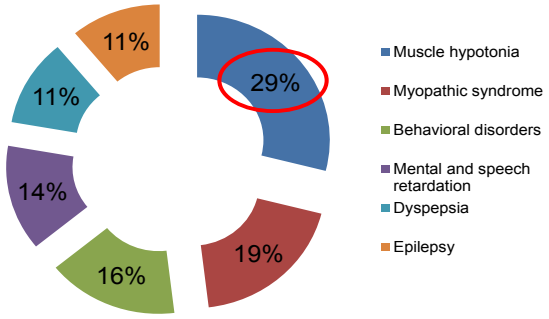
Haplogroup	Polymorphisms
H	3705 G/A, 8860G, 15326A/G, 14553 C/T
J	3624 A/G
X	3594 C/T, 14470 T/C, 17196 G/A
N	3336 T/C
C	3552 T/A
T	1888G/A, 8697 G/A, 8860G, 11251 A/G, 11719 G/A, 11812 A/G, 14766 C/T, 14905 G/A, 15326 A/G, 15452 C/A, 15607 A/G, 15928 G/A

Materials and methods

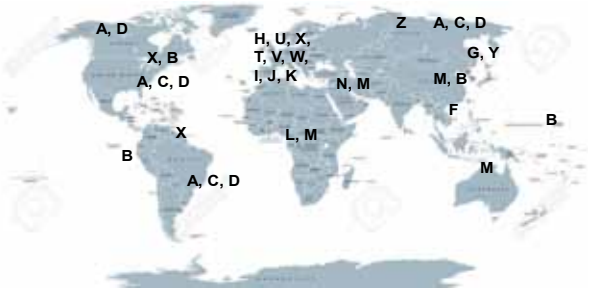
- About **38000** families are consulted in KhMSMGC-CR(O)D annually
- 203 patients with neurological disorders were examined
- 142 people in the control group
- 2445 molecular genetic tests of polymorphisms and 49 "point" mutations (*together with R.Matalon, B.Brendon, L.Peter, the University of Texas, USA; T. Schurr, University of Pennsylvania, USA*)

Methods: somatogenetic with syndromological analysis, genealogy, cytogenetic, biochemical, molecular genetic, mathematical and statistical.

Clinical features (n=203)



Haplogroups mtDNA



Polymorphisms mtDNA

Organopathy	Polymorphism tRNA-leu	Mutation de novo tRNA-leu	Polymorphism tRNA-lys	Mutation de novo tRNA-lys
Encephalopathy	3197 T/C 3336 T/C	3624 A/G 3594 C/T 3705 G/A 3505 A/G 3552 T/A	8697 G/A 8860 G/A 8856 G/A 8251 G/A 8701 A/G 8994 G/A 8337 T/C 8794 C/T 8584 G/A	8164 C/T 8610 T/C 8614 T/C
Muscle hypotonia, weakness	3197 T/C 3336 T/C	3594C/T 3624 A/G 3505 A/G 3552 T/A	8697 G/A 8860 G/A 8701 A/G 8556 G/A 8337 T/C 8794 C/T 8584 G/A	9018 C/T 8164 C/T 8836 A/G 8865 G/A

Haplogroups mtDNA (Ukrainian population)

Gaplogroup	Patients/frequency, %	Control/frequency, %
H	17/29.8	24/29.2
I	-	4/4.9
J	7/12.3	10/12.0
K	7/12.3	9/10.8
NV	3/5.3	-
T	9/15.7	5/6.0
U	10/17.5	26/31.0
V	2/3.5	1/1.2
W	1/1.8	-
X	1/1.8	4/4.9
Total	57/100	83/100

Organopathy	Polymorphism tRNA-leu	Mutation de novo tRNA-leu	Polymorphism tRNA-lys	Mutation de novo tRNA-lys
Dyspepsia	3997 T/C		8697 G/A 8860 G	
Skeletal disorders	3197 T/C		8697 G/A 8860 G 8860 A	
Ophthalmopathy	3336 T/C 3197 T/C	3705 G/A 3505 A/G	8860 G 8697 G/A 8448 T/C 8251 G/A	
Cardiopathy	3197 T/C	3705 G/A 3505 A/G	8251 G/A 8994 G/A 8860 G 8337 T/C 8794 C/T	8610 T/C 8614 T/C 8865 G/A 8592 G/A
Deafness			8448 T/C 8860 G	

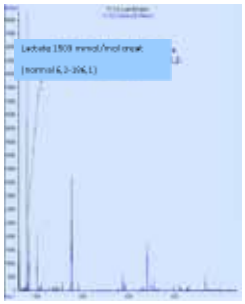
Polymorphisms mtDNA tRNA-lys

- 8697 G/A
- 8860 G
- 8701 G/A
- 8856 G/A
- 8860 A
- 8251 G/A
- 8472 C/T
- 8448 T/C
- 8994 G/A
- 8337 T/C
- 8794 C/T
- 8584 G/A
- 8701 A/G

→ multiple organ failure

Biochemical indicators

- Each **third** patient with a neurological pathology showed an increase in the level of blood lactate
- In **28%** of cases, hyperhomocysteinemia
- In the biochemical profile of blood, an increase in the level of aminotransferases was detected in 48% of patients



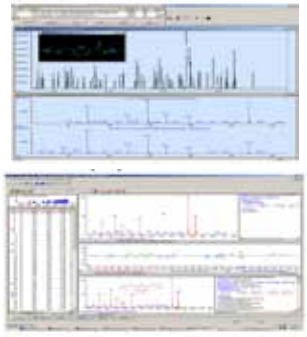
Mitochondrial deseases

Mutation	Deseases
T8993G gene ATP6	Leigh syndrome, NARP syndrome
A8344G gene tPHK lys	MERRF syndrome
A3243G gene tPHK leu	MELAS syndrome
Deletion of a large fragment of a mtDNA molecule	Kearns-Sayre syndrome, Pearson syndrome, Progressive external ophthalmoplegia

Gas chromatography/mass-spectrometry (urine)

- Identification of more than 150 metabolites
- The markers of the disorder of the energy metabolism, the methylation cycle: the increase of acids

methylmalonic (23%)
malonic (19%)
succinic (19%)
2-hydroxybutyric (35%)
glycerine (35%)

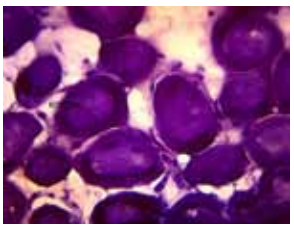


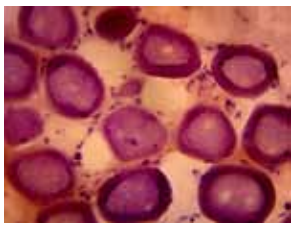
Polymorphisms MTHFR (C677T, A1298C, G1793A), MTRR (A66G), RFC-1 (G80A)

(*O.Grechanina, Y.Grechanina, V.Gusar, R.Matalone, 2011*)

Polymorphisms	Ukrainian n=199, %	Ashkenazi Jews n=155, %	African Americans n=97, %	Europeans n=159, %
MTHFR 677 C/C	52.26	31.00	77.30	45.90
MTHFR 677 C/T	40.70	42.60	21.60	42.80
MTHFR 677 T/T	7.04	26.50	1.00	11.30
MTHFR 1298 A/A	52.00	53.70	71.00	44.00
MTHFR 1298 A/C	39.50	38.30	26.80	47.20
MTHFR 1298 C/C	8.50	8.10	2.10	8.80
MTHFR 1793 G/G	95.38	97.40	93.80	86.80
MTHFR 1793 G/A	4.62	2.60	6.20	12.60
MTHFR 1793 A/A	0.00	0.00	0.00	0.60

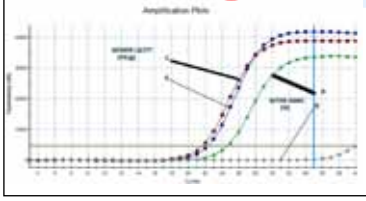
Muscle biopsy





Succinate dehydrogenase deficiency (32 years old)

Polymorphisms	Ukrainian n=199, %	Ashkenazi Jews n=155, %	African Americans n=97, %	Europeans n=159, %
MTRR 66 A/A	21.50	33.30	42.30	20.80
MTRR 66 A/G	43.00	47.20	47.40	49.70
MTRR 66 G/G	35.50	19.50	10.30	29.60
RFC-1 80 G/G	18.42	25.40	33.70	23.70
RFC-1 80 G/A	43.16	45.90	45.50	47.30
RFC-1 80 A/A	38.42	28.70	20.80	29.00



46XX/45X. Mitochondrial dysfunction. Polymorphysms mtDNA 8697G/A, 8860 G, MTHFR 677 C/T, MTRR 66 G/G

Conclusion

- 1. With the help of molecular genetic research, the characteristics of the main haplogroup mtDNA were complited, the calculation of the haplotype frequencies in the sample showed the presence of Eurospecific haplogroups: 95,6%.
- 2. Clinical features of carriers of polymorphisms of mtDNA were characterized by multiple organ failure, progressive flow, clinical polymorphism, genetic heterogeneity and preferential involvement of energetic organs. In 91 patients (45.31%), an element of synthropy was found, in which each of the "conglomerate diseases" retained its specificity.

- 3. It is noticed the most frequent inclusion in the pathological process of organs and systems in polymorphisms of mtDNA tRNA-lysine: 8697 G/A; 8860 G; 8701 G/A; 8856 G/A; 8860 A (CRS); 8251 G/A; 8472C/T; 8448 T/C; 8994 G/A; 8337 T/C; 8794 C/T; 8584 G/A; 8701 A/G; encephalopathies more often associated with the polymorphisms of tRNA-lysine and new mutations (tRNA-leucine)(3624 A/G; 3594 C/T; 3705 G/A; 3505 A/G; 3552 T/A).
- 4. The findings suggest the need to assess the state of the mitochondrial genome, metabolites of the Krebs cycle and folate-methionine cycle in patients with various neurological diseases, as pathways to pathogenetic therapy.

Acknowledges

Olena Grechanina, Professor of Department Medical Genetics of Kharkiv National Medical University, Ukraine

Kharkiv Interregional Specialized Medical Genetic Centre — Centre Rare (Orphan) Diseases, Ukraine

Yulia Grechanina, Professor, a Chief of Department Medical Genetics of Kharkiv National Medical University, Ukraine

Theodore Schurr, Professor of Department of Anthropology University of Pennsylvania, Philadelphia, USA

Reuben Matalon, Professor of Department of Pediatrics, University of Texas Medical Branch, Galveston, USA

THANK YOU FOR ATTENTION!

PARALLEL SYMPOSIUM 2
Supported by Sanofi/Genzyme

Moderator: Ivaylo Tournev

- **Pompe disease – a treatable myopathy**
  T. Chamova

- **Fabry disease – 10 years of experience of the Bulgarian expert centre (in Bulgarian)**
  E. Paskalev

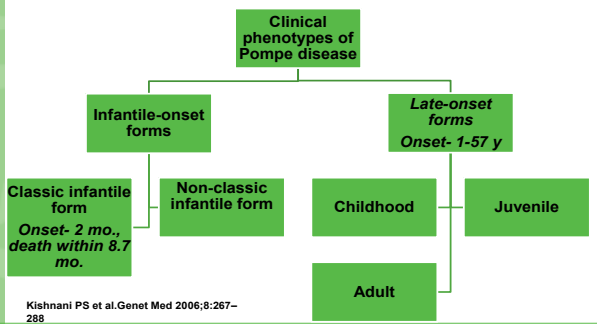
- **Myeloproliferative neoplasm in patients with Gaucher disease**
  Z. Stojanoski

POMPE DISEASE – TREATABLE MYOPATHY

Teodora Chamova

GZBG.XLSD.17.07.0051c09.2017

POMPE DISEASE- CLASSIFICATION



Kishnani PS et al.Genet Med 2006;8:267–288

SANOFI GENZYME Myozyme 5

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POMPE DISEASE- INTRODUCTION

- Progressive, multisystemic, debilitating, often fatal neuromuscular disease
- First described in 1932 by Dutch pathologist J.C. Pompe
- Also known as acid maltase deficiency (AMD), glycogen storage disease type II (GSD-II), or glycogenosis type II
- Characterised by progressive degeneration of skeletal, respiratory and, primarily in infants, cardiac muscle
- Encompasses a single disease continuum with variable rates of disease progression, ranging from a rapidly fatal phenotype in infants to slower progressive phenotypes in older children and adults

SANOFI GENZYME Myozyme 2

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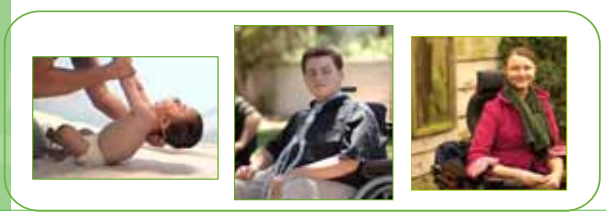
VARIABLE RATES OF DISEASE PROGRESSION



SANOFI GENZYME Myozyme 6

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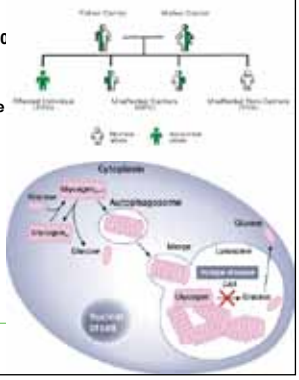
POMPE DISEASE- ETIOLOGY AND EPIDEMIOLOGY

- AR trait of inheritance
- Mutations in *GAA* gene (more than 300 currently known)
- Partial or total absence of the lysosomal enzyme acid α-glucosidase
- Poor genotype-phenotype correlations have been documented
- Combined incidence 1/40,000
- High incidence for:
  - Infantile form in African-Americans 1/50 000 and Chinese 1/14 000
  - Late-onset form in the Netherlands 1/57,000



SANOFI GENZYME

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POMPE DISEASE IN CHILDREN & ADULTS- CLINICAL FEATURES

Musculoskeletal
- Proximal muscle weakness
- Gait abnormalities
- Muscle pain
- Difficulty climbing stairs
- Frequent falls
- Scapular winging

Respiratory
- Respiratory failure/insufficiency
- Orthopnea
- Exertional dyspnea
- Respiratory infections
- Daytime somnolence
- Morning headache
- Nocturnal hypoventilation

Gastrointestinal
- Feeding and swallowing difficulties
- Poor weight gain/maintenance
- Difficulty chewing or jaw muscle fatigue

1.Hirschhorn R, Reuser AJJ. In: Scriver CR, Beaudet AL, et al, eds. The Metabolic and Molecular Bases of Inherited Disease. 8th ed. New York: McGraw-Hill; 2001:3389-3420;

2. Winkel LP, Hagemans ML, van Doorn PA, et al.J Neurol 2005; 252:875-84.

SANOFI GENZYME Myozyme 7

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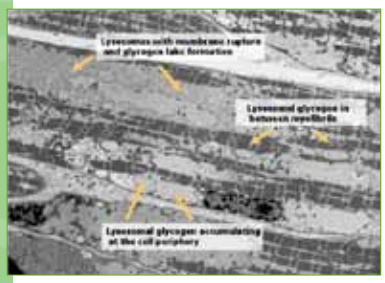
GLYCOGEN ACCUMULATION LEADS TO TISSUE DESTRUCTION



Electron microscopy in skeletal muscle of infantile patient. Magnification 6500x. Image courtesy of Genzyme Pathology.

- Healthy myofibrils are replaced by glycogen, gradually impairing muscle function
- Muscle pathology may precede symptomatology
- Glycogen accumulation begins before signs of clinical weakness, so early muscle damage may not be clinically detectable

SANOFI GENZYME Myozyme 4

GZBG.XLSD.17.07.0051c09.2017

POMPE DISEASE IN CHILDREN & ADULTS- CLINICAL FEATURES

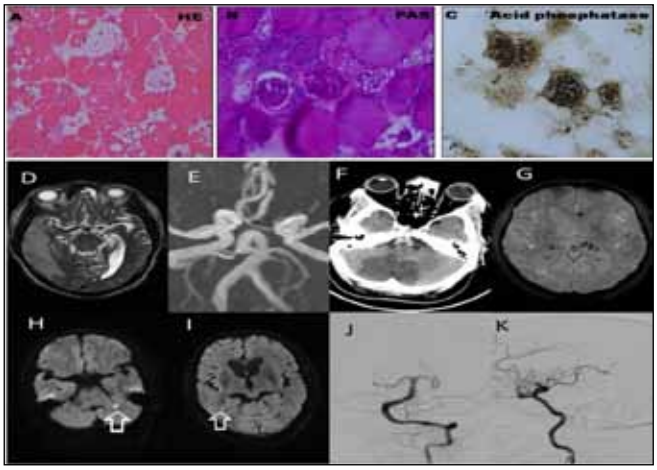
GAIT

SANOFI GENZYME Myozyme 8

GZBG.XLSD.17.07.0051c09.2017

POMPE DISEASE IN CHILDREN & ADULTS- CLINICAL FEATURES

GOWERS SIGN



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PROGRESSIVE LIMB-GIRDLE MUSCLE WEAKNESS IN POMPE DISEASE

- Predominant involvement of the lower limbs in comparison with the upper ones
- Early weakness of the axial and abdominal muscles with difficulties to stand up from lying position

>80% of the patients
50%-80% of the patients
<50% of the patients

Distribution of skeletal muscle weakness in 94 adults with Pompe disease. Adapted from Van der Beek et al. Orphanet J Rare Dis 2012;7(1):88, Fig. 2A, C

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DIAGNOSIS OF POMPE DISEASE

- **Labs**
  - Serum creatine kinase (CK)
  - Alanine and aspartate aminotransferase (ALT/AST) and lactate dehydrogenase (LDH)
  - Urinary hexose tetrasaccharide (Hex4)
- **Muscle**
  - Electromyography (EMG)/nerve conduction studies
  - Muscle strength testing
  - Muscle MRI
  - Histopathology
- **Cardiac**
  - Chest x-ray
  - Electrocardiography (ECG)
  - Echocardiography (Echo)
- **Pulmonary**
  - Pulmonary function testing (spirometry)
  - Chest X-ray
  - Pulse oximetry and capnography
  - Sleep studies

Kishnani PS, Steiner RD, Bali D, Berger K, et al. Genet Med 2006; 8:267-288;

Hirschhorn R, Reuser AJJ In: Scriver CR, Beaudet AL, et al, eds. The Metabolic and Molecular Bases of Inherited Disease. 8th ed. New York: McGraw-Hill; 2001:3389-3420

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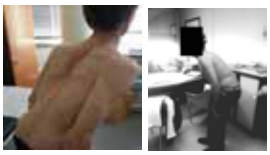
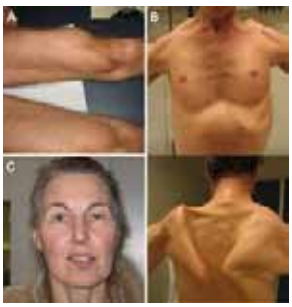
ATYPICAL FEATURES OF LATE-ONSET FORMS OF POMPE DISEASE





- Rigid spine
- Camptocormia
- Earlier involvement of the upper limbs
- Ptosis

Kostera-Pruszczyk et al., NMD 2006
Laforêt et al, NMD 2009;
Tasne N et al. Muscle and nerve, 2016;

Wens et al. Orphanet Journal of Rare Diseases 2013, 8:182;
Yanovitch TL et al. J Neuroophthalmol. 2010 Jun;30(2):165-6;

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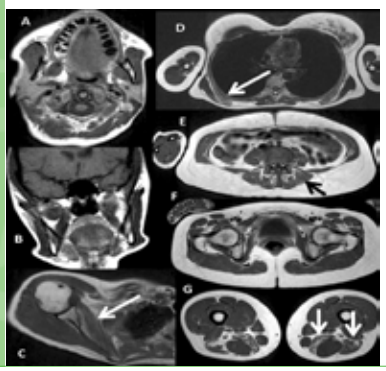
MUSCLE MRI IN POMPE DISEASE



Early involvement of

- Tongue muscles
- Axial muscles
- m. subscapularis
- Thigh muscles

ORIGINAL ARTICLE

Intracranial arterial abnormalities in patients with late onset Pompe disease (LOPD)

Federica Montagnese · Francesca Granata · Olimpia Musumeci · Corrado Badiali · Nicola Mondello · Emanuele Barca · Maria Ciolfetta · Anna Ciranni · Marcello Longa · Antonio Toscano

- Intracranial arterial abnormalities in 13/21 patients (62 %)
- 2/21 patients (9.5 %) with unruptured intracranial aneurysm (respectively 2 and 4 mm)
- 10/21 (47 %) with vertebrobasilar dolichoectasia (VBD)
- 1/21 a basilar artery fenestration
- Signs of lacunar encephalopathy (insular, capsular and frontal subcortical lesions) in 13/21 patients (62 %), correlating with the presence of respiratory impairment

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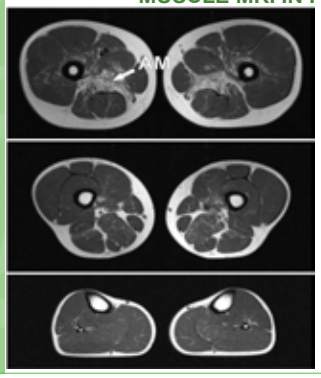
MUSCLE MRI IN POMPE DISEASE



Fibrosis and fatty degeneration of m. semimembranosus, m. semitendinosus, m. adductor magnus и m. biceps femoris

Gruhn K M et al. 2015; Mol Genet Metab Rep.;3:58-64

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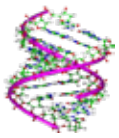
DIAGNOSIS OF POMPE DISEASE

- Confirmatory diagnosis requires quantitative enzyme (GAA) activity assay
- Now, minimally invasive blood tests can accurately quantify GAA activity
  - Dried blood spots
  - Mixed leukocytes
  - Lymphocytes
- Mutation Analysis
  - Especially for carrier testing (family/sibling)
  - Potential for prognostic value



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GZBG.XLSD.17.07.0051c/09.2017

Multidisciplinary approach in the treatment of Pompe disease

Kishnani et al. Genet Med 2006; 8:267-288.

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DIFFERENTIAL DIAGNOSIS OF LATE-ONSET FORM

Differential Diagnosis	Shared Signs & Symptoms
Limb girdle muscular dystrophy (LGMD)	Progressive muscle weakness in the pelvis, legs, or shoulders, abnormal gait, elevated creatine kinase (CK)
Becker/Duchenne muscular dystrophy	Progressive limb-girdle muscle weakness, respiratory impairment, difficulty walking, elevated CK
Glycogen storage diseases (GSD) III and IV	Hypotonia, hepatomegaly, muscle weakness, elevated CK
Mitochondrial myopathies	Hypotonia, hyporeflexia, hepatomegaly. Some forms with exercise intolerance, muscle weakness, headache, breathlessness, elevated CK
Carnitine deficiency	Muscle weakness in the hips, shoulders, and upper arms and legs

Continued on next slide

Kishnani PS, et al. Genet Med 2006;8:267-288. Gilbert-Barness E. Ann Clin Lab Sci 2004; 34:15-34. Roe CR, Ding J. New York, New York: McGraw-Hill; 2001. Gilchrist JM. Semin Respir Crit Care Med 2002; 23:191-200. Andres PL, et al. Neurol Clin 1987; 5:125-41.

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GZ

REVIEW

Enzyme replacement therapy in late-onset Pompe disease: a systematic literature review

Antonio Toscano · Benedikt Schoser

- Muscle strenght
- 6MWT
- Pulmonary function

- Alglucosidase alfa has a beneficial effect in LOPD patients as demonstrated by:
  - Improvements in survival and ambulation
  - Pevention of deterioration in respiratory function
  - Early treatment has a better outcome [1-8]

1.Merk T, et al. Eur J Neurol. 2009;16:274-7. 2. Bembi B, et al. J Inherit Metab Dis. 2010;33:727-35. 3.van Capelle CI, et al. Neuromuscul Disord. 2010;20:775-82. 4.Angelini C, et al. J Neurol. 2012;259:952-8. 5. Strothotte S, et al. J Neurol. 2010;257:91. 6.van der Ploeg AT, et al. N Engl J Med. 2010;362:1396-1406. 7. Regnery C, et al. J Inherit Metab Dis. [Epub ahead of print 2012 Jan 31]. 8.Grunch K M et al.Mol Genet Metab Rep. 2015 (3) 58-64

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DIFFERENTIAL DIAGNOSIS OF LATE-ONSET FORM

Differential Diagnosis	Shared Signs & Symptoms
Glycogen storage disease (GSD) V	Elevated CK, muscle cramps during exercise/exercise intolerance
Danon disease	Skeletal muscle myopathy, limb-girdle muscle weakness, scapuloperoneal muscular weakness, elevated CK
Polymyositis	Progressive, often symmetrical, muscle weakness, difficultly swallowing, elevated CK
Rheumatoid arthritis	Generalised weakness, stiffness, fatigue, musculoskeletal symptoms
Endocrine myopathies	Progressive, often symmetrical, muscle weakness

Continued on next slide

Kishnani PS, et al. Genet Med 2006;8:267-288. Gilbert-Barness E. Ann Clin Lab Sci 2004; 34:15-34. Roe CR, Ding J. New York, New York: McGraw-Hill; 2001. Gilchrist JM. Semin Respir Crit Care Med 2002; 23:191-200. Andres PL, et al. Neurol Clin 1987; 5:125-41.

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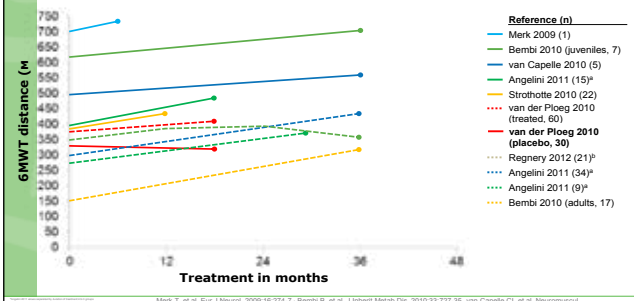
6MWT- 122 PATIENTS



Merk T, et al. Eur J Neurol. 2009;16:274-7. Bembi B, et al. J Inherit Metab Dis. 2010;33:727-35. van Capelle CI, et al. Neuromuscul Disord. 2010;20:775-82. Angelini C, et al. J Neurol. 2012;259:952-8. Strothotte S, et al. J Neurol. 2010;257:91. van der Ploeg AT, et al. N Engl J Med. 2010;362:1396-1406. Regnery C, et al. J Inherit Metab Dis. [Epub ahead of print 2012 ....

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GZBG.XLSD.17.07.0051c/09.2017

DIFFERENTIAL DIAGNOSIS OF LATE-ONSET FORM

Differential Diagnosis	Shared Signs & Symptoms
Spinal muscular atrophy	Asymmetrical muscle weakness, atrophy of voluntary muscles, elevated CK
Amyotrophic lateral sclerosis (ALS)	Progressive muscle weakness, respiratory impairment, elevated CK
Kennedy's disease	Bulbar muscle dysfunction/difficulty breathing and swallowing, elevated CK

Kishnani PS, et al. Genet Med 2006;8:267-288. Gilbert-Barness E. Ann Clin Lab Sci 2004; 34:15-34. Roe CR, Ding J. New York, New York: McGraw-Hill; 2001. Gilchrist JM. Semin Respir Crit Care Med 2002; 23:191-200. Andres PL, et al. Neurol Clin 1987; 5:125-41.

20

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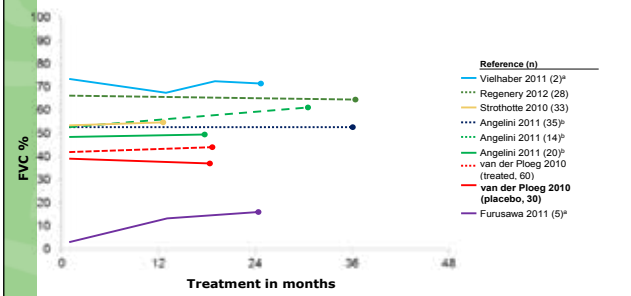
FVC %

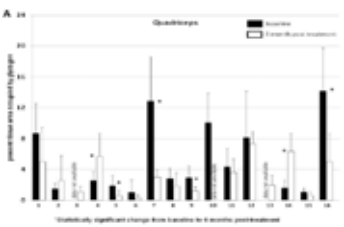


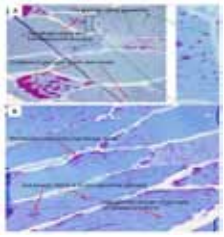
Vielhaber S,etal. Clin Neurol Neurosurg. 2011;113:350-7.Regnery C, et al. J Inherit Metab Dis. [Epub ahead of print 2012 Jan 31]. Strothotte S, et al. J Neurol 2010;257:91-7. Angelini C, et al. J Neurol 2012;259:952-8. van der Ploeg AT, et al. N Engl J Med 2010;362:1396-1406. Furusawa Y, e t al. J Inherit Metab Dis. 2012;35:301-10.

24

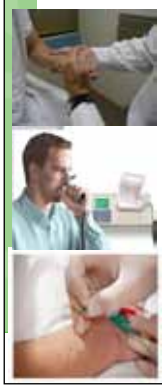
Molecular Genetics and Metabolism

Prospective exploratory muscle biopsy, imaging, and functional assessment in patients with late-onset Pompe disease treated with alglucosidase alfa: The EMBASSY Study





PREVALENCE STUDY OF POMPE DISEASE IN BULGARIA



After clinical evaluation

In case of low GAA activity in two consecutive tests

SHOULD PATIENTS WITH ASYMPTOMATIC POMPE DISEASE BE TREATED? A NATIONWIDE STUDY IN FRANCE

ANDONI ECHANIZ-LAGUNA, MD, PhD,[1] ROBERT-YVES CARLIER, MD,[2] KENZA LALOUI, MD,[3] PIERRE CARLIER, MD, PhD,[3] EMMANUELLE SALORT-CAMPANA, MD,[4] JEAN POUGET, MD, PhD,[4] and PASCAL LAFORET, MD, PhD[2]

- Earlier onset of male patients in comparison to female patients, carrying the same mutations
- Pompe disease may remain clinically silent for decades
- Asymptomatic, and affected patients should be monitored closely for overt myopathy using clinical examination, PFTs, and muscle MRI to determine when to start ERT

RESULTS OF PREVALENCE STUDY OF POMPE DISEASE IN BULGARIA

370 tested individuals
- 347 with myopathy
- 23 with asymptomatically elevated CK

9 with decreased activity of GAA on DBS

6 with genetically verified Pompe disease

PREVALENCE STUDY OF POMPE DISEASE IN BULGARIA

- **Retrospective study** of patients with undiagnosed myopathies included in the Registry of the Bulgarian National Genetic Laboratory Clinic of Neurology, University Hospital Alexandrovska, Sofia
- **Prospective study** of cohort of patients who are visiting university hospitals and EMG centers in Bulgaria
- Testing the asymptomatic sibs of the affected after informed consent and genetic counseling of the family
- Field studies
- Lectures and workshops, aiming to increase the awareness for Pompe disease among GP, pediatricians, neurologists, pulmonologists, cardiologists, medical students

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Current age (years)	9	16	50	44	49	62
Age at onset (years)	1.5	asymptomatic	35	40	40	51
Sex	male	female	female	male	male	female
Initial complaints	Muscle hypotony, delayed motor milestones	NA	Proximal muscle weakness in lower limbs	Proximal muscle weakness in upper limbs (R>L)	Low back pain, proximal muscle weakness in lower limbs	Proximal muscle weakness in lower limbs
CK (normal range 20-200 IU) at diagnosis	113	89	769	1394	928	193
Ventiliatory assessment at diagnosis	FVC=45.7% FEV1=54%	FVC=98% FEV1=92%	FVC=89% FEV1=102%	FVC=90%, FEV1=100%.	FVC=65%, FEV1=71 %	FVC=60% FEV1=69%
Molecular genetic analysis	g.-32-13T>G in intron 1 and c.1726G>A; p.(Gly576Ser) in exon 12 of GAA gene	g.-32-13T>G in intron 1 and c.1726G>A; p.(Gly576Ser) in exon 12 of GAA gene	g.-32-13T>G in intron 1 and c.1655T>C; p.(Leu552Pro) in exon 12	g.-32-13T>G in intron 1 and c.1655T>C; p.(Leu552Pro) in exon 12	g.-32-13T>G in intron 1 and c.1655T>C; p.(Leu552Pro) in exon 12	g.-32-13T>G in intron 1 and c.1655T>C; p.(Leu552Pro) in exon 12
Previous misdiagnoses	Congenital myopathy	NA	LGMD	_	Radiculopathy	Polimyositis

PREVALENCE STUDY OF POMPE DISEASE IN BULGARIA

Inclusion criteria

- Age > 8 years
- Male and female patients
- Signed informed consent by the patient or his/her legal representatives
- Patients with limb-girdle/axial muscle weakness with/without elevated CK
- Patients with limb-girdle/axial muscle weakness and/or respiratory insufficiency
- Patients with asymtomatically elevated CK
- Myopathic changes on EMG

Exclusion criteria

- Lack of informed consent
- Genetically verified muscular dystrophy or myopathy
- Previously tested patients with normal acid alpha-glucosidase activity
- Family history of X-linked trait of inheritance
- Family history of autosomal dominant trait of inheritance-not obligatory due to rare cases of pseudodominance

SUMMARY

- Pompe disease is a progressive, debilitating and often fatal neuromuscular disease
- Disease continuum with variable age of onset and rate of disease progression
- Diagnosis is often delayed
- Simple blood test is available
- Myozyme™ is the first and only specific treatment for Pompe disease
- Improvements in respiratory function, motor function, and quality of life in children and adults treated with Myozyme
- Early diagnosis and treatment may be critical to optimizing outcomes
- Screening in high risk patient population is an important tool

БОЛЕСТ НА ФАБРИ

Емил Паскалев

1

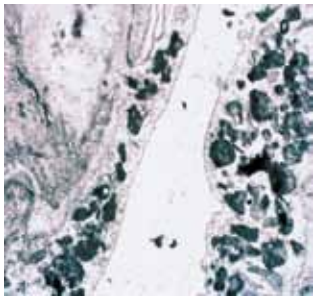
Болест на Фабри

- Болестта на Фабри е Х-свързано наследствено заболяване, засягащо мъже и жени[1,2].
- Налице е ранно и прогресивно натрупване на GL3, което води до нарушения в клетките и вторично потенциално възвратимо тъканно увреждане или органна недостатъчност[1,3].
- Клиничният спектър на заболяването е широк с различни прогресивни клинични прояви[1].
- Първите симптоми са през ранното детство. Заболяването може да прогесира в периода на юношеството и младежките години до клинични изяви, които се нуждаят от лечение, а по-нататък да доведът до преждевременна смърт[4-8].
- Ранното разпознаване на симптомите и диагностиката на пациентите в ранни стадии, когато промените имат потенциална обратимост, е от голямо значение[9].

1. Germain. Orphanet J Rare Dis 2010;5:30; 2. Wilcox et al. Mol Genet Metab 2008;93:112-28; 3. Rombach et al. Mol Genet Metab 2010;99:99-108; 4. Hopkin et al. Pediatr Res 2008;64:550-5; 5. Tøndel et al. Am J Kidney Dis 2008;51:767-76; 6. Schiffmann et al. Nephrol Dial Transplant 2009;24:2102-11; 7. MacDermot et al. J Med Genet 2001;38:750-60; 8. MacDermot et al. J Med Genet 2001;38:769-75; 9. Weidemann et al. Orphanet J Rare Dis 2013;8:116.

2

Vascular Endothelium



Electron-dense lysosomes containing undegraded glycosphingolipid

From R.J. Desnick, PhD, MD

02-026/05-2001

3

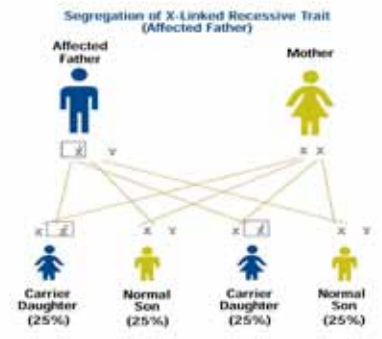
Болест на Фабри

- Повече от 600 вариации на GLA гена са свързани с болестта на Фабри, не всички от тях имат патогенетично значение.
- Акумулацията на GL3 стартира от много ранна възраст.
- Прогресивната акумулация на GL3 в подоцитите започва в много ранна възраст и прогресира, докато мезангиалната и ендотелиалната акумулация е зависима от възрастта и пола[1].
- Естественото развитие на болестта включва хронично бъбречно заболяване, мозъчно-съдово заболяване, кардиомиопатия и преждевременна смърт.

- *[1]B. Najafian, E. Svarstad, L. Bostad et al. Progressive podocyte injury and globotriaosylceramid (GL-3) accumulation in young patients with Fabry disease. Kidney Int 201; 79: 663-670.*

4

УНАСЛЕДЯВАНЕ

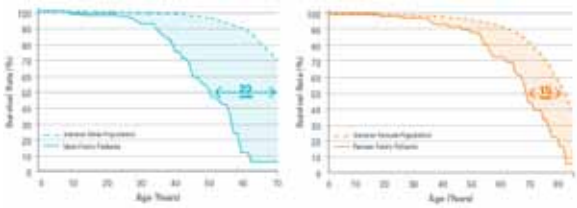


ЗАСЕГНАТИТЕ МЪЖЕ ПРЕДАВАТ ЗАБОЛЯВАНЕТО НА ВСИЧКИ СВОИ ДЪЩЕРИ, НО НЕ ПРЕДАВАТ НА СВОИТЕ СИНОВЕ.

ЗАСЕГНАТИТЕ ЖЕНИ ПРЕДАВАТ БОЛЕСТТА, КАКТО НА ДЪЩЕРИ ТАКА И НА СИНОВЕ, В 50%.

5

Скъсяване продължителността на живота



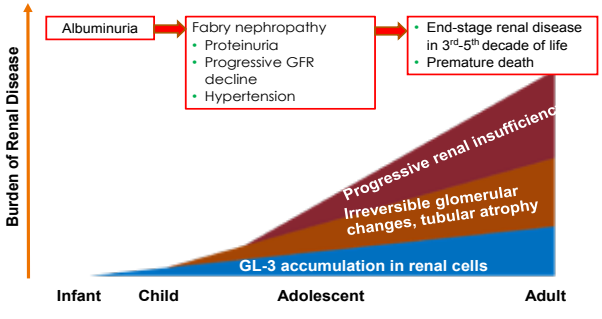
- Преживяемостта при мъже с болест на Фабри (лява фигура) и при жени (дясна) е съответно 50 и 70 г. (седно)[1,2]
  - Графиките показват 20 г. и 15 г. скъсяване на преживяемостта съответно при мъже и жени[1,2]

1. MacDermot et al. J Med Genet 2001;38:750-60.
2. 2. MacDermot et al. J Med Genet 2001;38:769-75. Figures adapted from these references.

Бъбречни, сърдечно-съдови и неврологични прояви

7

Бъбречни прояви при болестта на Фабри
Прогресия на бъбречното увреждане във времето

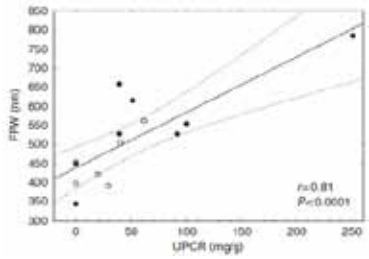


Tøndel et al. J Am Soc Nephrol 2013;24:137-48; Tøndel et al. Am J Kidney Dis 2008;51:767-76; Ramaswami et al. Clin J Am Soc Nephrol 2010;5:365-70; Ortiz et al. Nephrol Dial Transplant 2008;23:1600-7; Wanner et al. Clin J Am Soc Nephrol 2010;5:2220-8.

8

Бъбречни прояви

Ранните подоцитни увреждания рано водят до албуминурия/протеинурия



албуминурия/протеинурия:

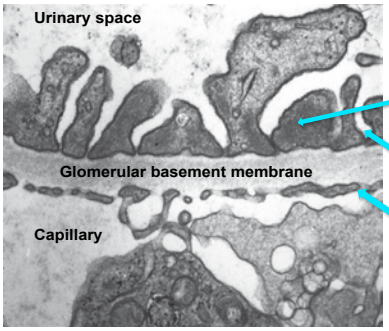
- Може да се развие рано - 1ва декада от живота[1,2].
- Показва прогресивна подоцитна увреда[3].
- Степента на протеинурията е свързана със степента на намаление на GFR[4,5].

Структурните промени в подоцитите (разширение на пространствата между подоцитните израстъци) корелира с възрастта и степента на протеинурия.

1. Tøndel et al. J Am Soc Nephrol 2013;24:137-48; 2. Tøndel et al. Am J Kidney Dis 2008;51:767-76; 3. Najafian et al. Kidney Int 2011;79:663-70 (Figure); 4. Ortiz et al. Nephrol Dial Transplant 2008;23:1600-7; 5. Wanner et al. Clin J Am Soc Nephrol 2010;5:2220-8.

9

Ранни прогресивни нарушения в подоцитите



Widened podocyte foot processes

Loss of integrity of slit diaphragms

Decreased endothelial cell fenestrations

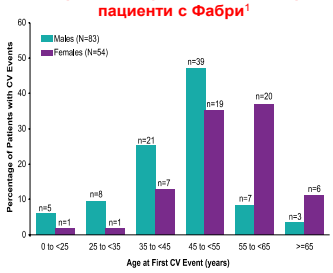
Image courtesy of M. Mauer, Minnesota.

10

Сърдечно-съдови (CV) прояви

- При нелекувани пациенти, 5.8% от мъжете и 3.7% от жените имат голямо CV събитие като[1]:
  - Миокарден инфаркт;
  - Сърдечна недостатъчност;
  - Сърдечно обусловена смърт.
- Първо CV събитие според възраст[1]:
  - 45 години при мъже;
  - 54 години при жени.

Възраст на първо CV събитие при пациенти с Фабри[1]

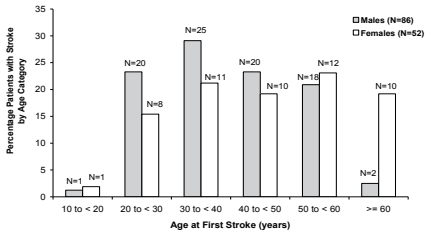


1. Patel et al. J Am Coll Cardiol 2011;57:1093-9.

11

Неврологични прояви - Мозъчни съдови прояви

Възраст на първи инсулт при пациенти с Фабри[1]



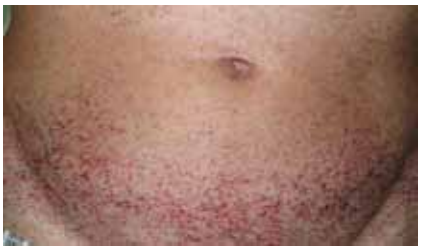
- **22% от нелекуваните Фабри пациенти и получили инсулт са под 30 годишна възраст[1]**
- **Средна възраст на първи инсулт е 39.8 г. за мъже и 45.7 г. за жени[1]**
- **Честотата на инсултите е значимо по-висока в сравнение с общата популация[1]**

1. Sims et al. Stroke 2009 ;40:788-94. Figure adapted from Sims et al. Stroke 2009;40:788-94.

ДРУГИ КЛИНИЧНИ ПРОЯВИ

13

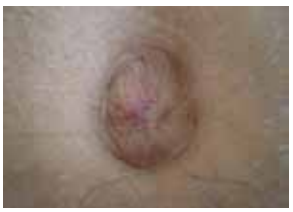
Ангиокератома



14

Ангиокератома

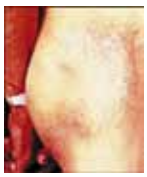
- Установява се при 37% от момчетата и 23% от момичетата, както и при 66% от възрастните мъже и 36% от жените[1]
- Пролиферация на дилатирани кръвоносни съдове в епидермиса[2]
- Червено-виолетови кожни лезии[2]
- Най-често умбиликално и седалищно[2]



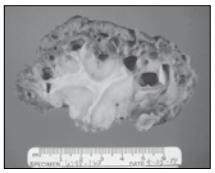
1. Orteu et al. Br J Dermatol 2007;157: 331-7; 2. Zampetti et al. Br J Dermatol 2012;166:712-20. Figure left: Germain. Orphanet J Rare Dis 2010;5:30. Figure right: Zampetti et al. Br J Dermatol 2012;166:712-20.

15

Fabry Disease



Angiokeratomas[1]



End stage Fabry kidney[2]

Cardiomyopathy[1]

"Whorllike" or "spokelike" corneal opacities[1]

1. Courtesy of R. J. Desnick, PhD, MD; 2. E. & L. Gilbert-Barness

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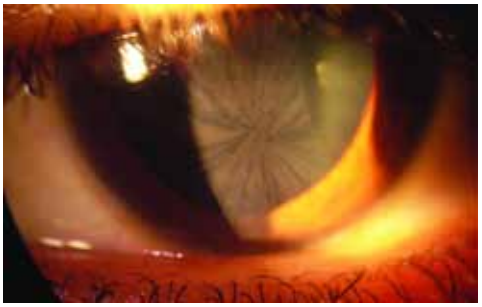
ОЧНИ ПРОМЕНИ



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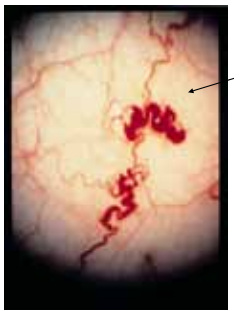
Conjunctival Involvement



Sausage-like and markedly dilated vessels

With permission, from R. J. Desnick, PhD, MD

05-005/05-2001

Диагноза

Болест на Фабри и лечение с фабразим 1mg/kg в България.

Дозата на ЕЗЛ е от решаващо значение[1-5]

1.Germain P.D et al. J Med Genet 2015; 52: 353-358; 2.Lidove O et al. Genet Med 2010; 12 (11): 668-679; 3.Germain P. D. et al Am Soc Nephrol 2007;18: 1547-1557; 4.Goker-Alpan O et al. JIMD Rep. 2016 ;25:95 -106; 5.Skurnes R. et al. Nephrol Dial Transplant (2016) 0: 1-6

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Болест на Фабри - лечение

Основното лечение при пациентите с болест на Фабри е заместване на ензимния дефицит. Клиниката има опит близо 10 години в провеждане на ензимозаместващо лечение (ЕЗЛ) в доза 1mg/kg (Фабразим) [6]

ЕЗЛ води до прекъсване на патогенетичните механизми на тъканното увреждане с възможности за възстановяване на част от промените. Това е лимитирано от етапа (възрастта) на започването на лечението и неговата непрекъснатост.

Освен намаление на натрупването на субстрат, дозата от 1mg/kg т.т. на всеки две седмици може да осигури и превенция на тежките органни нарушения и смърт при пациентите.[1-6]

1.Germain P.D et al. J Med Genet 2015; 52: 353-358; 2.Lidove O et al. Genet Med 2010; 12 (11): 668-679; 3.Germain P. D. et al Am Soc Nephrol 2007;18: 1547-1557; 4.Goker-Alpan O et al. JIMD Rep. 2016 ;25:95 -106; 5.Skurnes R. et al. Nephrol Dial Transplant (2016) 0: 1-6; 6.КХП Fabrazyme,03.08.2006,EU/1/01/188/001

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ЕНЗИМОЗАМЕСТИТЕЛНО ЛЕЧЕНИЕ

- Пациентите с болест на Фабри изчистват напълно глоботриаозилцерамид (GL3) от мезангиални и ендотелни клетки и частично изчистват подоцитите при лечение с agalsidasa-β в доза 1,0 г/кг т.т. на две седмици при продължително лечение.
- Реакумулация на GL3 в подоцити, но не и в мезангиум и ендотелиум се наблюдава след 3 години от преминаване към agalsidasa-α в доза 0,2 г/кг т.т. на две седмици.
- След повторно включване към терапия с agalsidasa-β в доза 1,0 г/кг т.т. на две седмици отново се установява редукция на GL3 в подоцитите при биопсично изследване след 2 години.

R. Skrunes, E. Svarstad, K. Kampevold, et al. Reacumulation of globotriaosiyceramid in podocytes after agalsidase dose reduction in young Fabry Patients. Nephrol Dial Transplant (2017) 32& 807-813.

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ЕНЗИМОЗАМЕСТИТЕЛНО ЛЕЧЕНИЕ

- В проучването се установява намаляване на ГФ с 18 мл/мин при лечение с agalsidasa-α, а след преминаване на лечение с agalsidasa-β ГФ се възстановява т.е. нормализира, и не се развива гломерулопатия.
- Промените в подоцитните израстъци намаляват и след 3 години на лечение с agalsidasa-β изчезват.
- Тези резултати допълват съществуващите данни, доказващи, че дозата има значение.

R. Skrunes, E. Svarstad, K. Kampevold, et al. Reacumulation of globotriaosiyceramid in podocytes after agalsidase dose reduction in young Fabry Patients. Nephrol Dial Transplant (2017) 32& 807-813.

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ЕНЗИМОЗАМЕСТИТЕЛНО ЛЕЧЕНИЕ

- В друго проучване се съобщава за сигнификантно намаляване на eGFR при лечение с ниска доза на agalsidasa-β – 0,3 г/кг т.т. или при директо преминаване от лечение с agalsidasa-β – 1,0 кг/т.т. към лечение с agalsidasa-α - 0,2 г/кг т.т.
- В същото време тези пациенти, които запазват режима си на лечение с agalsidasa-β – 1,0 кг/т.т. остават с непроменена висока eGFR.

- *M. Lenders, S. Canaan-Kuhl, J. Kramer et al. Patients with Fabry disease after enzyme replasement therapy dose reduction and swith-2-years follow-up. J Am Soc Nephrol 2016, 27; 952-962.*

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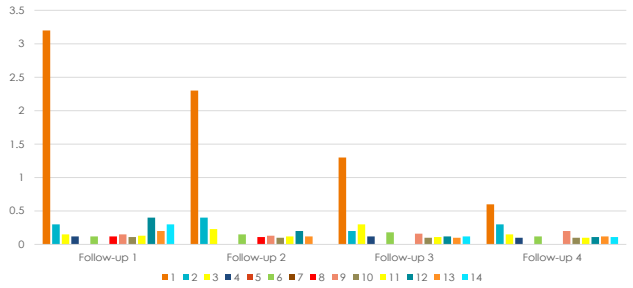
Ензимозаместително лечение

протеинурия



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Ензимозаместително лечение

- Пациентите са наблюдавани за период от 1 до 9 г.
- Всички пациенти имат запазена ГФ
- Един пациент е с гломерулопатия на фона на болестта на Фабри
- Един пациент е с диабетна нефропатия
- При жените от най-големият локус има подчертана белодробна симптоматика
- Един пациент на диализно лечение
- Един пациент с бъбречна трансплантация след предшестващо диализно лечение в продължение на 4 г.
- Всички пациенти са наблюдавани в Клиника по нефрология и трансплантация на УМБАЛ „Александровска", София, която е експертен център за болеста на Фабри за България.

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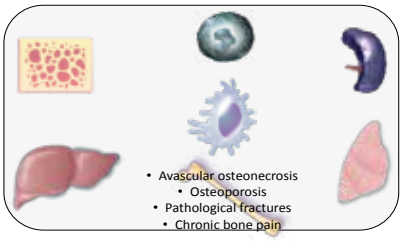
MYELOPROLIFERATIVE NEOPLASM IN PATIENT WITH GAUCHER DISEASE – CASE REPORT

Zlate Stojanoski

Clinical features

- Bone marrow infiltration
- Cytopenia



- Massive splenomegaly
- Cytopenia
- Hypermetabolic state: fatigue

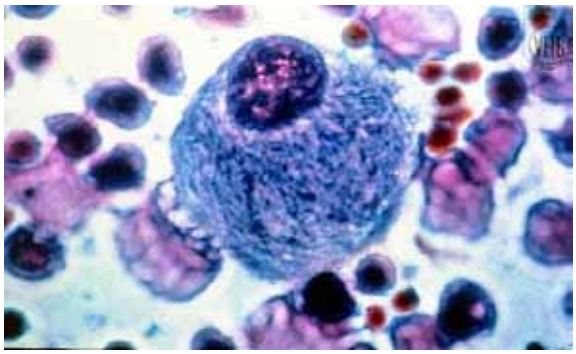
Hepatomegaly

- Avascular osteonecrosis
- Osteoporosis
- Pathological fractures
- Chronic bone pain

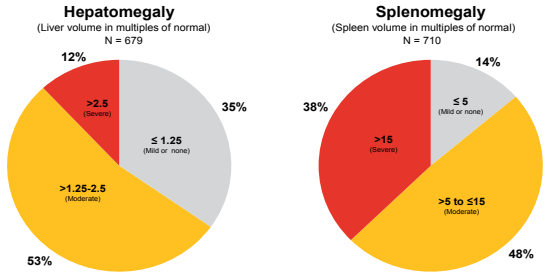
- Infiltrative lung disease

Although the actual defect is confined to the reticuloendothelial system, Gaucher disease is specifically a disease in which macrophages become engorged, causing the liver and spleen to become enlarged and this results in dysfunction of these organs. Other organs that may be affected by macrophage engorgement are bone marrow, lungs, and intestines. Gaucher disease often follows a progressive, symptomatic course. Symptoms are usually multisystemic, often debilitating or disabling, and can lead to death.

Gaucher cell



Visceral manifestations at the time of diagnosis for all disease subtypes



* "At diagnosis" is defined as the data point closest to the diagnosis date but no more than ± 2 years from diagnosis.

1. Gaucher Registry, 2006 Annual Report

Definition

Deficiency of the lysosomal enzyme acid β-glucosidase

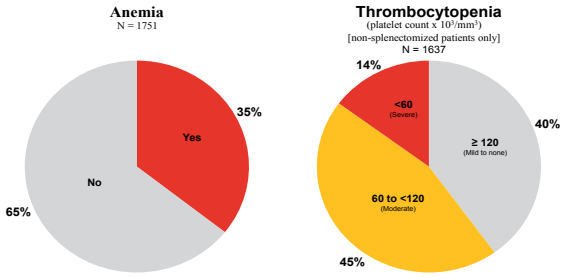
Storage of glucosylceramide primarily in cells of the monocyte/macrophage lineage

Progressive, multi-organ dysfunction primarily involving the reticulo-endothelial system

M.Gaucher is the most common genetic lysosomal storage disorder, affecting an estimated less than 10,000 individuals worldwide.

It is an autosomal recessive disorder defined by the presence of 2 mutant alleles for the acid-β-glucosidase gene, located on region q21 of chromosome 1. Gaucher disease is caused by deficient activity of the enzyme acid-β-glucosidase .

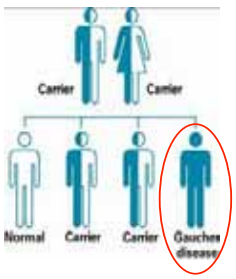
Hematologic manifestations at the time of diagnosis for all disease subtypes



* "At diagnosis" is defined as the data point closest to the diagnosis date but no more than ± 2 years from diagnosis.

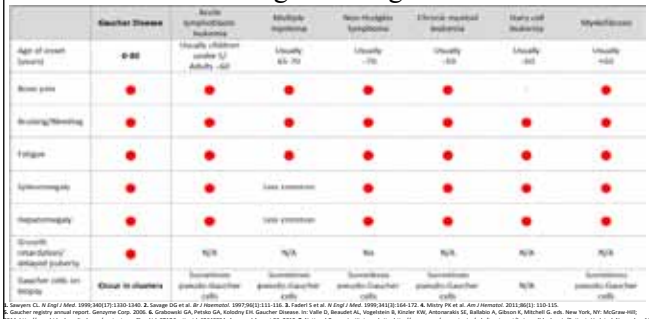
1. Gaucher Registry, 2006 Annual Report

Autosomal recessive disease



Gaucher disease is inherited in an autosomal recessive fashion, meaning that both parents of a child with Gaucher are carriers of a mutation in the acid-β-glucosidase gene. Carriers, or individuals with a single defective gene copy, do not develop the disease, presumably because the presence of one normal copy of the gene is able to make up for the deficiency. However, it should be noted that carrier couples (both mother and father are carriers) have a 1 in 4 or 25% risk of having an affected child with Gaucher disease with each pregnancy. All children born to an affected individual are obligate carriers.

Differential diagnosis and similar presenting symptoms of Gaucher disease and other hematological malignancies



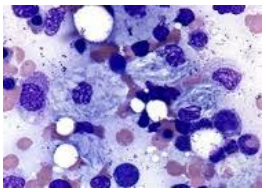
1. Sawyers CL. *N Engl J Med.* 1999;340(17):1330-1340. 2. Savage DG *et al. Br J Haematol.* 1997;96(1):111-116. 3. Fadeel S *et al. N Engl J Med.* 1999;341(2):164-172. 4. Mistry PK *et al. Am J Hematol.* 2011;86(1):110-115. 5. Gaucher registry annual report. Genzyme Corp. 2006. 6. Grabowski GA, Petsko GA, Kolodny EH. Gaucher Disease. In: Valle D, Beaudet AL, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A, Gibson K, Mitchell G. eds. New York, NY: McGraw-Hill; 2014. http://ommbid.mhmedical.com/content.aspx?bookid=971&Sectionid=62643884. Accessed August 03, 2015. 7. National Cancer Institute website. http://cancer.gov/cancertopics/pdq/treatment/hairy-cell-leukemia/Patient. Updated: November 11, 2014. Accessed July 20, 2015. 8. Hairy cell leukemia facts (FS16), Leukemia &Lymphoma Society, White Plains, NY; October 2013. 9. Hoffman R. et al. Hematology basic principles and practice, 6th edition. Philadelphia, PA: Saunders, an imprint of Elsevier Inc.: 2013. 10. O'Donnell M. http://www.cancernetwork.com/cancer-management/acute-leukemias. Accessed July 20, 2015. 11. Al Farsi K. *Oman Med J.* 2011; 26(1):3-11. 12. Shah D. http://emedicine.medscape.com/article/204369-clinical. Accessed July 20, 2015. 13. Myelofibrosis (FS14). Leukemia & Lymphoma Society, White Plains, NY; October 2013. 14. Tefferi A. *Am J Hematol.* 2013; 88(2):141-150. 15. National Cancer Institute website. http://cancer.gov/cancertopics/pdq/treatment/adult-non-hodgkins/Patient. Updated: April 25, 2014. Accessed July 20, 2015 16. Shankland KR et al. *Lancet.* 2012;380(9844):848-857. 17. Thiele J et al. *Histol Histopathol.* 2004;19(4):1277-1288.

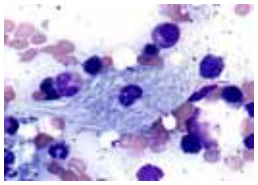
Replacement therapy

Gaucher disease is the first storage disorder successfully treated by enzyme replacement therapy.
In 1991, alglucerase (Ceredase, Genzyme Inc.), the placental derivative of glucocerebrosidase was FDA-approved.
In 1994, the human recombinant-form imiglucerase (Cerezyme, Genzyme Inc.) received FDA approval. Alglucerase is only available today for a handful of patients who are unable to tolerate imiglucerase.
In 2010, the FDA-approved velaglucerase alfa (VPRIV), a human fibroblast-derived glucocerebrosidase developed by Shire, Cambridge, Mass, USA for treatment of Gaucher disease.

Case report

Case report: male patient (S.L) initially presented (1991) at the age of 33 years with mild anemia, thrombocytopenia and splenomegaly. Gaucher disease type 1 (GD1) was diagnosed based on the findings of Gaucher cells in the bone marrow. Clinically, he has remained well over a period of 19 years. From year 2010 he received Cerezyme replacement therapy beacause of deep thrombocytopenia and nose bleeding.





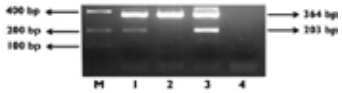
Cancer and Gaucher disease

Numerous studies have reported an increased risk of cancer in Gaucher disease patients. Compared to the general population, Gaucher patients have an increased risk of cancer in general (relative risk of 1.70) and multiple myeloma and hematological malignancies in particular (estimated risk between 25.0 and 51.1). Associated cancers include multiple myeloma, chronic lymphocytic leukemia, chronic myeloid leukemia, acute leukemia, large B-cell lymphoma, T-cell lymphoma, Hodgkin's disease, glioblastoma multiforme, lung cancer, dysgerminoma, hepatocellular carcinoma, and bone cancer. Of the aforementioned malignancies, multiple myeloma has been most frequently reported.

Francis Y. M. Choy and Tessa N. Campbell Gaucher Disease and Cancer: Concept and Controversy International Journal of Cell Biology Volume 11 (2011), Article ID 150450, 6 pages Review Article

Case report

Five years later (2015) he present elevation of Hb, RBC, Hct, WBC and Plt. The possibility of JAK2 mutation was considered as the cause of rising the level of blood count. PCR–based assay with allele-specific primers confirmed the presence of the V617F mutation. The diagnose of polycythemia rubra vera was established.



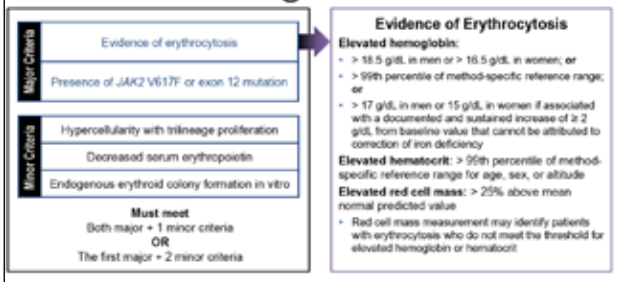
Cancer and Gaucher disease

Despite the consistent association between malignancies and GD, the underlying molecular and cellular bases of this association are not understood. Several potential mechanisms that may underlie predisposition to cancer are based on the current understanding of the pathophysiology of GD. These include:

- chronic inflammation
- chronic B-cell stimulation, abnormalities of T cell function
- aberrant polarization of macrophages
- potential role of splenectomy
- hyperferritinemia
- lysosomal dysfunction

Pramod K. Mistry, Tamar Taddei, Stephan vom Dahl, and Barry E. Rosenbloom
Gaucher Disease and Malignancy: A Model for Cancer Pathogenesis in an Inborn Error of Metabolism
Crit Rev Oncog. 2013; 18(3): 235–246.

Polycythemia Vera
2008 WHO Diagnostic Criteria

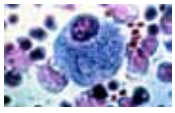


WHO: World Health Organization
Thiele J, et al. In: Swerdlow S, Campo E, Harris N, et al, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2008:40-43.

JAK2 kinase and Gaucher disease

The Janus kinase 2 (JAK2) V617F mutation is known to provide a growth and survival advantage to the affected clones of hematopoetic cells. It may result in clinical phenotypes of polycythemia vera, essential thrombocythemia, and primary myelofibrosis. Although constitutive JAK2 activation is largely responsible for developing myeloproliferative neoplasms (MPNs), an exaggeration of hematopoiesis might be beneficial to a preexisting cytopenic disorder.
Webb et al first reported the JAK2 V617F mutation in a GD1 patient who developed a myeloproliferative/myelodysplastic neoplasm that progressed rapidly to advanced myelofibrosis.

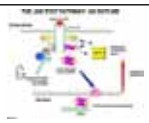
Webb BD, Weinreb NJ, Botti AC, Kirmse BM, Balwani M. JAK2V617F mutation and myeloproliferative malignancy in a patient with Type 1 Gaucher disease. Blood Cells Mol Dis. 2011;46(1):103-104.



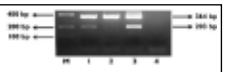
Case report

Hb: 201g/dL
RBC: 7.010 000
Hct: 0,62
WBC: 15,200
PLT: 476.000

Vein puncture of 350ml blood was introduced with aim to maintenance Hct level < 0,45. Also, therapy with ASA 100mg./ day for cardiovascular events prophylaxis. The patient doing well with this therapy.



Conclusion



In Macedonian Registry for Rare Disease we have registered 15 patients with Gaucher disease.
This is the first patient with malignant disease from our group of GD patients.
Testing for JAK2 V617F and other mutations associated with MPN may be useful for investigation of unexpected changes in patients with GD to confirm the cause when there are suggestive clinical features.

Thank you for your attention !

Cancer and Gaucher disease - Discussion

Several theories have been suggested to explain an association between Gaucher disease and cancer. One set of hypotheses focuses on the accumulation of glucocerebroside as the main culprit by impacting immune system regulation in a number of different ways.
Costello et al. postulated that exaggerated B-cell function may be secondary to stimulation by accumulated glucocerebroside, T-cell function dysregulation, augmented macrophage activation, and disruption of antigen presentation.
Moreover, it has been suggested that progressive accumulation of glucocerebroside may trigger macrophage activation, leading to chronic stimulation of the immune system. This could result in enhanced cytokine secretion and subsequent clonal B-cell expansion, setting the stage for eventual transformation. In support of this, elevated levels of specific cytokines, including IL-1, IL-6, IL-8, IL-10, and TNF-α, have been found in Gaucher's patients.

SESSION 3

Supported by Novartis

Moderator: Ana Pejcic

- **From cost to value in rare diseases**
  S. Popov

- **From cost to value in rare diseases**
  I. Petrov

- **Quality of life in acromegaly**
  S. Vandeva

- **Acromegaly – psychological consequences for the patient**
  N. Grigorova

FROM COST TO VALUE IN RARE DISEASES

Stamen Popov

NOVARTIS – Leader in R&D investments

Novartis Oncology

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The content herein includes information on investigational product(s) and/or investigational use(s). Efficacy and safety have not been established. There is no guarantee that the compound will become commercially available for use(s) under investigation. This is for your background and educational purposes only. The information presented should not be considered as recommendations for use.

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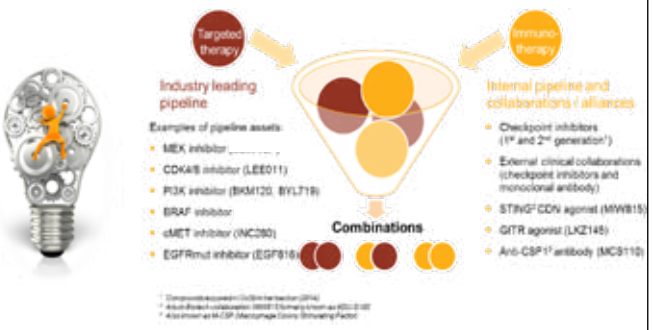
Novartis – Therapeutic Strategies



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Novartis – 4 major divisions

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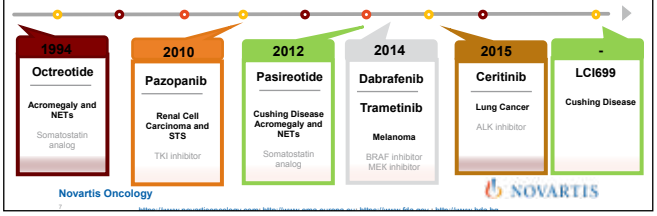
Novartis - Landmarks Achievements in Solid Tumors and Rare Diseases



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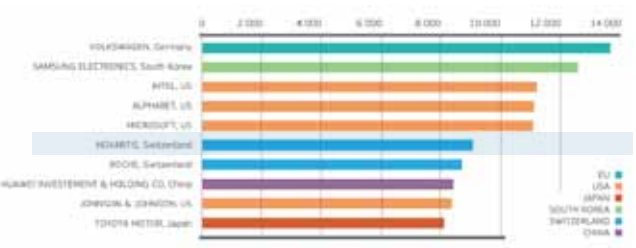
NOVARTIS – Leader in R&D investments



TOP 10 COMPANIES OF THE 2016 SCOREBOARD. Source: The 2016 EU Industrial R&D Investment Scoreboard. European Commission, JRC/DG RTD.

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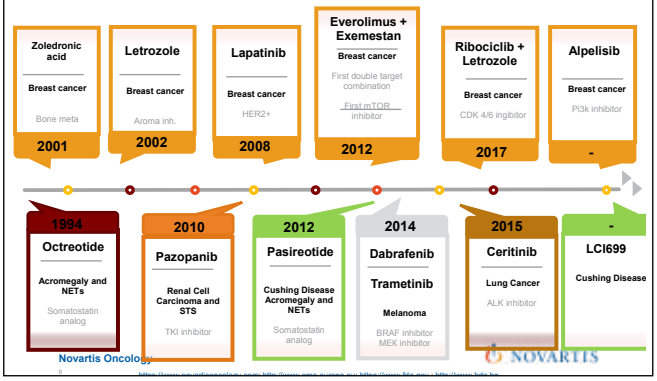
Novartis - Landmarks Achievements in Solid Tumors and Rare Diseases



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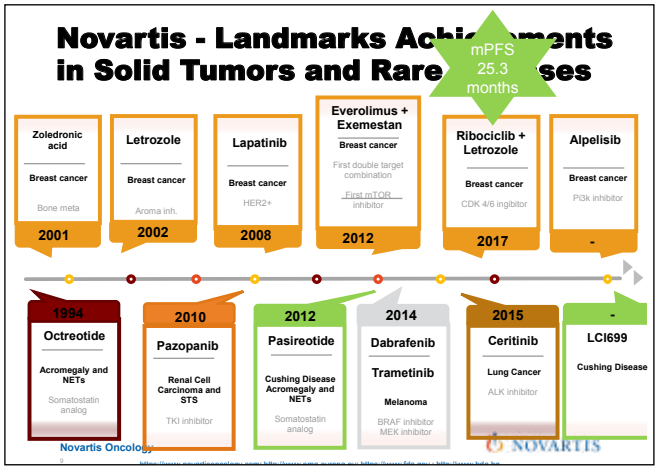
Novartis - Landmarks Achievements in Solid Tumors and Rare Diseases
mPFS 25.3 months
Zoledronic acid
Breast cancer
Bone meta
2001
Letrozole
Breast cancer
Aroma inh.
2002
Lapatinib
Breast cancer
HER2+
2008
Everolimus + Exemestan
Breast cancer
First double target combination
First mTOR inhibitor
2012
Ribociclib + Letrozole
Breast cancer
CDK 4/6 ingibitor
2017
Alpelisib
Breast cancer
PI3k inhibitor
-
1994
Octreotide
Acromegaly and NETs
Somatostatin analog
2010
Pazopanib
Renal Cell Carcinoma and STS
TKI inhibitor
2012
Pasireotide
Cushing Disease Acromegaly and NETs
Somatostatin analog
2014
Dabrafenib
Trametinib
Melanoma
BRAF inhibitor MEK inhibitor
2015
Ceritinib
Lung Cancer
ALK inhibitor
-
LCI699
Cushing Disease
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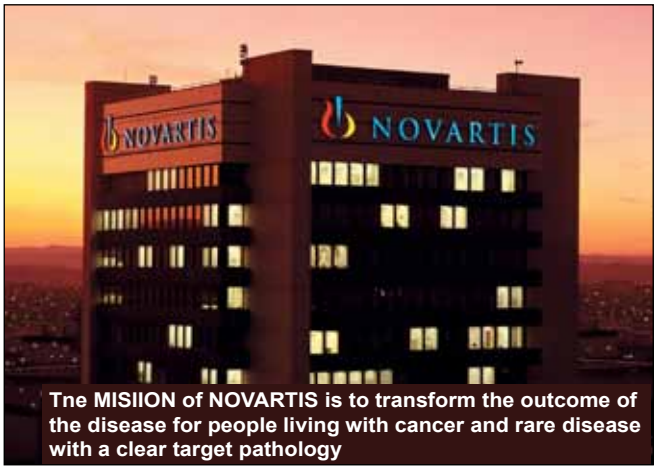
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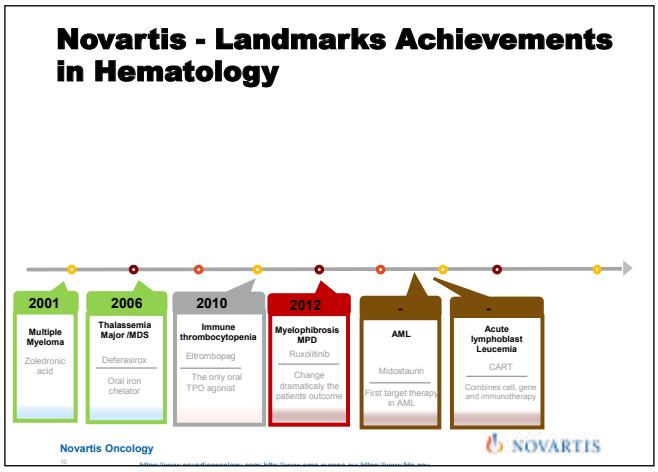
NOVARTIS
Tne MISIION of NOVARTIS is to transform the outcome of the disease for people living with cancer and rare disease with a clear target pathology


Novartis - Landmarks Achievements in Hematology
2001
Multiple Myeloma
Zoledronic acid
2006
Thalassemia Major /MDS
Deferasirox
Oral iron chelator
2010
Immune thrombocytopenia
Eltrombopag
The only oral TPO agonist
2012
Myelophibrosis MPD
Ruxolitinib
Change dramatically the patients outcome
-
AML
Midostaurin
First target therapy in AML
-
Acute lymphoblast Leucemia
CART
Combines cell, gene and immunotherapy
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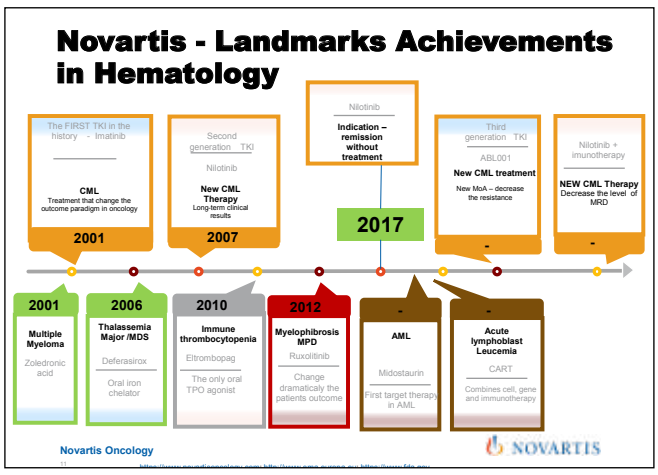
Novartis - Landmarks Achievements in Hematology
The FIRST TKI in the history - Imatinib
CML
Treatment that change the outcome paradigm in oncology
2001
Second generation TKI
Nilotinib
New CML Therapy
Long-term clinical results
2007
Nilotinib
Indication – remission without treatment
2017
Third generation TKI
ABL001
New CML treatment
New MoA – decrease the resistance
-
Nilotinib + imunotherapy
NEW CML Therapy
Decrease the level of MRD
-
2001
Multiple Myeloma
Zoledronic acid
2006
Thalassemia Major /MDS
Deferasirox
Oral iron chelator
2010
Immune thrombocytopenia
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Midostaurin
First target therapy in AML
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Combines cell, gene and immunotherapy
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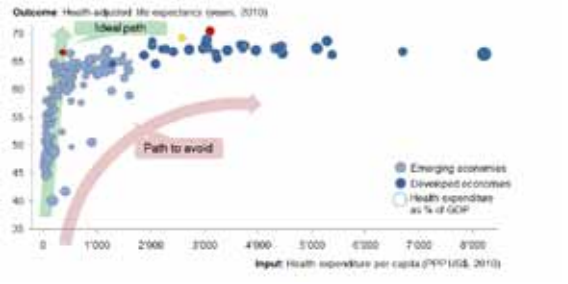
FROM COST TO VALUE IN RARE DISEASES

Ivaylo Petrov

Despite the rising costs improving outcomes is getting harder

Many countries struggle to translate healthcare spending into better health



Source: Boston Consulting Group

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Outline

- **Healthcare spending** is increasing faster than what the current economic climate can sustain.
- Many countries are concerned that higher spending does not lead to proportionally **better outcomes**.
- **Healthcare systems waste** a significant amount of resources that limits the fiscal space and hinders performance.
- Focusing on **value-based care**, reducing waste and directing investments to where greater value can be produced is critical for sustainability.
- **UHC aspirations** will be hard to materialize if we do not shift to value-based care and change policies to support this shift.
- This change requires **collaboration** of every member of the healthcare ecosystem and better public-private partnership.

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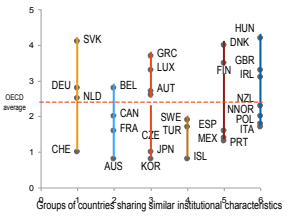
The health system type is not a good predictor of efficiency...

Potential gains in life expectancy (years, DEA)[1]



1. Derived from an output oriented DEA with per capita health care spending and composite indicator of socio-economic environment and lifestyle factors as inputs for 2007. Source: "Health Care Systems: Efficiency and institutions"; OECD Economics Department Working Paper n° 769, 2010

The OECD classified countries according to their institutional characteristics...

... and identified opportunities for efficiency improvements in all country groups

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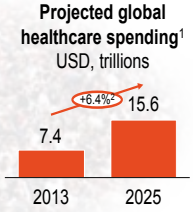
The world will be larger, older, sicker, and healthcare more costly...

From 2013-2025

Population will increase by ~1 billion

Number of additional >50 year olds >500 million

Chronic disease share of disease burden 70%



Projected global healthcare spending[1] USD, trillions

Nominal healthcare spending to double

Source: Projections from UN; WHO

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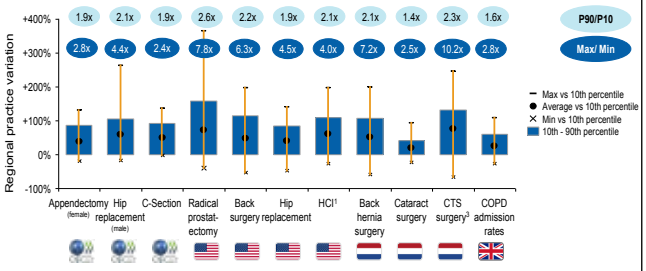
Practice variation found in all countries and diseases areas



1. The HCI index combines the number of days patients spent in the hospital and the average number of inpatient physician visits during the last two years of life . 2 Rates were adjusted for age, race, and sex using the U.S. Medicare population as the standard 3. CTS stands for carpal tunnel syndrome. Source: darthmouthatlas.org; Vektis report "Praktijkvariatierapport 7 Electieve zorg aandoeningen April 2014"; NHS CCG tools; OECD Health Working Papers, No. 61

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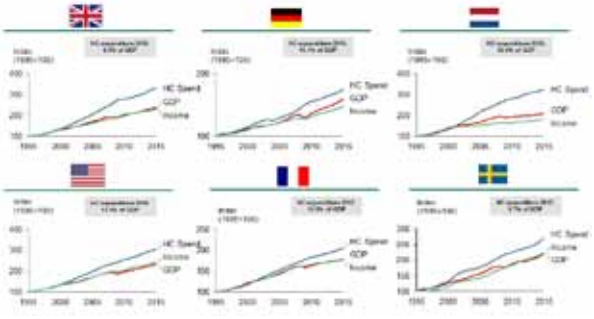
This growth is unsustainable in the current economic climate



Source: Boston Consulting Group

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3 out of 4 key sources of waste could be addressed by greater focus on outcomes

	Description
1 Overtreatment (Practice variation)	Care routed in outdated habits, ignoring scientific findings, motivated by something other than optimal care, etc.
2 Failures of care delivery and coordination (Practice variation)	Poor execution or lack of best practices, e.g. effective preventive care or patient safety, fragmented and disjointed care, low volume for specific treatments per hospital, etc.
3 Pricing, payment and incentives failures	Payments and incentives not rewarding valuable interventions, variation in pricing of services with equivalent outcomes
4 Administrative complexity	Waste created by inefficient or flawed rules and overly bureaucratic procedures across stakeholders

Could be addressed through outcomes focus

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What value-based care methods have in common

Several core, integrated capabilities are needed to drive success under VBHC models in Rare Disease – many of which are also valuable in terms of burden of illness

Theory Practice

1. **Diagnostic Delay** – *The pathway to diagnosis for patients with rare diseases is generally fraught with delay and misdiagnoses*
2. **Integrated Analytics & Health IT** – *Platforms that integrate clinical and financial insights to support decision-making, enable care coordination and drive quality improvement*
3. **Limited Treatments** – *The current treatments for most rare diseases are very limited or cumbersome.*
4. **Medical Costs** – *A rare disease imposes significant medical costs on patients and caregivers, as well as health systems*
5. **Diagnostic Trauma** – *Patients and caregivers described the experience of receiving a diagnosis for a rare disease as very traumatic*
6. **Patient's Quality of Life** – *Most rare diseases have a moderate to severe negative impact on quality of life*

Source: "Burden of Illness in Rare Diseases", August 17, 2017;

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5 key elements have been identified as successful at increasing HC value

1	Identify target population (e.g. disease groups)	• Identify patients based on their healthcare needs, behaviours, etc. to prevent and manage illness, rather than simply treat disease	Identify patients with common needs and highest costs
2	Define target outcomes to reduce costs	• That matter to patients and clinicians, balanced along full cycle of care - prevention and cure, comparable, linked to population	Identify which health outcomes are needed for healthy population
3	Measure and learn from variation	• Establish inter-operable data systems across providers, real-time measuring, transparency of outcomes, etc.	Improve to achieve target outcomes at minimum cost
4	Define treatment pathway with coordinated delivery	• New models need to be based on the patient along care chain, vs. single procedure or single episode of care	Whole-person focus (also reduce waste from coordination)
5	Align payments and incentives	• Payments aligned to providers' collective performance against target outcomes, instead of promoting price and volume. Ensure incentive design does not promote unwanted behaviors (e.g. hiding bad results...) • Gradual transfer of risk to providers	Align stakeholders to achieve previous goals

Source: "Accountable Care: Focusing Accountability on the Outcomes that Matter," WISH Accountable Care Report, 2013; BCG

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Mapping capabilities onto value-based care

The system core, integrated capabilities are needed to drive success under VBHC models

Theory Practice

Diagnostic Delay	Integrated provider network covering all levels of care provision
Integrated analytics & health IT	BG rare diseases comprehensive centers are connected in joint electronic evidence analytic system - allowing to share patient
Limited Treatments	As a non-profit organization earnings are reinvested into staff and clinical infrastructure
Medical Costs	The collaboration between clinics in a joint organization allows to build financial resources across provider organizations
Diagnostic Trauma	The BG clinics continuously refine their clinical system of treatment pathways for patients suffering an accident
Patient's Quality of Life	Patient engagement is an important element of rehabilitation, which lasts beyond hospital treatment and may even involve support by welfare workers

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Value-based care is fundamental to UHC aspirations

Value-based **care reduces** waste → Expands **fiscal space** – a larger "UHC cube"

Value-based care **maximizes outcomes** that matter for the system → Addresses the **missing dimension** of "UHC Cube" – **quality**

Value-based care **supports sustainability** of the system → Helps ensure **long-term funding** for UHC agenda and **political support** to the health system

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Focusing on outcomes in a cost-conscious way is at the core of the "Value" concept

$$\text{Value} = \frac{\text{Health outcomes}}{\text{Cost of delivering the outcomes}}$$

Improve outcomes	Reduce overall costs	Increase value
Starting point is to focus on improving patient outcomes	Better quality of care is often less expensive over the long-term	Better quality care at equal or lower cost leads to higher value in the system

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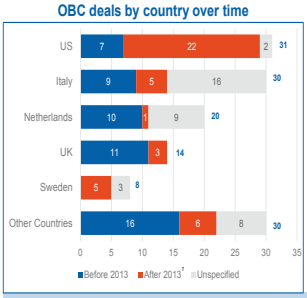
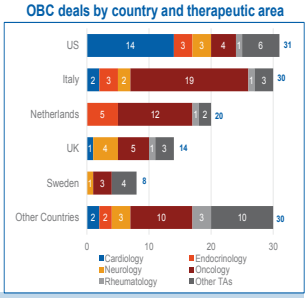
US and Italy are ahead of others in outcome-based deals

OBC deals by country over time



OBC deals by country and therapeutic area



Italy has established a clear focus for OBC deals in oncology, but cardiology in the US is also a clear trend

Source: Novartis commissioned research. The presented data may not be an exhaustive representation of the universe of outcome-based deals .

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Principles of value-based care

- **Value is created in caring for a patient's medical condition over the full cycle of care** – NOT by a hospital, a site, a specialty, an episode or an intervention
- **The most powerful single lever for reducing cost is improving outcomes**

Source: Porter, M. 'Value based health care delivery: strategy for health care leaders'. 2015 Health Forum Summit (link)

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Novartis has decided to embrace the drive towards outcomes and be on top of it

Past	Future
Treating signs & symptoms Hypertension, blood sugar levels	**Focus on overall clinical outcomes** Less heart failure exacerbations, fewer hospital admittances
Product offering The product itself	**Product plus offering** The product + measures to enhance outcomes: diagnostics, telehealth technology, apps
Selling medicines & devices Customers with fixed budget for pharmaceutical and device spending	**Partnering for better health** Partnering with customers to achieve good clinical outcomes against sustainable healthcare spending

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Moving ahead

- We, public and private sectors, should work together more effectively to transform the healthcare for greater sustainability of the ecosystem in which we operate.
- We are ready to be a winning partner in this transformation by offering better products and services for the tough healthcare challenges.
- We are ready to be rewarded proportionally to the real world outcomes that we deliver for the healthcare system.
- We want to make our tangible contribution to making UHC aspirations reality for everyone in the country.

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The winning way of looking beyond the present

... and what we can achieve on the way

Source: Profitable ways of looking beyond the balance sheet; A. Edmans; 25th May 2016

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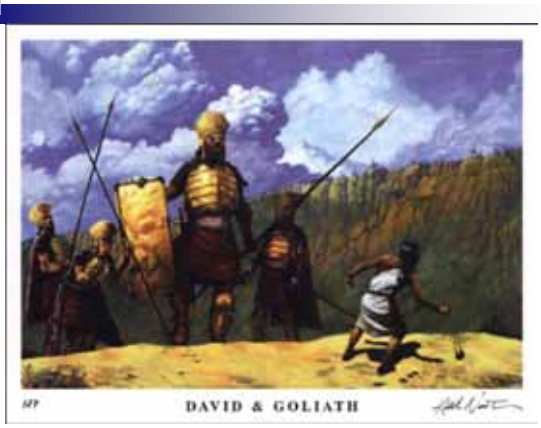
QUALITY OF LIFE IN ACROMEGALY

Silvia Vandeva

Treatment goals

- Biochemical control
  GH – random <1 µg/l; nadir in OGTT <0.4 µg/l
  IGF-1 – age-adjusted
- Reduction and control of tumor size
- Management of co-morbidities
- Normal life expectancy
- Improvement in health- related quality of life



Treatment possibilities

- Surgery
  - TSA
- Pharmacological therapy
  - Dopamine agonists
    - Cabergoline
  - Somatostatin analogs
    - Sandostatin LAR
    - Somatuline autogel
    - Pasireotide LAR
  - Growth hormone receptor blocker
    - Pegvisomant
- Radiotherapy

ACROQOL

- Acromegaly Quality of Life Questionnaire – the first disease-specific questionnaire (Webb SM et al. Acromegaly Quality of Life Questionnaire (ACROQOL) a new health-related quality of life queistionnaire for patients with acromegaly: development and spychometric properties. *Clin Endocrinol* 2002, 57:251-258)
- 22 questions - physical and psychological scales
- Physical scale
  - My legs feel weak
  - I get depressed
  - I have problems carrying out my usual activities
  - The illness affects my performance at work or in my usual tasks
  - My joints ache
  - I am usually tired
  - I feel like a sick person
  - I feel weak

- Increased morbidity and mortality due to
  - Cardiovascular diseases
  - Neoplasms
  - Respiratory diseases
- Decreased health-related quality of life

ACROQOL

- Psychological scale
- Appearance subscale
  - I feel ugly
  - I look awful in photographs
  - I look different in the mirror
  - Some parts of my body (nose, feet, hands…) are too big
  - I have problems doing things with my hands, for example, sewing or handling tools
  - I snore at night
  - It is hard for me to articulate words due to the size of my tongue

ACROQOL

- Psychological scale
- Personal relations subscale
  - I avoid going out very much with friends because of my appearance
  - I try to avoid socializing
  - I feel rejected by people because of my illness
  - People stare at me because of my appearance
  - I have problems with sexual relationships
  - The physical changes produced by my illness govern my life
  - I have little sexual appetite

Studying of factors influencing AcroQoL

- Multivariate linear regression analysis
  - age
  - gender
  - disease control
  - duration of active disease since diagnosis/duration of remission
  - presence of hypopituitarism
  - prior radiotherapy
  - number of surgeries (1 or ≥2)
  - dopamine agonist (DA) treatment
  - somatostatin analog (SSA) treatment

ACROQOL

- Answers
  - Frequency of occurrence
    - Always
    - Most of the time
    - Sometimes
    - Rarely
    - Never
  - Degree of agreement
    - Completely agree
    - Moderately agree
    - Neither agree nor disagree
    - Moderately disagree
    - Completely disagree
  - Max. score of each scale – 100, minimum - 0

Multivariate linear regression analysis of factors determining QoL in the cross-sectional group

	Variables	Total Score		Physical score		Appearance score		Personal relations score		Psychological score	
		B	p	B	p	B	p	B	p	B	p
All patients (n=212)	Age	-0.69	**<0.001**	-0.76	**<0.001**	-0.58	**<0.001**	-0.7	**<0.001**	-0.643	**<0.001**
	Gender (f vs. m)	-8.8;	**0.011**	-10.5	**0.004**	-6.83	0.063	-8.8	**0.023**	-7.84	**0.028**
	Radiotherapy	-13.8;	**0.007**	-11.85	**0.03**	-13.0	**0.017**	-16.4	**0.004**	-14.9	**0.005**
	Lack of disease control	-3.74	Ns	-0.52	Ns	-7.39	**0.041**	-3.78	Ns	-5.59	Ns

Bulgarian experience with AcroQoL

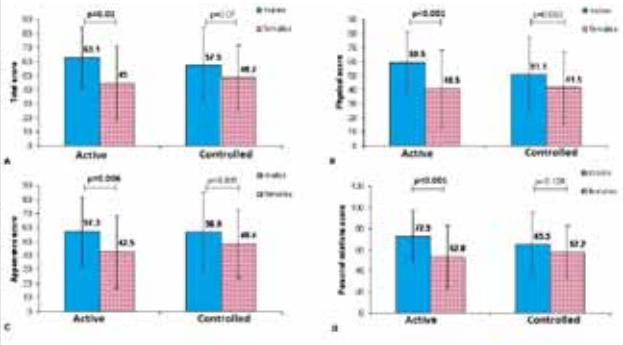
- Aim
  - to evaluate QoL in patients with acromegaly
  - To assess the influence of biochemical disease control and treatment approach
- Study – 212 patients with acromegaly over a 6-year period of time
  - Cross-sectional branch
    - 100 with active disease
    - 112 with controlled disease
  - Longitudinal branch – 70 patients with active acromegaly at baseline
    - 45 patients in remission at the time of reevaluation

Vandeva et al. Disease control and treatment modalities have impact on quality of life evaluated by Acromegaly Quality of Life (AcroQoL) Quesnnaire. Endocrine 2015

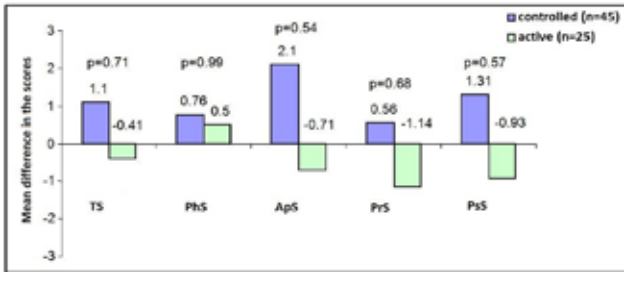
Multivariate linear regression analysis of factors determining QoL in the cross-sectional group

	Variables	Total score		Physical score		Appearance score		Personal relations score		Psychological score	
		B	p	B	p	B	p	B	p	B	p
Controlled patients (n=112)	Age	-0.55	**0.010**	-0.74	**0.002**	-0.35	Ns	-0.556	**0.017**	-0.452	**0.04**
	Duration of remission	-1.17	0.057	-0.99	Ns	-0.74	Ns	-1.585	**0.014**	-1.164	0.055

Comparison of AcroQoL scores between men and women in the cross-sectional group of patients.



Comparison of the mean individual change of scores between active and controlled patients at the time of re-evaluation



Comparison of the mean individual change of scores depending on the treatment at the time of re-evaluation.

	Operated patients (n=11)	Patients under DA (n=14)	Patients under SSA (n=37)	Patients under Pegvisomant (n=7)	P value
TS change	3.51±26.1	-5.76±15.3	2.39±14.2	-3.25±6.8	0.359
PhS change	1.43±34.5	-3.12±18.9	2.36±20.5	-3.13±11.4	0.842
AS change	2.93±24.6	-7.39±18.9	4.83±15.5	-7.14±10.3	0.096
PrS change	6.5±25.7	-7.14±15.7	-0.001±13.7	0.51±9.9	0.227
PsS change	4.71±24.2	-7.27±15.7	2.41±12.5	-3.32±6.0	0.147

Summery of factors with some influence on different AcroQoL scores

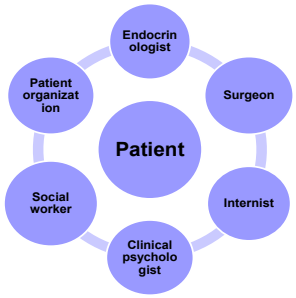
- General
  - Age
  - Gender
- Disease-specific
  - Disease control
  - Radiotherapy
  - SSA
  - Hypopituitarism

Studying of factors influencing improvement in AcroQoL in the longitudinal group

- Multivariate logistic regression analysis
- A model with application of SSA
  - SSA predicted improvement in
  - Total score - OR 3.14 (95% CI 1.035-9.515), **p=0.043**
  - Appearance score - OR 4.16 (95% CI 1.29-13.44), **p=0.017**

Conclusion

- Quality of life is a multifactorial issue
- Multidisciplinary approach is necessary



Multivariate logistic regression analysis of factors predicting improvement of the AcroQoL scores at re-evaluation.

Variables	TS OR (95% CI), p value	PhS OR (95% CI), p value	AS OR (95% CI), p value	PrS OR (95% CI), p value	PsS OR (95% CI), p value
Remission (yes vs. no)	3.08 (0.94-10.1); 0.062 *4.09 (1.17-14.2), **0.026**	Ns	Ns	Ns	Ns
Corresponding baseline score	0.98 (0.95-1.0), 0.071	0.95 (0.35-3.76), **0.001**	0.97 (0.95-0.996), **0.022**	Ns	Ns
Hypopituitarism (no vs. yes)	4.96 (0.90-27.2), 0.065	Ns	7.33 (1.19-45.3), **0.032**	Ns	Ns

Thanks for your attention!

ACROMEGALY – PSYCHOLOGICAL CONSEQUENCES FOR THE PATIENT

Nataliya Grigorova

Diagnose

Most of the counselled patients report that the most traumatic period for them was the period of diagnosing and setting up the brain surgery to remove the pituitary adenoma.

Most common reaction upon diagnosing are:

- depression
- anxiety
- anger

Most likely after the brain surgery a patient will develop post traumatic stress disorder.

Within a project for Extra care for patients with rare disease, in a period of July 2016 – April 2017 in the University Specialized Hospital for Active Treatment of Endocrinology "Academician Ivan Penchev" was provided psychological counseling for

60 people diagnosed with Acromegaly, from who 36 men and 24 women aged between 24 and 71 years old.

36 people from them also filled out Quality of life Questionnaire for people with Acromegaly.

In the following presentation we will summarize some observations upon the psychological state of the patients made during our work with them.

Coping with diagnose

After the initial shock from the diagnosis, the patient usually experiences a period of acceptance of the consequences and the changes in their life resulting from it.

The condition that patients with Acromegaly experience after they are already diagnosed is close to the

condition of a person in mourning or experiencing severe loss.

It is estimated that One in Five people have a pituitary adenoma (tumor).[1]

But only in 60 out of 1 Million Acromegaly disease occurred.

Most common symptoms of the disease are enlargement of limbs aggravation and change of facial features of affected persons. [2]

Mourning

This condition is usually composed of experiences that can be divided into five phases[3]:

1. **Denial and isolation**: "this can not happen to me"
2. **Anger:** "why me ?!"
3. **Bargaining**: "if I behave, maybe it will disappear on its own"
4. **Depression**: reactive or preparatory, the patient may not go into the next phase
5. **Acceptance**: "I accept that this happens to me, I am ready to cope and live with it"

Diagnosis

Diagnosis completely changes the patient's life.

- chronicity
- long-term medical therapy
- painful medical interventions

Coping strategies in patients with Acromegaly

If the patient does not go to the acceptance of diagnosis phase, then he / she is most likely to have

Disadaptive style of coping:

- these are patients who more often report the occurrence of multiple recurrences and failed drug therapy of the disease;
- experience constant depressive or mixed depressive-anxiety symptoms,
- have unrealistic beliefs about the disease, such as the belief that the disease will disappear miraculously that the disease is a fate, punishment, trial, and the like
- an external control site or absolute conviction for exceptional external control of the disease
- In these patients there is no further supporting family and / or social environment

Coping strategies

If the patient goes to the acceptance phase, then he / she is most likely to have:

Adaptive style of coping:

- these are patients who have been able to "integrate" the disease and its therapy into their everyday lives so much that there is no illness experience,
- accept realistically the disease,
- there are no uncomfortable psychic experiences,
- these patients usually have a supportive family and / or social environment.

Other psychological aspects

- **problems with adapting to life with the disease:** staying in the depression phase and being unable to accept the disease as part of the life path,
- **a change in the body's self-image:** the real change in appearance leads to a change in self-perception and instability of patient's identity.

"I will never be like I used to."

Clinical interview

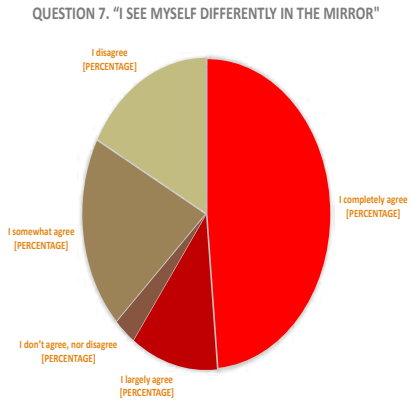
From the clinical interview, including questions about the presence of stressful events before diagnosis, how to accept and deal with the illness and the patient's vision for his / her own future, the following observations can be highlighted:

Most of the interviewed patients have reported

Experiencing a traumatic event – the decease of loved ones, car incidents, severe birth or

experiencing chronic stress – alcohol abuse in the family, domestic violence, workplace bullying, mental illnesses in the family,

which happened from **1 to 4 years before diagnosis** of the disease.



Clinical interview

"My disease is a punishment"

This is the most common explanation that patients give for their illness.

Most patients believe that their illness is a punishment or trial for something bad that they have done, in reality or imaginative, of other life transgressions or punishment from God.

This belief brings to the patient

a strong sense of guilt,

which in turn provokes

depressive experiences and mental discomfort.

Patients diagnosed with Acromegaly

suffer physically, emotionally and psychologically

as consequences of their disease which inevitably worsens their life quality,

their social adaptation and the success of the disease's therapy.

This makes it necessary for **psychosocial help**

of patients to be a part of a multidisciplinary care for them,

as such support would increase significantly the quality of

patient care and their complex condition..

Other psychological aspects

Other symptoms causing a mental disorder seen in consulted patients with Acromegaly are:

- **anxiety and / or depressive thoughts and experiences**: inability to see oneself in the future, low self-esteem, social exclusion,
- **Post-traumatic stress symptoms**: Most often patients report having been induced after brain surgery, most often they have nightmares or flashbacks about the traumatic event

Special thanks

to NOVARTIS, University Specialized Hospital for Active Treatment of Endocrinology, prof.Zaharieva and her team and Dr. Stoyanov, without whom this projects would not be possible.

NOVARTIS

PARALLEL SYMPOSIUM 3
Supported by Shire

Moderator: Valeria Kaleva

- **Hemophilia**
  V. Kaleva, T. Beleva

- **New opportunities for personalized prophylaxis in patients with hemophilia A with Advate [in Bulgarian]**
  V. Kaleva

- **Feiba – review of clinical trials (Pro-FEIBA, PROOF, FENOC) [in Bulgarian]**
  S. Goranov

- **Overview of thromboembolic events in congenital hemophilia, reported with Feiba over the last four decades [in Bulgarian]**
  D. Stoyanova

ХЕМОФИЛИЯ

Валерия Калева, Тодорка Белева

Глобално разпространение

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За Shire

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C-APROMBGI/0011; DOP: 07/2017

Терапевтични области

Хематология $3,627m Всички продажби през 2015 г. Хемофилия A & B, Хемофилия с инхибитори, Придобита хемофилия, Болестта на Вон Вилебранд, Основна тромбоцитопения	**Имунология**[1] $2,516m Всички продажби през 2015 г. Имунни недостатъци, Критична грижа, Шок и травма, Невроложично и белодробно заболяване	**HAE & LSD**[2] $2,399m Всички продажби през 2015 г. Наследствен ангиоедем, Синдром на Хънтър, Болест на Гоше, Болест на Фабри
Неврология $2,200m Всички продажби през 2015 г. ADHD - Синдром на дефицит на вниманието и хиперактивност, Психично разстройство Конвулсивни припадъци при деца	**GI & IM**[3] $1,501m Всички продажби през 2015 г. Синдром на късото черво, Хипопаратиреоидизъм, Надбъбречна недостатъчност, Язвен колит, Хроничен запек	**Онкология** $87m Всички продажби през 2015 г. Остра лимфобластна левкемия

1. Отразява продажбата на имунологични средства на Baxalta
2. HAE & LSD - Наследствен ангиоедем и нарушения на съхранението на лизозоми
3. GI & IM - стомашно-чревна и вътрешна медицина

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Нашите основни характеристики

В момента ние сме водещата световна биотехнологична компания, съсредоточена върху обслужването на пациенти с редки заболявания и други високо специализирани състояния.

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3

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Водещи продукти на пазара

Advate, Adynovate, Feiba, Hemofil M, Obizur, Recombinate, Rixubis **Хематология** $3,627m Всички продажби през 2015 г.	**Aralast, Buminate, Ceprotin, Flexbumin, Glassia, Hyqvia, Gammagard/Kiovig** **Имунология** [1] $2,516m Всички продажби през 2015 г.	**Cinryze, Elaprase, Firazyr, Kalbitor, Replagal, Vpriv** HAE & LSD[2] $2,399m Всички продажби през 2015 г.
Adderall Xr, Buccolam, Equasym, Intuniv, Vyvanse **Неврология** $2,200m Всички продажби през 2015 г.	**Gattex/Revestive, Fosrenol, Lialda/ Mezavant, Natpara, Pentasa, Xagrid** GI & IM[3] $1,501m Всички продажби през 2015 г.	**Oncaspar** **Онкология** $87m Всички продажби през 2015 г.

1. Отразява продажбата имунологични средства на Baxalta
2. HAE & LSD - Наследствен ангиоедем и нарушения на съхранението на лизозоми
3. GI & IM - стомашно-чревна и вътрешна медицина

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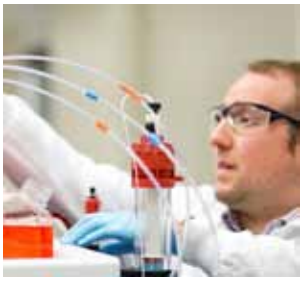
Кратък обзор

- Основана преди повече от 30 години
- Наскоро обединена с Baxalta за да се превърне в водещата биотехнологична компания с фокус върху редки заболявания и други високо специализирани състояния
- Растеж чрез научно изследване, стратегически придобивания и иновативни лицензионни споразумения
- Иновативни нови терапии в процес на изследване
- Основни офиси в Lexington & Cambridge (Масачузетс, САЩ), Bannockburn (Илинойс, САЩ), Дъблин (Ирландия) и Цуг (Швейцария)
- Продукти, предлагани в повече от 100 страни
- С котировки на Лондонската фондова борса и NASDAQ



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Непрекъсната иновация

Иновацията е "жизнената сила на нашия настоящ и бъдещ успех".

Ние съсредоточаваме нашите нововъведения в области, в които има нерешени медицински нужди. За целта ние се стремим да разширим експертните знания в областта на редките болести и предлагане чрез изследване и партньорства, и да разширим съществуващото портфолио от продукти към нови индикации и терапевтични области.

Към 28 юли 2016 г.

2016 40+ Клинични програми в процес на изследване

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Лидер в индустрията за биотехнологии

Shire е обявена за най-достойна компания за здравеопазване в САЩ и е 31-ва най-достойна компания в САЩ, съгласно 2015 U.S. RepTrak® 100

Въз основа на общественото мнение за компаниите на базата на седем показателя: иновации, лидерство, управление, гражданство, работно място, финансово представяне и продукти / услуги.

Флеминг Орнсков е сред 100-те най-добре представящи се изпълнителни директори в света

От "Харвардския бизнес преглед", ноември 2015.

Harvard Business Review

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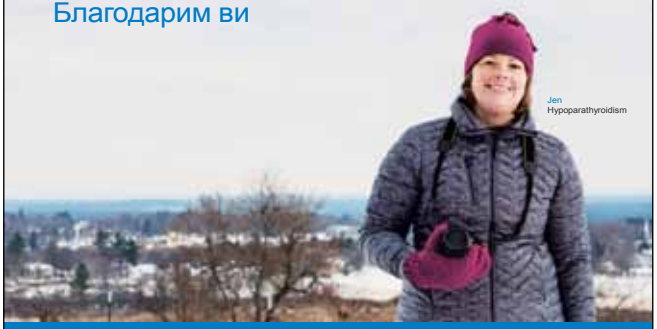
Благодарим ви



Jen
Hypoparathyroidism

Shire

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НОВИ ВЪЗМОЖНОСТИ ЗА ПЕРСОНАЛИЗИРАНЕ НА ПРОФИЛАКТИКАТА ПРИ ПАЦИЕНТИ С ХЕМОФИЛИЯ А

Валерия Калева

Декларация

- Декларирам, че съм получавала хонорар, пътни разходи и настаняване от следните компании: *Shire, Bayer, Novartis, Novo Nordisk, Pfizer, Sobi*.
- Вижданията и мненията, представени в следващите слайдове, са на лектора и не трява да бъдат разбирани и цитирани като изработени от *Shire*.

ХЕМОФИЛИЯ А

- **боледуват само мъже; жените са носителки на хемофилна наследственост**
- **синовете на болните от хемофилия мъже са винаги здрави; дъщерите – винаги носителки**
- **при жени се среща изключително рядко – дъщеря на баща-хемофилик и майка, носителка на гена за хемофилия**

www.wfh.org, accessed Feb 23, 2017

ХЕМОФИЛИЯ А

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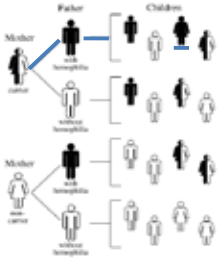
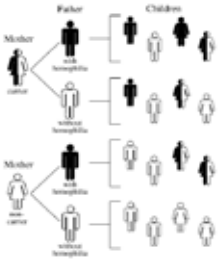


www.wfh.org, accessed Feb 23, 2017

ХЕМОФИЛИЯ А

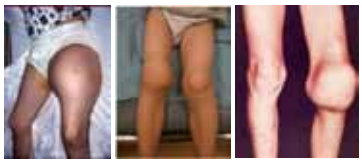
- **генетично заболяване – липса или недостатъчен синтез на коагулационен фактор VIII (FVIII)**
- **честота – 1 на 5 000 до 1 на 10 000 мъжко население; над 400 000 болни**
- **мутация в гена на фактор VIII, разположен на половата Х хромозома**
- **класически пример за наследствена болест, предаваща се по полово-рецесивен път**
- **може да възникне спонтанно (30%)**



www.wfh.org, accessed Feb 23, 2017

ХЕМОФИЛИЯ А

- **Клинична характеристика[1]:**
  - **спонтанни кръвоизливи**
  - **продължително кървене**
  - **кръвоизливи в мускули и стави**
  - **хронична хемофилна артропатия**
- **Класификация[2]: три степени на тежест**
- **Лечение: заместително приложение на концентрат на FVIII:**
  - **„при нужда“**
  - **профилактика**
- **Най-сериозното усложнение: неутрализиращи антитела срещу FVIII (инхибитори срещу FVIII)**



Степен на тежест	Активност на FVIII (%)	Честота на кръвоизливи	Причини за кръвоизлив
Тежка хемофилия	< 1%	24-48	спонтанни
Средно тежка хемофилия	1- ≤ 5%	4-6	малки травми
Лека хемофилия	> 5% - < 40%	много рядко	големи травми/хирургия

[2]Таблицата е адаптирана от White, G C et al. Thromb Haemost 2001; 85: 560

Pictures from: http://www.stepwards.com/?page_id=956
https://www.slideshare.net/csnvittal/hemophilia-34850996
http://us.bp.api.bmj.com/best-practice/monograph/468/resources/image/bp/3.html?locale=en_GB

[1] www.wfh.org, accessed Feb 23, 2017. [2] White, G C et al. Thromb Haemost 2001; 85: 560

ХЕМОФИЛИЯ А

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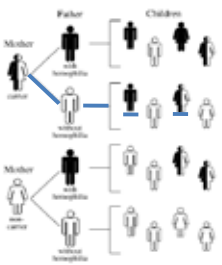

www.wfh.org, accessed Feb 23, 2017

ФАКТОРИ, ДОПРИНАСЯЩИ ЗА ПОЯВА НА КРЪВОИЗЛИВИ В СТАВИ И ХЕМОФИЛНА АРТРОПАТИЯ

[1] Soreff J et al. *Acta Paediatr Scand.* 1980; 69: 667-73. [2] Valentino LA. *Haemophilia.* 2014;20:607-15. [3] Soucie M et al. Blood 2004; 103: 2467-73. [4] Gringeri A et al. *Hemophilia.* 2014; 20:459-63. [5] Srivastava A et al. *Haemophilia.* 2013; 19: e1-47. [6] Valentino L et al. *Haemophilia.* 2007;13(Suppl.3):10-3. [7] Acharya SS et al. *Blood.* 2011; 117: 2484-93. [8] Carcao M, Srivastava A. *Semin Hematol.* 2016; 53: 3-9

ЦЕЛТА Е ПОСТИГАНЕ НА НУЛЕВО КЪРВЕНЕ

Намаляване честотата на кървене

Общ брой на ставни кръвоизливи		≥ 4	3	0–2
Оценка на ставите (score)	Физик. изсл.	3–7	0–2	0
	Рентген	7–12	0–3	0
	МРТ	3–8	2	0

Ставно здраве

тежки увреждания

средно тежки увреждания

нормални стави

Последици за пациента

C-APROM/BG//0018; DOP: 09/2017

Funk M et al. *Haemophilia*. 2002; 8: 98-103.

ПРОФИЛАКТИКА

РЕЖИМИ:

- **Стандартен (високодозов, шведски)[1]:**
  20-40 E/kg FVIII, 3 x седм./през ден
- **Интермедиерен (холандски)[2]:**
  15-25 E/kg FVIII, 2-3 x седм.
- **Дозово-ескалиращ (канадски)[3]:**
  15-25 E/kg FVIII веднъж седм.; ескалиране до 30 E/kg FVIII 2 x седм;
  25 E/kr FVIII 3 x седм./през ден

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[1] Nilsson IM, et al. *J Intern Med* 1992; 232: 25-32. [2] Van den Berg HM, et al. *Br J Haematol* 2001; 115: 561-565
[3] Feldman BM, et al. J Thromb Haemost 2006; 4: 1228–36.

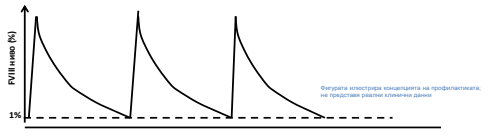
ПРОФИЛАКТИКА

ДЕФИНИЦИЯ[1]

- **Редовни инфузии на коагулационен фактор за поддържане на постоянно минимално ефективно ниво на дефицитния фактор с цел предотвратяване:**
  - кръвоизливи с последваща инвалидизираща артропатия или
  потенциално животозастрашаващо кървене

ИСТОРИЯ[2]

- **1958 г. в Малмьо, Швеция**
- **наблюдение на проф. Инга Нилсън:**
  пациенти със средна форма на хемофилия (FVIII 1-5%) имат много по-малко ставни увреждания
- **хипотеза:**
  трансформирането на болестта от тежка в средна форма чрез профилактично прилагане на коагулационен концентрат ще намали честотата на ставните увреждания
- **минималните прагови нива на FVIII за профилактика: ≥ 1% (1 IU/dL)**



Фигурата илюстрира концепцията на профилактиката; не представя реални клинични данни

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[1] https://www.wfh.org/en/abd/prophylaxis/what-is-prophylaxis. [2] Nilsson IM, et al. *J Intern Med* 1992; 232: 25-32

ПРОФИЛАКТИКА

ПРОФИЛАКТИКА ПРИ ДЕЦА С ХЕМОФИЛИЯ:
рандомизирани проучвания

- ***Copola A, et al.*** **Prophylaxis in people with haemophilia. *Thromb Haemost* 2009; 101 (4): 674-81.**
- ***Blanchette S.*** **Prophylaxis in the haemophilia population. *Haemophilia* 2010; Suppl 5: 181-8.**
- ***Ljung R.*** **Prophylactic therapy in haemophilia. *Blood Rev* 2009; 23 (6): 267-74.**
- ***Manco-Johnson MJ, et al.*** **Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; 357 (6): 535-44.**
- ***Gringeri A, et al.*** **A randomized clinical trial of prophylaxis in children with hemophilia A (the ESPRIT Study). *J Thromb Haemost* 2011; 9 (4): 700-10.**

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ПРОФИЛАКТИКА

ПРОФИЛАКТИКА ПРИ ДЕЦА:

- **50 години в Европа и САЩ**
- **първи избор на лечение по препоръка на СЗО и Световната федерация по хемофилия (СФХ)[1, 2]**
- **цел:**
  -- намаляване на епизодите на кървене
  -- предпазване от хемофилна артропатия
  -- подобряване на качеството на живот и социално благополучие[3-6]

ПРОФИЛАКТИКА ПРИ ВЪЗРАСТНИ:

- **цел:**
  -- намаляване на прогресията на хемофилна артропатия
  -- подобряване на мобилността
  -- подобряване на качеството на живот и социална реализация[7, 8]

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[1] NHF. *MASAC Recommendation Concerning Prophilaxis* 2012; http://www.hemophilia.org; [2] WFH. *Guidelines for the Management of Hemophilia* 2008; http://www.wfh.org ; [3] Berntorp E, et al. *Bull World Health Organ* 1995; 73: 691; [4] Petrini P, et al. *Am J Pediatr Hematol Oncol* 1991;13:280; [5] Royal S, et al. *Haemophilia* 2002;78:44; [6] Fischer K, *et al. Haemophilia* 2003;9:376; [7] Schobess R, et al. *Thromb Haemost* 2008;99:71; [8] Tagliaferri A, et al. *Haemophilia* 2008;14:945

ПРОФИЛАКТИКА

ПРОФИЛАКТИКА ПРИ ДЕЦА С ХЕМОФИЛИЯ:
рандомизирани проучвания

Редовната профилактика при деца, започнала в ранна възраст:

- **По-малко хемартрози**
  По-малко артропатия
- **По-малко мускулни кръвоизливи**
  По-малък риск от церебрално кървене
- **По-малко хоспитализации**
  По-малко активно наблюдение
- **По-малко отсъствие от училище и работа**
  По-малко ортопедични операции
  По-добро качество на живот

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ПРОФИЛАКТИКА

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[1] Nilsson IM, et al. *J Intern Med* 1992; 232: 25-32. [2] Van den Berg HM, et al. *Br J Haematol* 2001; 115: 561-565

ПРОФИЛАКТИКА

ПРОФИЛАКТИКА ПРИ ВЪЗРАСТНИ С ХЕМОФИЛИЯ:
мащабни проучвания

- ***Aledort L, et al.*** **A longitudinal study of orthopaedic outcomes for severe factor-VIII deficient haemophiliacs. The Orthopaedic Outcome Study Group. *J Intern Med* 1994; 236: 391-9.**
- ***Collins P, et al.*** **Efficacy and safety of secondary prophylactic vs. on-demand sucrose-formulated recombinant factor VIII treatment in adults with severe hemophilia A: results from a 13-month crossover *study. J Thromb Haemost* 2010 Jan; 8 (1): 83-89.**
- ***Manco-Jojnon MJ, et al.*** **Randomized, controlled, parallel-group trial of routine prophylaxis vs. on-demand treatment with sucrose-formulated recombinant factor VIII in adults with severe hemophilia A (SPINART). *J Thromb Haemost* 2013; 11 (6): 1119-1127.**
- ***Noone D, et al.*** **A survey of the outcome of prophylaxis, on-demand treatment or combined treatment in 18-359year old men with severe haemophilia in six countries. *Haemophilia* 2013; 19: 44-50.**
- ***Valentino LA, Mamonov V, Hellmann A, et al.*** **A randomized comparison of two prophylaxis regimens and a paired comparison of on-demand and prophylaxis treatments in hemophilia A management. *J Thromb Haemost* 2012; 10: 359–67.**

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ПРОФИЛАКТИКА

ПРОФИЛАКТИКА ПРИ ВЪЗРАСТНИ С ХЕМОФИЛИЯ: мащабни проучвания

Профилактиката при възрастни:

- **"[…] Резултира с по-добри ортопедични резултати и забавя прогресията на артропатия в сравнение с "лечение при нужда"**
- **" В интервал от 6 месеца драматично редуцира броя на ставни кръвоизливи"**
- **"[…] Води до сигнификантна редукция на кървенето в сравнение с "лечение при нужда"**
- **" Качеството на живот е стабилно по-високо в сравнение "лечение при нужда"**
- **възраст 7-59 години: "[…] Сигнификантно редуцира честотата на кървене в сравнение с "лечение при нужда."**

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ПЕРСОНАЛИЗИРАНЕ НА ПРОФИЛАКТИКАТА

"one size fits all"?

[1] Valentino LA. *Haemophilia* 2014; 20: 607–615; [2] Khair K. *Haemophilia* (2014), 20, 601–603; [3] Carcao M and Srivastava A. *Semin Hematol*. 2016; 53 (1):3-9 ;[4]Carcao M and Iorio A. *Semin Thromb Hemost* 2015; 41(8):864-71; [5] Ar MC et al. *Expert Rev Hematol* 2016; 9 (12): 1203-1208; [6] Margaglione M et al. *Haematologica* 2008; 93 (5): 722-8..

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ПРОФИЛАКТИКА

- **Въпреки профилактиката, пациентите все още получават кръвоизливи (> 50% вкл. и в клинични проучвания)[1,5,6]**
- **Праговото ниво на FVIII 1% не е критичния праг, над който могат да бъдат предотвратени всички кръвоизливи или под който неизбежно се появяват кръвоизливи[4]**
- **Различни проучвания:**
  - **пациенти с различен начин на живот се нуждаят от различни прагови нива на FVIII[1]**
  - **пациенти с поддържани прицелни прагови нива > 1% продължават да имат кръвоизливи[1]**
  - **пациенти изискват минимални прагови нива над FVIII > 3% (дори над 15%)[2]**
  - **пациенти със средно тежка хемофилия А също имат кръвоизливи, които се нуждаят от профилактика[1]**
  - **10-15% от пациентите с прагово ниво < 1% имат много рядко кръвоизливи[3, 7]**

[1] Valentino LA. Haemophilia (2014), 20, 607–615. [2] Den Uijl, IEM et al. Haemophilia (2011)17(6): 849-853. [3] Aledort LM, et al. J Intern Med (1994); 236, 391–399. [4] Valentino LA et al. *J Thromb Haemost* 2012; 10 (3): 359–67. [5] Jiménez-Yuste V et al. *Blood Transfus* 2014; 12 (3): 314–19. [6] Mahlangu. *Blood* 2014. [7] Jayandharan GR, et al. Semin Thromb Hemost 2008; 34 (1): 128-141.

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ПЕРСОНАЛИЗИРАНЕ НА ПРОФИЛАКТИКАТА

[1] Ar MC, Vaide I, Berntorp E, et al. Methods for individualising factor VIII dosing in prophylaxis. *Eur J Haematol* 2014; 93 (Suppl 76): 16–20.

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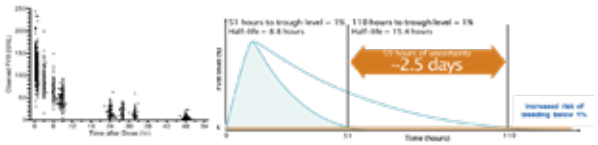
ПРОФИЛАКТИКА

Въпреки профилактиката, пациентите все още получават кръвоизливи (> 50% вкл. и в клинични проучвания)[1, 2, 3]

- **Различна фармакокинетика (ФК) на FVIII при различните индивиди[4, 5]**



[1] Valentino LA et al. *J Thromb Haemost* 2012;10(3):359–67. 2. Jiménez-Yuste V et al. *Blood Transfus* 2014;12(3):314–19. 3. Mahlangu. *Blood* 2014. [4] Björkman S et al. *Blood*. 2012;119(2):612-618.. [5] Collins PW et al. *Haemophilia*. 2011;17(1):2-10.

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ПЕРСОНАЛИЗИРАНЕ НА ПРОФИЛАКТИКАТА

2001 [1] Petrini P. What factors should influence the dosage and interval of prophylacti treatment in patients with severe haemophilia A and B? *Haemophilia* 2001; 7: 99–102

2006 [2] Feldman BM, et al. Tailored prophylaxis in severe hemophilia A: interim results from the first 5 years of the Canadian hemophilia primary prophylaxis study. *J Thromb Haemost* 2006; 4: 1228–1236

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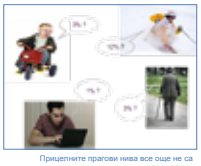
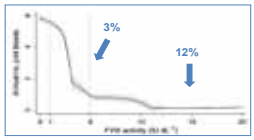
ПРОФИЛАКТИКА

Въпреки профилактиката, пациентите все още получават кръвоизливи (> 50% вкл. и в клинични проучвания)[1, 2, 3]

- **Различната активност и други индивидуални особености на пациента, които изискват различни най-ниски прагови нива на FVIII: начин на живот, професионални изисквания, коморбидност и др.[1, 6]**



Прицелните прагови нива все още не са определени



[1] Valentino LA et al. *J Thromb Haemost* 2012;10(3):359–67. 2. Jiménez-Yuste V et al. *Blood Transfus* 2014;12(3):314–19. 3. Mahlangu. *Blood* 2014. [4] Björkman S et al. *Blood*. 2012;119(2):612-618.. [5] Collins PW et al. *Haemophilia*. 2011;17(1):2-10. [6] Den Uijl, IEM et al. *Haemophilia* 2011; 17 (6): 849-853.

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2006 [2] Feldman BM, et al. Tailored prophylaxis in severe hemophilia A: interim results from the first 5 years of the Canadian hemophilia primary prophylaxis study. *J Thromb Haemost* 2006; 4: 1228–1236

[3] Hilliard P, Zourikian N, Blanchette V, et al. *J Thromb Haemost* 2013; 11: 460–466.

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ПЕРСОНАЛИЗИРАНЕ НА ПРОФИЛАКТИКАТА

[1] Carlsson M, et al. Pharmacokinetic dosing in prophylactic treatment of haemophilia A. *Eur J Haematol* 1993; 51: 247 — 1993

[2] Carlsson M, et al. Improved cost-effectiveness by pharmacokinetic dosing of factor VIII in prophylactic treatment of haemophilia A. *Haemophilia* 1997; 3: 96 — 1997

Оптимизиране на дозирането

Дозиране в зависимост от ФК

- подход за спестяване на разходи[1]
- 30% намаление на 6-месечна консумация на FVIII без увеличение на броя на кръвоизливите[2]

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ПЕРСОНАЛИЗИРАНЕ НА ПРОФИЛАКТИКАТА

Modified from Iannazzo et al 2016

Оптимизиране на дозирането

- **ФК-базираната профилактика е за предпочитане пред стандартния режим от 30 IU/kg с по-ниска СГЧК и по-ниска цена[1]**
- **Генерира спестяване от около 5 000 € за пациент на година и нарастване на съотношението разходи/ ефективност с приблизително 30 000 € за избегнат кръвоизлив[1]**

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[1] Iannazzo S et al. Blood Coagulation and Fibrinol. ysis 2016. Epub ahead of print.

ПЕРСОНАЛИЗИРАНЕ НА ПРОФИЛАКТИКАТА

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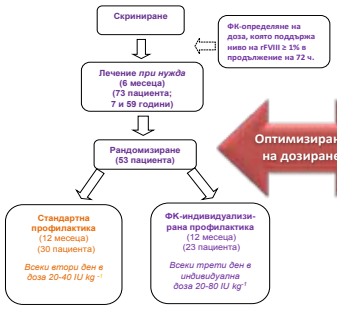

Оптимизиране на дозирането

Многоцентрово, рандомизирано, проспективно клинично проучване за сравнение на безопасността и ефикасността на три терапевтични режима с *rFVIII*:
- лечение *при нужда*,
- стандартна профилактика и
- **ФК-индивидуализирана профилактика**

Modified from Valentino et al 2012

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ПЕРСОНАЛИЗИРАНЕ НА ПРОФИЛАКТИКАТА

Modified from Iannazzo et al 2016

Оптимизиране на дозирането

Заключение

ФК-базирана профилактика, отчитаща индивидуалната ФК вариабилност на пациента, е обещаваща стратегия за подобряване на резултатите с ефикасно използване на наличните ресурси при пациенти с тежка хемофилия А [1, 2]

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[1] Iannazzo S et al. Blood Coagulation and Fibrinol. ysis 2016. Epub ahead of print. [2] Gringeri A et al. *Haemophilia* 2016; 22 (Suppl 4): 37.

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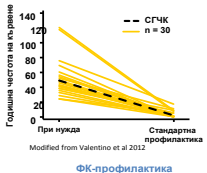
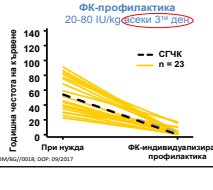
Стандартна профилактика
20-40 IU/kg всеки 2ри ден



Modified from Valentino et al 2012

ФК-профилактика
20-80 IU/kg всеки 3ти ден



Оптимизиране на дозирането

Едногодишна профилактика с *rFVIII*:

- **41.5% (22/53) от пациентите нямат нито един епизод на кръвене (42% за стандартна и 40% за ФК-базирана)**
- **Средната годишна честота на кръвене СГЧК е намалена от 44 на 1 кръвоизлив – 98% намален риск за кървене**

Modified from Valentino et al 2012

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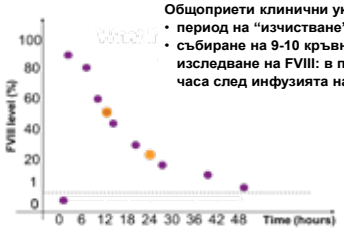
Създаването на индивидуална ФК-крива за FVIII е ограничено от практически затруднения

Общоприети клинични указания за ФК[1-5]:

- **период на "изчистване"**
- **събиране на 9-10 кръвни проби за изследване на FVIII: в продължение на 48 часа след инфузията на проучваната доза**



Графиката е илюстративна и не е базирана на резултати от клинични проучвания

[1] Björkman S. *Haemophilia* 2010; 16 (4): 597-605; [2] Morfini M, Lee M, Messori A. The design and analysis of half-life and recovery studies for factor VIII and factor IX. *Thrombos Haemostas* 1991; 66: 384–6. [3] Morfini M. Comparative pharmacokinetic studies in haemophilia. *Haemophilia* 2002; 2: 30–3. [4] Lee M, Morfini M, Negrier C, Chamouard V. The pharmacokinetics of coagulation factors. *Haemophilia* 2006; 3: 1–7. [5] M. Lee, M. Morfini, S. Schulman, J. Ingerslev and the Factor VIII/Factor IX Scientific and Standardization Committee of the International Society for Thrombosis and Haemostasis https://c.ymcdn.com/sites/www.isth.org/resource/group/d4a6f49a-f4ec-450f-9e0f-7be9f0c2ab2e/official_communications/fviiipharmaco.pdf

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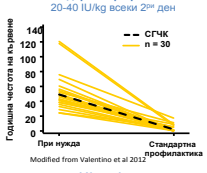
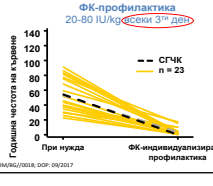
Стандартна профилактика
20-40 IU/kg всеки 2ри ден



Modified from Valentino et al 2012

ФК-профилактика
20-80 IU/kg всеки 3ти ден



Оптимизиране на дозирането

Заключение

ФК-базираната профилактика всеки трети ден е алтернатива на стандартната профилактика по отношение на превенция на кръвоизливи

Modified from Valentino et al 2012

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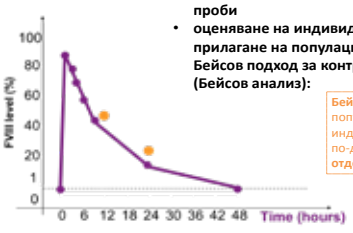
Преодоляване на затрудненията, свързани със създаване на индивидуална ФК-крива за FVIII

Препятствията могат да бъдат преодолени[1-4]:

- **редуциране на броя на вземаните кръвни проби**
- **оценяване на индивидуалната ФК на FVIII чрез прилагане на популационен ФК-модел и Бейсов подход за контрол на дозовия режим (Бейсов анализ):**

Бейсовият подход взема предвид популационния профил и индивидуалните характеристики за по-добро **предсказване на ФК за отделния пациент**.



Фигурата е илюстративна графика не представя реални клинични данни

[1] Björkman S. *Haemophilia* 2010; 16: 597–605. [2] Björkman S. *Haemophilia* 2011; 17: e239–e240. [3] Björkman S, et al. *Blood* 2012; 119: 612–618. [4] Björkman S, Collins P. *J Thromb Haemost* 2013; 11 (1): 180–182.

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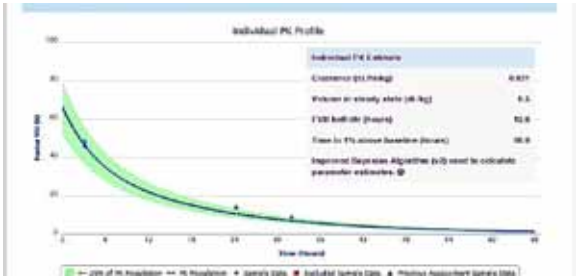
ТРАДИЦИОННА И ПОПУЛАЦИОННА ФАРМАКОКИНЕТИКА

- **Традиционна ФК**
  - **Оценяване на ФК параметри на пациента чрез обичайно измерване на лекарствени нива (обикновено след приложена доза), без да се прилага популационен модел**
- **Популационна ФК**
  - **Определяне на лекарствен дозов режим на базата на оценени ФК-параметри на пациента, адаптирани към индивидуалните му характеристики (напр. тегло, възраст, пол, серумен креатинин)**

C-APROM/BG/0018; DOP: 09/2017

[1] Bayesian principles in pharmacokinetics. http://www.rxkinetics.com/bayes.html. Accessed February 13, 2015

ПЕРСОНАЛИЗИРАНА ПРОФИЛАКТИКА



C-APROM/BG/0018; DOP: 09/2017

[1] myPKFiT User Manual. Version 2.0. Vernon Hills, IL: Baxalta Incorporated; 2016

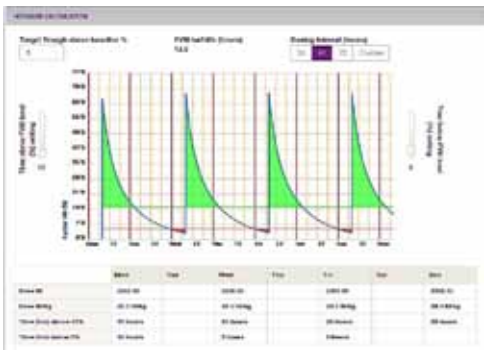
БЕЙСОВ ПОДХОД

- Първо се прилага популационен ФК-модел като начална оценка за всеки индивид (начална прогноза)
- След това тя се коригира въз основа на измерени нива на лекарството при пациента, като се вземат предвид вариабилността на параметрите на популацията и тези на серумното ниво

C-APROM/BG/0018; DOP: 09/2017

[1] Bayesian principles in pharmacokinetics. http://www.rxkinetics.com/bayes.html. Accessed February 13, 2015

ПЕРСОНАЛИЗИРАНА ПРОФИЛАКТИКА



C-APROM/BG/0018; DOP: 09/2017

[1] myPKFiT User Manual. Version 2.0. Vernon Hills, IL: Baxalta Incorporated; 2016

ПЕРСОНАЛИЗИРАНА ПРОФИЛАКТИКА

Медицински софтуер за персонализирана профилактика с rFVIII при пациенти с ХА

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[1] myPKFiT User Manual. Version 2.0. Vernon Hills, IL: Baxalta Incorporated; 2016

ПОСЛАНИЯ ЗА ВКЪЩИ

- **Артропатията е едно от най-сериозните усложнения при тежка хемофилия и може да бъде предотвратена или забавена чрез профилактично прилагане на коагулационен фактор.**
- **Профилактиката е стандарт за терапевтично поведение при тежка хемофилия, а стандартните профилактични режими са базирани на килограм т.м. и се прилагат два или три пъти седмично в доза 20-40 IU/kg.**
- **Въпреки стандартната профилактика пациентите получават кръвоизливи.**
- **Съвременната профилактика изисква персонализиран подход, включващ оценка на комплекс от параметри, най-важните от които са: възраст, фенотип на кървене, индивидуална фармакокинетика, ставно здраве, съпътстващи заболявания и придържане към терапията.**
- **Персонализирана профилактика се дефинира като индивидуализирана заместителна терапия с коагулационен фактор според реалните нужди и очаквания на пациента и се определя чрез персоналния модел на кървене и индивидуална фармакокинетика.**
- **Ефективността на персонализираната профилактика може да бъде повишена чрез употреба на електронни медицински устройства.**

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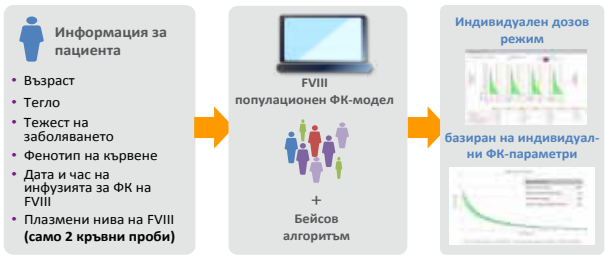
ПЕРСОНАЛИЗИРАНА ПРОФИЛАКТИКА



C-APROM/BG/0018; DOP: 09/2017

[1] myPKFiT User Manual. Version 2.0. Vernon Hills, IL: Baxalta Incorporated; 2016

ADVATE® 500 IU powder and solvent for solution for injection octocog alfa (recombinant human coagulation factor VIII)

- **Лекарственият продукт се отпуска по лекарско предписание**
- **Последно актуализирана КХП[1] е на разположение в заседателната зала или може да бъде намерена на: http://www.ema.europa.eu/docs/bg_BG/document_library/EPAR_-_Product_Information/human/000520/WC500022467.pdf**
- **Дата на последна актуализация: 08/07/2015**
- **Притежател на разрешението за употреба: Baxter AG, Vienna, Австрия**
- **Адрес, на който медицинските специалисти могат да получат пълна информация:** Баксалта България, ул. «Съборна» 2А, ет.2, 1000 София, тел.: 02/926 43 48, факс: 02/926 43 50
- **Съобщаването на подозирани нежелани реакции след разрешаване на употреба на лекарствения продукт е важно. Това позволява да продължи наблюдението на съотношението полза/риск за лекарствения продукт. От медицинските специалисти се изисква да съобщават всяка подозирана нежелана реакция.**
- **Моля, съобщете всяка информация за подозирана нежелана реакция на местния представител на ПРУ,** pharmacovig@bda.bg **или изпратете на: drugsafety@shire.com.**
- **Преди да изпишете продукта, моля прочетете последната одобрена кратка характеристика на продукта**

Advate® ((octocog alfa) EU Summary of Product Characteristics. July 2015

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ADVATE 500 IU прах и разтворител за инжекционен разтвор Съкратена кратка характеристика на продукта[1]

КАЧЕСТВЕН И КОЛИЧЕСТВЕН СЪСТАВ Всеки флакон съдържа номинално количество 500 IU човешки коагулационен фактор VIII (р-ДНК), октоког алфа (octocog alfa). След разтваряне всеки ml инжекционен разтвор ADVATE съдържа приблизително 250 IU човешки коагулационен фактор VIII (р-ДНК), октоког алфа. Октоког алфа (човешки коагулационен фактор VIII (р-ДНК)) е пречистен протеин от 2332 аминокиселини. Той е получен по рекомбинантна ДНК технология в овариални клетки на Китайски хамстер (CHO). Приготвен е без прибавяне на каввито и да са протеини от човешки или животински произход (екзогенни) в процеса на клетъчно култивиране, пречистването или производство на крайния продукт. **4.1 Терапевтични показания** Лечение и профилактика на кръвоизливи при пациенти с хемофилия А (вроден дефицит на фактор VIII). ADVATE е показан при всички възрастови групи. **4.2 Дозировка и начин на приложение** Дозировка Дозата и продължителността на заместителната терапия зависят от тежестта на дефицита на фактор VIII, локализацията и степента на кръвоизлива, както и от клиничното състояние на пациента. *Лечение при необходимост* Изчисляването на необходимата доза на фактор VIII се прави въз основа на емпирично правило, че 1 IU фактор VIII на kg телесна маса повишава плазмената активност на фактор VIII с 2 IU/dl. Необходимата доза се определя по следната формула: Необходим брой единици (IU) = телесно тегло (kg) x желаното повишаване на фактор VIII (%) x 0,5. Дозата и честотата на въвеждане трябва да са съобразени с клиничното проявения ефект при всеки отделен пациент. При определени обстоятелства (напр. наличие на инхибитор в ниски титър) може да са необходими по-високи от изчислените по формулата дози. По време на лечението се препоръчва по подходящ начин да се определя плазменото ниво на фактор VIII, което да служи при определяне на дозата, която ще се прилага, и на честотата на повтаряне на инжекцията. Особено в случаи на големи хирургични операции, прецизният контрол на заместителната терапия чрез определяне на плазмената активност на фактор VIII 95 е задължителен. Ефектът на фактор VIII може да показва индивидуални различия между отделните пациенти, тъй като се постигат различни нива на възстановяване *in vivo* и са установени различни стойности на полуелиминиране. *Профилактика* За дългосрочна профилактика на кръвоизливи при пациенти с тежка форма на хемофилия А, обичайните дози на фактор VIII са 20 до 40 IU на kg телесно тегло през интервали от 2 до 3 дни. *Педиатрична популация* дозирането не се различава от това при възрастните пациенти. За профилактично лечение при пациенти на възраст под 6 години се препоръчват дози от 20 до 50 IU фактор VIII на kg телесно тегло, прилагани 3-4 пъти седмично. Приложението формите от 2 ml не е доказано при педиатрични пациенти на възраст < 2 години. Начин на приложение: ADVATE трябва да се прилага интравенозно. Когато се прилага от лица, които не са медицински специалисти,те трябва да бъдат подходящо обучени. Скоростта на приложение трябва да се определя така, че да не предизвиква неприятни усещания у пациента и да не надвишава 10 ml/min. След разтваряне, разтворът е бистър, безцветен, без видими частици и е с pH от 6,7 до 7,3. **4.3 Противопоказания** Свръхчувствителност към активното вещество или някое от помощните вещества посочени в точка 6.1, или към миши или хамстеров протеин. **4.4 Специални предупреждения и предпазни мерки при употреба** *Свръхчувствителност* При употребата на ADVATE се съобщават алергичен тип реакции на свръхчувствителност. Продуктът съдържа следи от миши и хамстеров протеин. При поява на симптоми на свръхчувствителност, пациентите трябва да бъдат предупредени незабавно да прекратят употребата на продукта и да се свържат с лекаря си. Пациентите трябва да бъдат информирани за ранните симптоми на реакциите на свръхчувствителност, които включват уртики, генерализирана уртикария, стягане в гърдите, задух, хипотония и анафилаксия. В случай на анафилактичен шок, трябва да се започне стандартно лечение на шока. Поради намаляването на инжекционния обем на ADVATE, разтворен в 2 ml стерилизирана вода за инжекции, при проява на реакции на свръхчувствителност има по-малко време за реакция чрез спиране на инжектирането. Затова се препоръчва повишено внимание по време на инжектирането на ADVATE, разтворен в 2 ml стерилизирана вода за инжекции, особено при деца. *Инхибитори* Образуването на неутрализиращи антитела (инхибитори) срещу фактор VIII е познато усложнение при лечението на лица, страдащи от хемофилия А. Тези инхибитори обикновено са имуноглобулини IgG, насочени срещу прокоагулантната активност на фактор VIII, и се определят количествено в Бетезда единици (BU) на ml плазма, като се използва модифицираният тест. При пациентите развитието на инхибитори на фактор VIII може да се прояви като недостатъчен клиничен отговор.

Advate® ((octocog alfa) EU Summary of Product Characteristics. July 2015

ADVATE 500 IU прах и разтворител за инжекционен разтвор Съкратена кратка характеристика на продукта[1]

В тези случаи се препоръчва да се потърси помощта на специализирани центрове за лечение на хемофилия. Рискът от развитие на инхибитори зависи от степента на експозиция на фактор VIII, като този риск е най-висок през 96 първите 20 дни на експозиция, а също и от други генетични фактори и фактори на средата. В редки случаи, инхибитори може да се образуват и след първите 100 дни на експозиция. Случаи на повторно развитие на инхибитори (ниськ-титър) след повече от 100 дни експозиция са били наблюдавани при пациенти, след преминаване от един продукт, съдържащ рекомбинантен фактор VIII към друг и с миналата анамнеза за развитие на инхибитори. Поради това се препоръчва да се следят внимателно всички пациенти за поява на инхибитор след всяка смяна на продукта. Най-общо, всички пациенти лекувани с коагулационен фактор VIII трябва внимателно да се следят за развитието на инхибитори, посредством подходящо клинично наблюдение и лабораторни тестове. Ако не бъдат достигнати очакваните нива на плазмена активност на фактор VIII, или ако кървенето не се контролира с подходяща доза, трябва да се проведе тест за наличие на инхибитор на фактор VIII. При пациенти с високи нива на инхибитор, заместващото лечение с фактор VIII може да не е ефективно и трябва да бъдат обсъдени други варианти на лечение. Лечението на подобни пациенти трябва да се води от лекари с опит в грижите за пациенти с хемофилия и инхибитори на фактор VIII. *Неправилно приложение на ADVATE* При ADVATE, разтворен в 2 ml стерилизирана вода за инжекции, неправилното приложение (интраартериално или паравенозно) *може да доведе до лези*, кратковтрайни реакции на мястото на инжектиране, като например посиняване и еритем. **4.6 Фертилитет, бременност и кърмене** Не са провеждани проучвания при животни за влиянието на фактор VIII върху репродукцията. Въз основа на редките случаи на хемофилия А при жени, няма достатъчно опит от употребата на фактор VIII по време на бременност и кърмене. Затова фактор VIII трябва да се прилага по време на бременност и кърмене, само ако е строго показан. **4.8 Нежелани лекарствени реакции** *Резюме на профила на безопасност.* Клинични проучвания с ADVATE включват 418 лица с поне една експозиция на ADVATE, като се съобщават общо 93 нежелани лекарствени реакции (НЛР). НЛР, възникнали с най-голяма честота са развитие на неутрализиращи антитела към фактор VIII (инхибитори), главоболие и висока температура. Реакции на свръхчувствителност или алергични реакции (които могат да включват ангиоедем, парене и щипане на мястото на инфузия, втрисане, зачервяване, генерализирана уртикария, главоболие, копривна треска, хипотония, летаргия, гадене, безпокойство, тахикардия, стягане в гърдите, изтръпване, гадене, задух) са наблюдавани рядко и могат в някои случаи да еволюират до тежка анафилаксия (включително шок). Може да се наблюдава развитие на антитела към протеин от мишка и/или хамстер със съответните реакции на свръхчувствителност. Пациенти с хемофилия А могат да развият неутрализиращи антитела (инхибитори) спрямо фактор VIII. Ако има такива инхибитори, то състоянието ще се прояви като недостатъчен клиничен отговор. В такива случаи се препоръчва свързване със специализиран център по хемофилия. *Резюме на нежеланите реакции Чести (≥ 1/100 до < 1/10):* Нарушения на кръвоносната и лимфна системи - инхибиране на фактор VIII; Нарушения на нервната система – главоболие; Общи нарушения и ефекти на мястото на приложение – фебрилитет. *Нечести (≥ 1/1 000 до < 1/100):* Инфлуенца, ларингит, лимфангит, световъртеж, нарушена пакет, синкоп, тремор, мигрена, дисгезия, възпаление на очите, палпитации, хематом, горещи вълни, бледност, диспнея, диария, коремна болка в горната част, гадене, повръщане,сърбеж, обрив,хиперхидроза, уртикария, периферни отоци, гръдна болка, гръден дискомфорт, втрисане, чувствате се необичайно, хематом на мястото на пункция на съда, намален брой моноцити, понижено ниво на коагулационен фактор VIII, понижен хематокрит, Отклонения в резултатите от лабораторни тестове, усложнения след интервенцията, кръвоизлив след интервенцията, реакции на мястото на интервенция. *Описание на определени нежелани реакции: Развитие на инхибитори* Съобщава се за развитието на инхибитори при лекувани преди това пациенти (PTPs) и при нелекувани преди това пациенти (PUPs). За по-подробна информация направете справка в точки 5.1 (Фармакодинамични свойства) и 4.4 (Специални предупреждения и предпазни мерки при употреба). *HIP: специфични за остатъците от производствения процес* От 229-мата лекувани пациенти, изследвани за наличие на антитела срещу клетъчен протеин от овариални клетки на китайски хамстер (CHO) клетъчен протеин), 3-ма са показали статистически значима тенденция на нарастване на титрите, 4-ма са имали устойчиви пикове или преходни островърхи максимуми, а при един от пациентите са налице и двете, но без проява на каввито и да са други клинични симптоми. От 229-мата лекувани пациенти, изследвани за оценка на антитела срещу миши IgG, 10-на показват статистически значима 99 тенденция на нарастване, 2-ма имат устойчив пик или преходен островрък максимум, а при един се наблюдават и двете.

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ADVATE 500 IU прах и разтворител за инжекционен разтвор Съкратена кратка характеристика на продукта[1]

Четирима от посочените пациенти съобщават за изолирани епизоди на уртикария, сърбеж, обрив и леко повишен брой на еозинофилите в периода на повтарящи се експозиции на проучвания продукт. *Свръхчувствителност* Алергичният тип реакции включват анафилаксия и са били проявени като замайване, парестезии, обрив, зачервяване, подуване на лицето, уртикария и сърбеж. *Педиатрична популация* Освен развитието на инхибитори при нелекуваните до момента педиатрични пациенти (PUP) и свързаните с катетъра усложнения, при клиничните проучвания не са отбелязани възрастово специфични разлики в НЛР. **4.9 Предозиране** Няма съобщения за симптоми на предозиране на рекомбинантния човешки коагулационен фактор VIII. **5.1 Фармакодинамични свойства** Фармакотерапевтична група: антихеморагични средства; коагулационен фактор VIII; АТС код: B02BD02. Комплексът от фактор VIII и фактор на von Willebrand се състои от две молекули (фактор VIII и фактор на von Willebrand), които имат различни физиологични функции. ADVATE съдържа рекомбинантен коагулационен фактор VIII (октоког алфа), гликопротеин, който е биологично еквивалентен на фактор VIII, гликопротеин, който се съдържа в човешката плазма. *Развитие на инхибитор* Имуногенността на ADVATE е оценена при лекувани преди това пациенти. По време на клиничните проучвания с ADVATE при 233 педиатрични и възрастни пациенти [педиатрични пациенти (възраст 0 –16 години) и възрастни пациенти (възраст над 16 години)] с установена тежка форма на хемофилия А (фактор VIII < 1%), с предходящо излагане на концентрати на фактор VIII ≥ 150 дни за възрастните и големите деца и ≥ 50 дни за децата на възраст < 6 години, един пациент е развил инхибитор с ниськ титър (2.4 BU при модифицирания метод на Bethesda) след 26 дни на експозиция на ADVATE. Контролните изследвания за инхибитор при този пациент след излекуването му от проучването са били отрицателни. При всички проучвания, средната експозиция на ADVATE е била 97,0 дни на експозиция на лице (диапазон от 1 до 709) при лекувани преди това пациенти. Общата честота на развитие на каквато и да е инхибитор на фактор VIII (ниськ или висок) е била 0,4% (1 от 233). В завършеното неконтролирано проучване 060103, 16 от 45 (35,6%), от не лекуваните преди това пациенти с тежка форма на хемофилия А (ФVIII< 1%) и с поне 25 експозиции на ФVIII развиват инхибитори срещу ФVIII: 7 (15,6%) лица, развиват висок титър на инхибитори, а 9 (20%) лица развиват ниськ титър на инхибитори, един от които е класифициран като преходен инхибитор. Рисковите фактори свързани с развитието на инхибитори в това проучване включват етнически произход непринадлежащ към бялата раса, фамилна анамнеза за инхибитори и интензивно лечение с високи дози при първите 20 експозиции. При 20-те лица, които не са носители на тези рискови фактори, не се наблюдава развитие на инхибитор. При пациентите с инхибитори са събрани данни относно предизвикването на имунна толерантност (ITI). В хода на подпроучване на PUP проучване 060103 е документирано ITI лечение при 11 PUP. Извършен е ретроспективен преглед на картоните на 30 пациенти на ITI (изпитване 060103), като събирането на данни от регистъра продължава. В проучването 060201 се сравняват две дългосрочни схеми на профилактично лечение при 53 пациенти, лекувани преди това (PTP): индивидуализиран и ръководен от фармакокинетиката режим на дозиране (в диапазон от 20 до 80 IU фактор VIII на kg телесно тегло на интервали от 72 ± 6 часа, n=23) и стандартен профилактичен режим на дозиране (20 до 40 IU/kg на всеки 48 ± 6 часа, n=30). Ръководеният от фармакокинетиката режим на дозиране (по определена формула) цели да подържа най-ниските нива на фактор VIII ≥ 1% при интервал на дозите от 72 часа. Данните на това проучване показват, че двата режима на дозиране са сравними по отношение на намаляване честотата на кървене. **6.2 Несъвместимости** При липса на проучвания за несъвместимости този лекарствен продукт не трябва да се смесва с други лекарствени продукти или разтворители. **6.3 Срок на годност** 2 години. След разтваряне, от микробиологична гледна точка, продуктът трябва да се използва незабавно. Все пак е доказана химическа и физична стабилност в периода на използване за 3 часа при 25 °C на готовия за употреба разтвор. В рамките на срока на годност, този продукт може да се съхранява при стайна температура (до 25 °C) за еднократен период до 6 месеца. Краят на 6-те месеца от датата на преместването му на стайна температура трябва да се отбелязви върху картонената опаковка на продукта. Продуктът не трябва да се прибира обратно в хладилник. **6.4 Специални условия на съхранение** Да се съхранява в хладилник (2 °C до 8 °C). Да не се замразява. ADVATE с приспособление ВАХЈЕСТ II: Флаконът на продукта да се съхранява в оригиналната опаковка, за да се предпази от светлина. **6.6 Специални предпазни мерки при изхвърляне и работа** ADVATE трябва да се прилага интравенозно след разтваряне на продукта. Приготвеният разтвор трябва да се прегледа визуално за видими частици и/или промяна на цвета. След разтваряне разтворът трябва да е бистър, безцветен, без съдържание на видими частици. Не използвайте разтвори, които са мътни или имат утайка.

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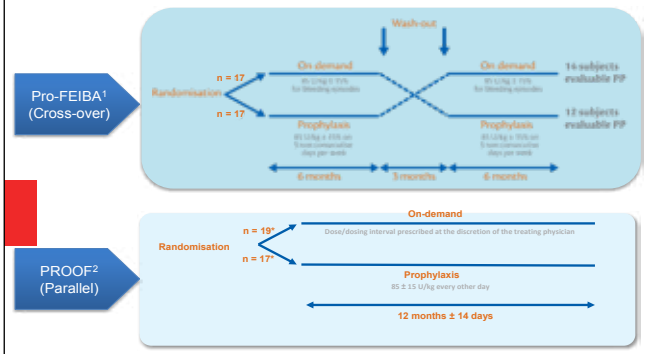
ФЕЙБА (АКТИВНОСТ, ЗАОБИКАЛЯЩА ИНХИБИТОРА НА ФАКТОР VIII)- ОБЗОР НА КЛИНИЧНИТЕ ПРОУЧВАНИЯ

Стефан Горанов

Pro-FEIBA и PROOF дизайн на клиничните проучвания



PP = Per Protocol; *ITT = intent to treat

1. Leissinger C, et al. *N Engl J Med*. 2011;365:1684-1692. 2. Antunes S, et al. Haemophilia. Published online August 1, 2013; DOI:10.1111/hae.12246.

Конфликт на интереси

С настоящото декларирам, че съм получил хонорар от Shire.

Възгледите и мненията, изразени в презентацията са изцяло на автора. Те не трябва да се приемат или цитират като направени от името на компания Shire.

Pro-FEIBA – Резултати от клиничното проучване

Сравнени с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- **лечение при нужда,** ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- **профилактика 85 U/kg ± 15% прилагана 3 непоследователни дни седмично**:

- Редукция на кървене 62% (*P*<0.001)
- Редукция на ставно кървене 61% (*P*<0.001)
- Редукция на кървене в таргетни стави 72% (*P*<0.001)
- Редукция на всякакво кървене при good responders‡ 84% (*P*<0.001)

При 10 от 26 пациенти с<50% редукция в честотата на кървене се наблюдава 28% понижаване на епизодите на кървене (P<0.02)

* Определено като ≥3 haemarthroses в отделна става за 6-месечен период на лечение.

‡ **Good responders:** ≥50% редукция на всякакво кървене по време на FEIBA профилактика сравнено с лечение при нужда.

Leissinger C, et al. *N Engl J Med*. 2011;365:1684-1692.

Опит с FEIBA (Активност, заобикаляща инхибитора на фактор VIII) профилактика

Изследовател и данни за пациентите n=54	Профилактика Продължителност (мес.)	Ефикасност	Безопасност
1.Ettingshausen[1] (n=7) • Възраст: 1.5 – 11.8 години (средно 6 години)	9.6-205.2 (средно: 82.8)	• **Средна годишна честота на кървене 1.5** • Запазена ставна цялост и подобрено качество на живот • Pettersson скала среден резултат 4, сравним с пациентите без инхибитори получаващи профилактика с фактор FVIII	• Не са наблюдавани тромботични усложнения и сериозни странични реакции
2.Valentino[2] (n=6) • 3 с ITI • Възраст: 3.7 – 24.1 години (средно 8.4 години)	1.3-51.0 (средно: 26.9)	• **84% средна редукция на кървене** • Средна годишна честота на кървене по време на профилактика **1.81** • Повечето пациенти са с подобрение в ортопедичния статус и качество на живот	• Не са наблюдавани странични реакции
3.Leissinger[3] (n=5) • 2 с ITI • Възраст: 3 – 16 години (средно 7 години)	6.0-24.0 (средно: 15.0)	• Ортопедичен статус съхранен при 4 пациенти и подобрен при 1 със съществуваща артропатия • **73%-83% редукция на честотата на кървене**	• Не са наблюдавани странични реакции и тромботични усложнения
4.DiMichele and Negrier[4] (n=14) • Възраст: 3 – 61 години (средно 25)	0.25 – 26.0 (средно: 19.5)	• Подобрен или стабилизиран ортопедичен статус при 11 от 13 пациенти (85%)	• Не са наблюдавани тромботични усложнения
5.Kreuz[5] (n=22) • Всички с ITI • Възраст: 0.1 – 6 години	Не е упоменото	• Средната годишна честота на ставно кървене, е била 1 (0-6) • ITI е била успешна при 18/22 пациенти • Радиологична оценка при 8 пациенти; липса на ставно увреждане при 6/8 пациенти	• Не са наблюдавани странични реакции и тромботични усложнения

1. Ettingshausen CE, et al. *Haemophilia*. 2010;16(1):90-100. 2. Valentino LA. *Haemophilia*. 2009;15(3):733-742. 3. Leissinger CA, et al. *Haemophilia*. 2007;13(3):249-255. 4. DiMichele D, et al. *Haemophilia*. 2006;12(4):352-362. Kreuz W, et al. *Blood*. 2000;96 (Suppl):266a. Abstract 1141.

PROOF – Резултати от клиничното проучване

Сравнено с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- **on-demand лечение,** ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- **профилактика 85 ± 15 U/kg през ден:**

- Редуцирана средна годишна честота на кървене 72.5% (*P*=0.0003)
- Редуцирана средна годишна честата на кървене в стави 73.8% (*P*=0.0006)
- Редуцирана средна годишна честата на кървене в нови таргетни стави * 100% (*P*=0.0271)

* ≥4 епизоди на кървене по време на 6-месечен период в една и съща става, която не е била таргетна в началото на проучването

Antunes S, et al. *Haemophilia*. Published online August 1, 2013; DOI:10.1111/hae.12246.

Опит с FEIBA профилактика по време и извън индукция на имунен толеранс

Brackmann - 22
Kreuz - 22
DiMichele - 14
Lambert - 13
Escuriola - 7
Hilgartner - 7
Schino - 7
Ewing - 7
Valentino - 6
Eminez-Yuste- 5

Рандомизирани пациенти – 110
Средно време на профилактика – 21 месеца-
Обощо регистрирани епизоди на кървене преди профилактиката - над 4000 !

Pro-FEIBA проучване: първото рандомизирано клинично проучване за профилактика с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)

Leissinger C, et al. *N Engl J Med* 2011

Pro-FEIBA проучване – преглед

- Инициирано от изследователи, проспективно, рандомизирано, кръстосано проучване, обхващащо 16 центрове за лечение на хемофилия в Европа и САЩ.
- Основна цел /Primary objective/
  - Да се определи дали профилактика с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- понижава съществено епизодите на кървене сравнено с терапия при нужда при пациенти, които са били и на двата режима.

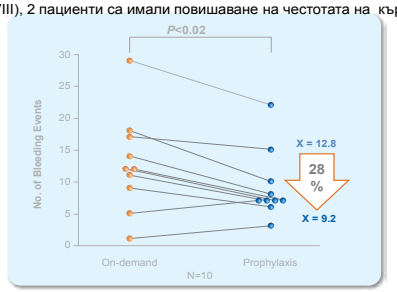
Критерии за включване /Inclusion criteria/

- Пациенти с тежка форма на хемофилия А, с висок титър на инхибитори към фактор FVIII (>5 BU)
- Възраст: >2 години и ≥ 6 епизоди на кървене, изискващи бай-пас терапия 6 месеца преди да бъдат включвани в проучването
- Средна възраст на пациентите: 28.7 години (обхват: 2.8 - 67.9)

Leissinger C, et al. *N Engl J Med.* 2011;365(18):1684-1692.

Намаляване на епизодите на кървене при пациенти с <50% редукция на кръвоизливите

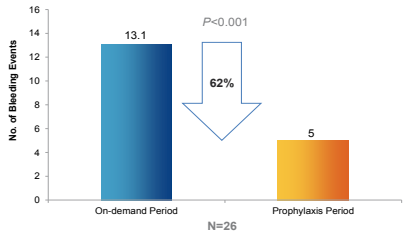
По време на профилактика с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII), 2 пациенти са имали повишаване на честотата на кървене



X = среден брой на епизоди на кървене
N = брой пациенти, завършили и двата режима на проучването

Leissinger C, et al. *N Engl J Med.* 2011;365:1684-1692.

Основна цел /Primary objective/: Намаляване на общия брой епизоди на кървене

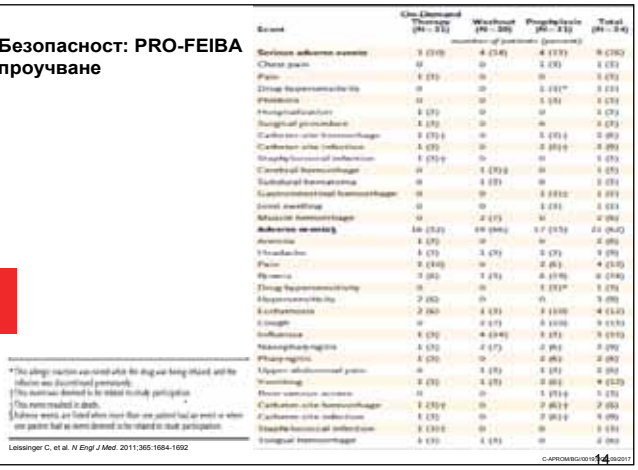


Среден брой на епизодите на кървене –режим профилактика и лечение при нужда

N = общ брой на пациентите завършили лечението на двата терапевтични режима.

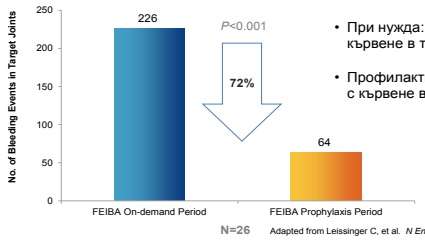
Leissinger C, et al. *N Engl J Med.* 2011;365:1684-1692.

Безопасност: PRO-FEIBA проучване



Leissinger C, et al. *N Engl J Med.* 2011;365:1684-1692

Вторична цел /Secondary Outcome/: Намаляване на епизодите на кървене в таргетни стави



Adapted from Leissinger C, et al. *N Engl J Med.* 2011

- При нужда: 18 пациенти с кървене в таргетни стави
- Профилактикаs: 11 пациенти с кървене в таргетни стави

N = общ брой на пациентите завършили лечението на двата терапевтични режима

Таргетна става: ≥3 haemarthroses в отделна става по време на 6-месечния период на проучването.

Leissinger C, et al. *N Engl J Med.* 2011;365:1684-1692.

Безопасност

Безопасността е оценена при 34 пациенти

Тромбоемболични усложнения не са наблюдавани

Едно сериозно странично действие към изпитваното лекарство;

Алергична реакция към FEIBA

Два смъртни случая несвързани с проучването и изпитваното лекарство

Intracranial haemorrhage (during washout period)

Gastrointestinal haemorrhage (свързан с диабетна кома; по време на профилактичния период)

При трима пациенти (9%) са наблюдавани събития свързани с поставянето на централен венозен катетър, а именно:

Инфекция

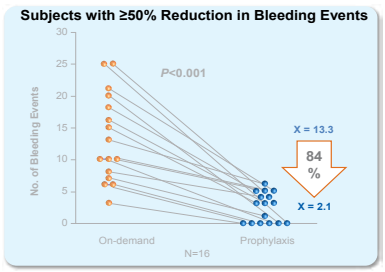
Кървене

Изместване на катетъра и отстраняване

Leissinger C, et al. *N Engl J Med.* 2011;365:1684-1692.

Намаляване на епизодите на кървене при пациентите с добър отговор /Good Responders/

6 (38%) пациенти не са имали никакво кървене по време на профилактичното лечение



X = среден брой епизоди на кървене
N = общ брой пациенти, завършили и двата терапевтични режима

Пациенти с добър отговор /Good responders/ пациенти с ≥50% намаляване на епизодите на кървене на профилактика с FEIBA в сравнение с лечение при нужда

Leissinger C. et al. *N Engl J Med.* 2011;365:1684-1692.

PROOF Study:

Нови доказателства за ефикасността на профилактика с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- Hemophilia, 2014 Jan; 20(1): 65–72., Antunes SV et al

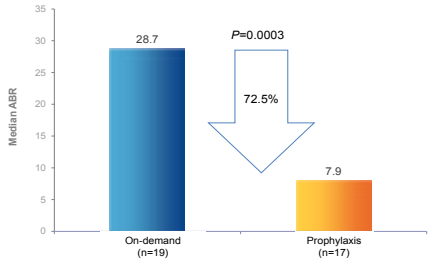
PROOF проучване-преглед

- Фаза 3, проспективно, рандомизирано, мултицентрово, отворено, паралелно проучване в 17 центъра в света
- Основна цел /Primary objective/
  - Намаляване на средната годишна честота на кървене при пациенти на профилактика в сравнение с лечение при нужда по време на 12-месечен период
- Критерии за включване:
  - Haemophilia A или B с висок титър на инхибитори (>5 BU) или нисък титър на инхибитори (≤5 BU) рефрактерни на покачване на дозата на FVIII или FIX за 12-месечен период
  - ≥4 и ≤65 години
  - ≥12 епизода на кървене по време на 12 месечен период преди периода на проучването
  - Включени пациенти /Study population/
  - 36 лица (33 с hemophilia A и 3 с hemophilia B)
  - Средна възраст на пациентите в intent-to-treat (ITT) групата-23.5 години

Hemophilia, 2014 Jan; 20(1): 65–72., Antunes SV et al

Основен резултат /Primary Outcome/: Намаляване на Средната Годишна Честота на Кървене

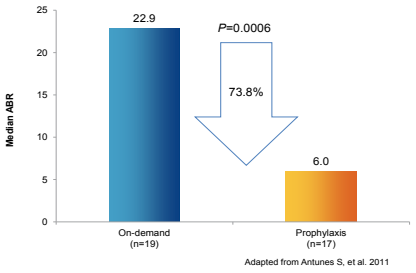
При 3/17 (17.6%) пациенти в intent-to-treat рамо не се наблюдава никакво кървене. Двама от тези 3 пациенти са завършили пълния 12-месечен курс на проучването



Hemophilia, 2014 Jan; 20(1): 65–72., Antunes SV et al

Основен резултат /Primary Outcome/: Намаляване на средната годишна честота на кървене в стави

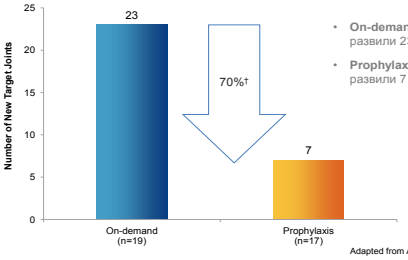
Профилактиката значимо намалява кървенето в стави в сравнение с терапията при нужда



Adapted from Antunes S, et al. 2011

Hemophilia, 2014 Jan; 20(1): 65–72., Antunes SV et al

Вторичен резултат /Secondary Outcome/: Развитие на нови таргетни стави*



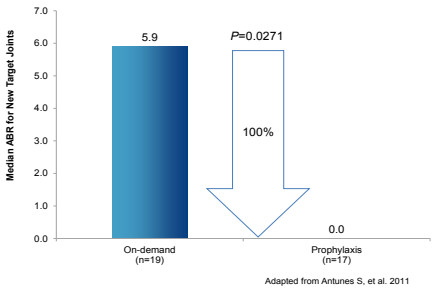
Adapted from Antunes S, et al. 2011

- **On-demand**: 11/19 пациенти са развили 23 нови таргетни стави
- **Prophylaxis**: 5/17 пациенти са развили 7 нови таргетни стави

- Нова таргетна става: ≥4 haemarthroses в отделна става, която не е била засегната в началото на проучването, по време на 6-месечен период
- †Редукцията не е статистически значима.

Hemophilia, 2014 Jan; 20(1): 65–72., Antunes SV et al.

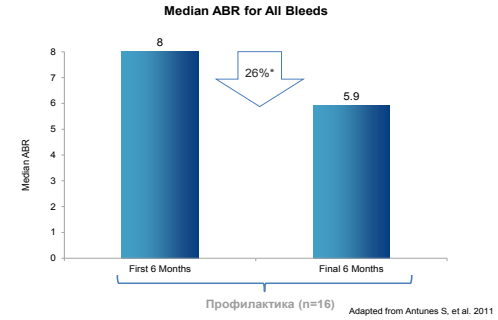
Вторичен резултат /Secondary Outcome/: Намаляване на средната годишна честота на кървене в нови таргетни стави



Adapted from Antunes S, et al. 2011

Hemophilia, 2014 Jan; 20(1): 65–72., Antunes SV et al.

Епизоди на кървене през първите 6 месеца от проучването в сравнение с последните 6 месеца, профилактичен режим

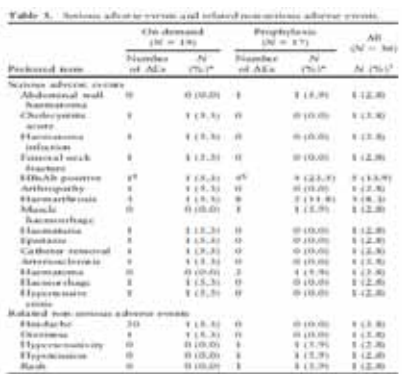


Adapted from Antunes S, et al. 2011

* Редукцията не е статистически значима

Hemophilia, 2014 Jan; 20(1): 65–72., Antunes SV et al.

Безопасност - PROOF проучване



Hemophilia, 2014 Jan; 20(1): 65–72., Antunes SV et al

Безопасност

Безопасността е оценена при 36 пациенти.

Тромбоемболични усложнения не са наблюдавани.

Не са идентифицирани значими проблеми с безопастността.

4 сериозни странични реакции свързани с позитивен HBsAb.

Клинични или лабораторни симптоми/изследвания за активна HBV инфекция не са наблюдавани.

Позитивният HBsAb е най-вероятно в резултат на "пасивен трансфер" от плазма.

1 пациент е прекъснал лечението поради реакция на свръхчувствителност (вероятна връзка).

1 смъртен случай, несвързан с проучването и изпитваното лекарство; свързан с атеросклеротична и хипертонична кардиоваскуларна болест.

HBsAb = hepatitis B surface antibodies; HBV = hepatitis B virus; CVD = cardiovascular disease.

Antunes S, et al. *Haemophilia*. Published online August 1, 2013; DOI:10.1111/hae.12246.

Основни изводи и заключения

- Повтарящото се ставно кървене води до прогресираща ставна болест[1]
- Наличието на инхибитори повишава риска от развитие на трудно за контролиране кървене и артропатия и затова:
  - Предотвратяването или намаляването на епизодите на кървене е от изключителна важност за пациентите с инхибиторна хемофилия.
- Опитът показва, че профилактика с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) може да намали честотата на епизодите на кървене при пациентите с инхибиторна хемофилия. [4-8]
- Резултатите от 2 проспективни, рандомизирани, клинични проучвания дават неоспорими доказателства, че профилактика с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- 3 - 4 пъти седмично:
  - Значимо понижава общата честота на кървене и ставно кървене[9,10]
  - Понижава развитието на нови таргетни стави в сравнение с лечение при нужда[10]
  - Показва тенденция за подобряване на качеството на живот.*[10,11]

*Не е статистически значимо

1. Pergantou H, et al. *Haemophilia*. 2006;12(3):241-247. 2. Kasper CK. World Federation of Hemophilia: 2004. No. 34. 3. Leissinger CA. *Haemophilia*.1999;5 (suppl 3):25-32.
4. Ettingshausen C, et al. *Haemophilia*. 2010;16(1):90-100. 5. Valentino LA. *Haemophilia*. 2009;15(3):733-742. 6. Leissinger, CA. *Haemophilia*. 2007;13(3):249-255.
7. DiMichele D, et al. *Haemophilia*. 2006;12(4):352-362. 8. Kreuz W. et al. *Blood*. 2000;96(Suppl):266a. Abstract 1141. 9. Leissinger C, et al. *N Engl J Med*. 2011;365:1684-1692.
10. Antunes S, et al. *Haemophilia*. Published online August 1, 2013; DOI:10.1111/hae.12246. 11. Gringeri A, et al. Haemophilia. Published online April 14, 2013; DOI:10.1111/hae.12178.

FEIBA NF 500 U, 20 ml powder and solvent for solution for injection

(Factor VIII Inhibitor Bypassing Activity)

Съкратена кратка характеристика на продукта[1]

Активно вещество: Активност, заобикаляща инхибитора на Фактор VIII. Активното вещество, съдържащо се в 1 ml ФЕЙБА е 25 U. Опаковка с 500 U съдържа 500 U активност, заобикаляща инхибитора на Фактор VIII в 200-600 mg човешки плазмен протеин. ФЕЙБА съдържа също и факторите II, IX и X, предимно в неактивирана форма, както и активиран фактор VII. Кръвосъсиряващият антиген на фактор VIII (F VIII C:Ag) присъства в концентрация до 0,1 U/1 U ФЕЙБА. Факторите на каликреин-кининовата система присъстват само в следи или изобщо не се съдържат. 1U ФЕЙБА скъсява активираното парциално тромбопластиново време (аРТТ) на плазма, съдържаща инхибитор на фактор VIII до 50% от изходната стойност (бланкетна стойност). **4.1 Терапевтични показания** Лечение на кръвоизливи при пациенти с хемофилия А с инхибитори. Лечение на кръвоизливи при пациенти с хемофилия В с инхибитори, ако не се предлага друго специфично лечение (вж. точка 5.1). Лечение на кръвоизливи при пациенти без хемофилия с придобити инхибитори срещу фактор VIII. Профилактика на кръвоизливи при пациенти с хемофилия А с инхибитори, които са имали значително кървене или са високо рискови за значителен кръвоизлив. **4.2 Дозировка и начин на приложение** *Дозировка* Дозировката и продължителността на лечението зависят от тежестта на хемостатичното нарушение, локализацията и степента на кръвоизлива и клиничното състояние на пациента. Дозировката и честотата на приложение трябва винаги да се определят според клиничната ефикасност във всеки отделен случай. Както общо правило се препоръчва доза от 50-100 U ФЕЙБА на килограм телесно тегло (т.т.). Еднократна доза от 100 U/kg т.т. и максимална дневна доза от 200 U/kg т.т. не трябва да се превишават, освен ако тежестта на кръвоизлива не дава основание и не налага използването на по-високи дози. Вижте точка 4.4. *Педиатрична употреба (деца)* Опитът при деца на възраст под 6 години е ограничен; същата дозировка като при възрастни трябва да се адаптира към клиничното състояние на детето. **1) Спонтанен кръвоизлив Ставни, мускулни и мекотъканни кръвоизливи** При леки до умерено силни кръвоизливи се препоръчва доза от 50-75 U/kg т.т., приложена през 12-часови интервали. Лечението трябва да продължи до появата на явни признаци за клинично подобрение като отзвучаване на болката, намаляване на отока или раздвижване на ставата. При големи мускулни и мекотъканни кръвоизливи, като например ретроперитонеален кръвоизлив, се препоръчва доза от 100 U/kg т.т., приложена през 12-часови интервали. **Кръвоизливи от лигавици** Препоръчва се доза от 50 U/kg т.т., която се прилага на всеки 6 часа при внимателно мониториране на пациента (визуален контрол на кръвоизлива, повторно определяне на хематокрита). Ако кръвоизливът не се овладява, дозата може да бъде увеличена до 100 U/kg т.т., без да се надвишава максималната дневна доза от 200 U/kg т.т. **Други тежки кръвоизливи** При тежки кръвоизливи, като кръвоизлив в ЦНС, се препоръчва доза от 100 U/kg т.т., приложена през 12-часови интервали. В отделни случаи ФЕЙБА може да се приложи и през интервали от 6 часа до постигане на ясно изразено клинично подобрение. (Да не се надвишава максималната дневна доза от 200 U/kg т.т.). **2) Хирургически операции** При хирургическа интервенция може да се приложи начална доза от 100 U/kg телесно тегло предоперативно, а последваща доза от 50-100 U/kg телесно тегло може да се приложи след 6-12 часа. Като постоперативна поддържаща доза, 50-100 U/kg телесно тегло може да се прилага през интервали от 6-12 часа; дозировката, интервалите на дозиране и продължителността на пери- и постоперативната терапия се определят от хирургическата интервенция, общото състояние на пациента и клиничната ефикасност при всеки отделен случай. (Не трябва да се надвишава максимална дневна доза от 200 U/kg телесно тегло). **3) Профилактика при пациенти с хемофилия А с инхибитори Профилактика на кръвоизливи при пациенти с висок инхибиторен титър и чести кръвоизливи, при които ITI (терапия за имунен толеранс) е неуспешна или не е обсъждана:** препоръчва се доза от 70-100 U/kg т.т. през ден. Ако е необходимо, тази доза може са бъде увеличена до ежедневна доза от 100 U/kg т.т., ако пациента продължава да кърви, или постепенно да бъде намалена. **Профилактика на кръвене при пациенти с висок инхибиторен титър, подлежащи на ITI (терапия за имунен толеранс):** ФЕЙБА може да се прилага едновременно с фактор VIII концентрати, в доза от 50-100 U/kg т.т., двукратно дневно, докато инхибитора на фактор VIII бъде редуциран до <2 B.U. **4) Употреба на ФЕЙБА при специални групи пациенти** Вижте точка 5.1 за информация относно пациенти с хемофилия В с инхибитор на фактор IX. В комбинация с концентрат на фактор VIII, ФЕЙБА е използвана и за продължително лечение за постигане на пълно и постоянно отстраняване на инхибитора на фактор VIII.

Основни изводи и заключения

На базата на клинични проучвания и огромен опит, може да се заключи, че профилактика с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) може да помогне на пациентите с хемофилия с висок титър на инхибитори да нямат кървене:

- При 6 пациенти от Pro-FEIBA проучването не се наблюдава кървене през 6-те месеца профилактика с продукта.[1]
- При 2-ма пациенти от рамото с профилактичен режим в PROOF проучването не се наблюдава кървене през 6-те месеца профилактика с продукта.[1]

•ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) е лекарство с доказана ефикасност:

- ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) е одобрена в >60 страни и е показана за профилактика на пациенти с инхибиторна хемофилия
- Към датата на проучването* няма съобщения за трансмисия на вирусите на hepatitis A, B, и C или HIV дефинитивно свързани с ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)
- Тромбоемболични усложнения не са наблюдавани по време на Pro-FEIBA and PROOF проучванията[1,2]

1. Leissinger C, et al. *N Engl J Med*. 2011;365:1684-1692. 2. Hemophilia, 2014 Jan; 20(1): 65–72., Antunes SV et al

* August 2013

FEIBA NF 500 U, 20 ml powder and solvent for solution for injection

(Factor VIII Inhibitor Bypassing Activity)

- Лекарственият продукт се отпуска по лекарско предписание
- Последно актуализирана КХП може да бъде намерена на: **http://www.bda.bg/images/stories/documents/bdias/2016-11-23-6716.pdf**
- Дата на последна актуализация: 03/11/2016
- Притежател на разрешението за употреба: Baxter AG, Vienna, Австрия

- Адрес, на който медицинските специалисти могат да получат пълна информация: Баксалта България, ул. «Съборна» 2А, ет.2, 1000 София, тел.: 02/926 43 48, факс: 02/926 43 50
- Съобщаването на подозирани нежелани реакции след разрешаване на употреба на лекарственния продукт е важно. Това позволява да продължи наблюдението на съотношението полза/риск за лекарственния продукт. От медицинските специалисти се изисква да съобщават всяка подозирана нежелана реакция.
- **Моля, съобщете всяка информация за подозирана нежелана реакция на Изпълнителна агенция по лекарствата, София, ул. Дамиан Груев 8, тел. +359 2 8903417 Е-мейл :** pharmacoviq@bda.bg, www.bda.bg **и на притежателя на разрешението за употреба на Е-мейл : drugsafety@shire.com..**
- **Преди да изпишете продукта, моля прочетете последната одобрена кратка характеристика на продукта.**

FEIBA NF 500 U, 20 ml powder and solution for injection

(Factor VIII Inhibitor Bypassing Activity)

Съкратена кратка характеристика на продукта[1]

Начин на приложение Пригответе продукта както е описано в точка 6.6 и приложете като бавна интравенозна инфузия. Не трябва да се надвишава скорост на инфузия от 2 U/kg т.т. на минута. **4.3 Противопоказания** В зависимост от лечебните алтернативи, ФЕЙБА не трябва да се използва в следните случаи: Свръхчувствителност към активното вещество или към някое от помощните вещества. Дисеминирана интраваскуларна коагулация (ДИК). Остра тромбоза или емболия (включително миокарден инфаркт). ПРЕДУПРЕЖДЕНИЯ Реакции на свръхчувствителност ФЕЙБА може да предизвика реакции на свръхчувствителност от алергичен тип, които включват уртикария, ангиоедем, стомашно-чревни прояви, бронхоспазъм и хипотония. Тези реакции могат да бъдат тежки и системни (като например анафилаксия с уртикария и ангиоедем, бронхоспазъм и циркулаторен шок). Има съобщения и за други инфузионни реакции като втрисане, гърчове и хипертония. При първата проява или симптом на инфузионна реакция/реакция на свръхчувствителност, приложението на ФЕЙБА трябва да се спре и да се предприемат съответните медицински грижи. Когато се обсъжда повторно приложение на ФЕЙБА при пациенти със съмнение за свръхчувствителност към продукта или към някои от неговите съставки, трябва да бъдат внимателно преценени очакваната полза и риска от повторна експозиция, като се отчитат известните или подозирани типове (алергични или неалергични) свръхчувствителност на пациента, включително и за потенциални лечебни и/или профилактични терапии или алтернативни лекарствени продукти. Тромботични и тромбоемболични събития Тромботични и тромбоемболични събития, включително дисеминирана интраваскуларна коагулация (ДИК), венозна тромбоза, белодробен емболизъм, инфаркт на миокарда и инсулт, са възниквали в хода на лечението с ФЕЙБА. Някои от тези събития възникват при дози над 200 U/kg/ден или при пациенти с други рискови фактори включително ДИК, напреднало перооперативно заболяване, травма със смазване или асептичемия) за тромбоемболични събития. Съпровождащото лечение с рекомбинантен Фактор VIIa може да повиши риска от развитие на тромбоемболично събитие. Рискът от тромботични и тромбоемболични събития може да се увеличи при високи дози ФЕЙБА. ФЕЙБА трябва да се прилага с особено внимание и само при липсата на терапевтични алтернативи при пациенти с повишен риск за тромбоемболични усложнения. Това включва, но не се ограничава до пациенти с анамнеза за коронарна болест на сърцето, чернодробно заболяване, ДИК, артериална или венозна тромбоза, следоперативно обездвижване, пациенти в напреднала възраст и новородени. При първите прояви или симптоми на тромбоемболични събития, инфузията трябва да бъде преустановена незабавно и да се предприемат необходимите диагностични и терапевтични мерки. Единична доза от 100 U/kg телесно тегло и дневна доза от 200 U/kg телесно тегло не трябва да се надвишават, освен ако тежестта на кръвенето не го налага и обосновава използването на по-високи дози. Значение на броя на тромбоцитите В случай на неадекватен отговор към лечението с ФЕЙБА се препоръчва да бъде изследван броя на тромбоцитите, тъй като за ефективното действие на ФЕЙБА са необходими достатъчно високи broй функционално интактни тромбоцити.

FEIBA NF 500 U, 20 ml powder and solution for injection

(Factor VIII Inhibitor Bypassing Activity)

Съкратена кратка характеристика на продукта[1]

ПРЕДПАЗНИ МЕРКИ Тромботични и тромбоемболични усложнения При следните ситуации, ФЕЙБА трябва да се прилага само, ако не може да се очаква реакция към лечението с подходящи концентрати на фактор на кръвосъсиряването, като например в случай на висок инхибиторен титър и животозастрашаващ кръвоизлив или риск от кървене (например посттравматично или перооперативно): Дисеминирана вътресъдова коагулация (ДИК): лабораторни данни и/или клинични симптоми. Чернодробно увреждане: поради забавен клирънс на активирани фактори на коагулация, пациентите с увредена чернодробна функция са с повишен риск за развитие на ДИК. Коронарна болест на сърцето, остра тромбоза и/или емболия. Пациентите, които получават ФЕЙБА, трябва да бъдат наблюдавани за развитието на ДИК, остра коронарна исхемия и за признаци и симптоми на други тромботични или тромбоемболични събития. При първите признаци или симптоми на тромботични и тромбоемболични събития, инфузията трябва да се прекрати незабавно и да се предприемат съответни диагностични и терапевтични мерки. Анамнестични отговори Приложението на ФЕЙБА при пациенти с инхибиторни нива до доведе до първоначално „анамнестично" повишаване на нивата на инхибитора. При продължително приложение на ФЕЙБА, нивата на инхибиторите могат да се понижат с времето. Клинични и публикувани данни показват, че ефективността на ФЕЙБА не се понижава. Педиатрична употреба Отделни съобщения и ограничените клинични данни посочват, че ФЕЙБА може да се използва при деца по-малки от 6-годишна възраст. Същият режим на дозиране, както при възрастните трябва да бъде адаптиран към клиничното състояние на детето. **4.6 Фертилитет, бременност и кърмене** Няма адекватни данни за употребата на ФЕЙБА при бременни и кърмещи жени. Лекарите следва да балансират потенциалните рискове и да предписват ФЕЙБА само, ако е категорично необходимо, като вземат предвид, че бременността и периодът след раждането са с повишен риск от тромбоемболични събития и редица усложнения на бременността, които са свързани с повишен риск от ДИК. Не са провеждани проучвания с ФЕЙБА при животни за репродуктивна токсичност, а ефектите на ФЕЙБА върху фертилитета не са установени в контролирани клинични изпитвания. За информация относно инфекция с парвовирус B19 вижте точка 4.4. **4.8 Нежелани лекарствени реакции** ФЕЙБА може да предизвика реакции на свръхчувствителност от алергичен тип, които включват уртикария, ангиоедем, стомашно-чревни прояви, бронхоспазъм и рязко понижаване на артериалното налягане; тези реакции могат да бъдат тежки и системни (като например анафилаксия с уртикария и ангиоедем, бронхоспазъм и циркулаторен шок). Вижте също точка 4.4 Реакции на свръхчувствителност. Чести нежелани реакции (≥ 1/100 до <1/10): Свръхчувствителност, главоболие, замаяност, хипотония, обрив, положителен хепатит В повърхностен антиген. НПР с неизвестна честота (от наличните данни не може да бъде направена оценка): ДИК, повишен титър на инхибитора (анамнестичен отговор), уртикария, анафилактична реакция, парастезия, хиперестезия, тромботичен удар, емболичен удар, сънливост, нарушение на вкуса, тромбоза (венозна , артериална), емболия, хипертония, зачервяване, белодробна емболия, бронхоспазъм, хрипове, кашлица, диспнея, повръщане, диария, коремен дискомфорт, гадене, чувствителност и изтръпване на лицето, ангиоедем, уртикария, пруритус, болка на мястото на инжектиране, неразположение, чувство за парене, втрисане, пирексия, болка и дискомфорт в гърдите, рязко понижаване на артериалното налягане. Реакции на класа Други симптоми на реакции на свръхчувствителност към получени от плазма продукти включват летаргия и безпокойство.

FEIBA NF 500 U, 20 ml powder and solvent for solution for injection

(Factor VIII Inhibitor Bypassing Activity)

Съкратена кратка характеристика на продукта[1]

Активно вещество: Активност, заобикаляща инхибитора на Фактор VIII. Активното вещество, съдържащо се в 1 ml ФЕЙБА е 25 U. Опаковка с 500 U съдържа 500 U активност, заобикаляща инхибитора на Фактор VIII в 200-800 mg човешки плазмен протеин. ФЕЙБА съдържа също и факторите II, IX и X, предимно в неактивирана форма, както и активиран фактор VII. Кръвосъсиряващият антиген на фактор VIII (F VIII C:Ag) присъства в концентрация до 0,1 U/1 U ФЕЙБА. Факторите на каликреин-кининовата система присъстват само в следи или изобщо не се съдържат. 1U ФЕЙБА скъсява активираното парциално тромбопластиново време (аРТТ) на плазма, съдържаща инхибитор на фактор VIII до 50% от изходната стойност (бланкетна стойност). **4.1 Терапевтични показания** Лечение на кръвоизливи при пациенти с хемофилия А с инхибитори. Лечение на кръвоизливи при пациенти с хемофилия В с инхибитори, ако не се предлага друго специфично лечение (вж. точка 5.1). Лечение на кръвоизливи при пациенти без хемофилия с придобити инхибитори срещу фактор VIII. Профилактика на кръвоизливи при пациенти с хемофилия А с инхибитори, които са имали значително кървене или са високо рискови за значителен кръвоизлив. **4.2 Дозировка и начин на приложение** *Дозировка* Дозировката и продължителността на лечението зависят от тежестта на хемостатичното нарушение, локализацията и степента на кръвоизлива и клиничното състояние на пациента. Дозировката и честотата на приложение трябва винаги да се определят според клиничната ефикасност във всеки отделен случай. Както общо правило се препоръчва доза от 50-100 U ФЕЙБА на килограм телесно тегло (т.т.). Еднократна доза от 100 U/kg т.т. и максимална дневна доза от 200 U/kg т.т. не трябва да се превишават, освен ако тежестта на кръвоизлива не дава основание и не налага използването на по-високи дози. Вижте точка 4.4. *Педиатрична употреба (деца)* Опитът при деца на възраст под 6 години е ограничен; същата дозировка като при възрастни трябва да се адаптира към клиничното състояние на детето. **1) Спонтанен кръвоизлив Ставни, мускулни и мекотъканни кръвоизливи** При леки до умерено силни кръвоизливи се препоръчва доза от 50-75 U/kg т.т., приложена през 12-часови интервали. Лечението трябва да продължи до появата на явни признаци за клинично подобрение като отзвучаване на болката, намаляване на отока или раздвижване на ставата. При големи мускулни и мекотъканни кръвоизливи, като например ретроперитонеален кръвоизлив, се препоръчва доза от 100 U/kg т.т., през 12-часови интервали. **Кръвоизливи от лигавици** Препоръчва се доза от 50 U/kg т.т., която се прилага на всеки 6 часа при внимателно мониториране на пациента (визуален контрол на кръвоизлива, повторно определяне на хематокрита). Ако кръвоизливът не се овладява, дозата може да бъде увеличена до 100 U/kg т.т., без да се надвишава максималната дневна доза от 200 U/kg т.т. **Други тежки кръвоизливи** При тежки кръвоизливи, като кръвоизлив в ЦНС, се препоръчва доза от 100 U/kg т.т., приложена през 12-часови интервали. В отделни случаи ФЕЙБА може да се приложи и през интервали от 6 часа до постигане на ясно изразено клинично подобрение. (Да не се надвишава максималната дневна доза от 200 U/kg т.т.). **2) Хирургически операции** При хирургическа интервенция може да се приложи начална доза от 100 U/kg телесно тегло предоперативно, а последваща доза от 50-100 U/kg телесно тегло може да се приложи след 6-12 часа. Като постоперативна поддържаща доза, 50-100 U/kg телесно тегло може да се прилага през интервали от 6-12 часа; дозировката, интервалите на дозиране и продължителността на пери- и постоперативната терапия се определят от хирургическата интервенция, общото състояние на пациента и клиничната ефикасност при всеки отделен случай. (Не трябва да се надвишава максимална дневна доза от 200 U/kg телесно тегло). **3) Профилактика при пациенти с хемофилия А с инхибитори Профилактика на кръвоизливи при пациенти с висок инхибиторен титър и чести кръвоизливи, при които ITI (терапия за имунен толеранс) е неуспешна или не е обсъждана:** препоръчва се доза от 70-100 U/kg т.т. през ден. Ако е необходимо, тази доза може са бъде увеличена до ежедневна доза от 100 U/kg т.т., ако пациента продължава да кърви, или постепенно да бъде намалена. **Профилактика на кръвене при пациенти с висок инхибиторен титър, подлежащи на ITI (терапия за имунен толеранс):** ФЕЙБА може да се прилага едновременно с фактор VIII концентрати, в доза от 50-100 U/kg т.т., двукратно дневно, докато инхибитора на фактор VIII бъде редуциран до <2 B.U. **4) Употреба на ФЕЙБА при специални групи пациенти** Вижте точка 5.1 за информация относно пациенти с хемофилия В с инхибитор на фактор IX. В комбинация с концентрат на фактор VIII, ФЕЙБА е използвана и за продължително лечение за постигане на пълно и постоянно отстраняване на инхибитора на фактор VIII.

FEIBA NF 500 U, 20 ml powder and solvent for solution for injection

(Factor VIII Inhibitor Bypassing Activity)

Съкратена кратка характеристика на продукта[1]

4.9 Предозиране При употребата на високи дози ФЕЙБА може да се увеличи риска от тромботични или тромбоемболични събития (включително ДИК, миокарден инфаркт, венозна тромбоза и белодробна емболия). Някои от съобщените тромбоемболични събития са настъпили при дози надвишаващи 200 U/kg или при пациенти с други рискови фактори за тромбоемболични събития. Ако се наблюдават признаци или симптоми на тромботично и тромбоемболично събитие, инфузията трябва да се спре незабавно и да се предприемат подходящи диагностични и терапевтични мерки. Вижте точка 4.4. **5.1 Фармакодинамични свойства** Фармакотерапевтична група: фактори на кръвосъсирването, **АТС код:** B02BD03. Въпреки че ФЕЙБА е разработен в началото на 70те и неговата активност, заобикаляща инхибитора на фактор VIII е доказана in vitro и in vivo, активната му съставка е все още обект на научен дебат. ФЕЙБА, както се установява от анализа на активността, се състои от протромбинов комплекс цимогени, които са едновременно прокоагуланти (протромбин FVII, FIX, FX) и антикоагуланти (протеин С) в относително еднакви количества спрямо единица за мощност на ФЕЙБА, но неговото относително съдържание е относително ниско. Така ФЕЙБА съдържа преензимите на факторите на протромбиновия комплекс, но само много малко количество от техните активационни продукти, като съдържанието на FVIIa е най-високо. Въпреки това, най-новите разработки показват ролята на специфичните компоненти от активирания протромбинов комплекс - зимоген протромбин (FII) и активирания фактор X (FXa) комплекс, в начина на действие на ФЕЙБА. ФЕЙБА контролира кървенето чрез индукция и улесняване на тромбинового генериране, процес, при който формираното на протромбиновия комплекс е от съществено значение. Няколко in vitro и in vivo проучвания показват, че FXa и протромбинът играят критична роля в активността на ФЕЙБА. Установено е, че протромбиназният комплекс е основно целево място за ФЕЙБА. Освен протромбин и FXa, ФЕЙБА съдържа и други протеини на протромбиновия комплекс, които също могат да улеснят хемостазата при пациенти с хемофилия с инхибитори. **5.2 Фармакокинетични свойства**Тъй като механизмът на действие на ФЕЙБА все още се обсъжда, не е възможно да се даде окончателно становище по отношение на фармакокинетичните свойства на ФЕЙБА. **6.2 Несъвместимости** Този лекарствен продукт не трябва да се смесва с други лекарствени продукти с изключение на разтворителя посочен в точка 6.6. Както при всички продукти за кръвосъсиряване, ефикасността и поносимостта на лекарствения продукт може да бъде нарушена, като се смесва с други лекарствени продукти. Препоръчва се да се изплаче общият венозен достъп с подходящ разтвор, напр. изотоничен разтвор на натриев хлорид, преди и след приложението на ФЕЙБА. Факторите на кръвосъсирване, получени от човешка плазма, са възможно да адсорбират по-голяма или по-малка степен върху вътрешните повърхности на някои видове изделия за инжектиране/инфузия. Ако това се случи, то може да доведе до провал на лечението. Ето защо, трябва да се използват само одобрени пластмасови изделия за инфузия с ФЕЙБА. 6.3 **Срок на годност** Две години. Данните за физико-химичната стабилитност потвърждават, че готовият разтвор е стабилен за 3 часа при стайна температура (до 25°C). От микробиологична гледна точка, освен ако методът на разтваряне изключва риска от микробно замърсяване (контролирани и валидирани асептични условия), продуктът трябва да се използва незабавно. Ако готовият за употреба разтвор не се използва незабавно, потребителят е отговорен за условията и времето на съхранението му. Готовият за употреба разтвор не трябва да се замразява. **6.4 Специални условия за съхранение** Да се съхранява под 25°C. Да не се замразява. Да се съхранява във външната картонена опаковка, за да се предпази от светлина. **6.6 Специални предпазни мерки при изхвърляне и работа** ФЕЙБА трябва да се разтвори непосредствено преди употреба, след което разтворът трябва да се използва веднага (разтворът не съдържа консерванти). Разклатете внимателно докато целият материал се разтвори. Уверете се, че ФЕЙБА е разтворен напълно, в противен случай, по-малко количество единици от предвиденото ще бъдат приложени през филтъра. След разтваряне продуктът трябва да се провери за наличие на неразтворени частици и за промяна на цвета. Да не се използват разтвори, които са мътни или имат утайки. Само за еднократна употреба. Продуктът да не се използва, ако системата за стерилност е нарушена или опаковката е повредени, или показва признаци на замърсяване.

ФЕЙБА (АКТИВНОСТ, ЗАОБИКАЛЯЩА ИНХИБИТОРА НА ФАКТОР VIII) – ПРОФИЛ НА БЕЗОПАСНОСТ

Денка Стоянова

В България

- 14.4% от децата с тежка хемофилия са с инхибитори
- 80% от тях с висок титър.

Данни на автора

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Инхибиторна хемофилия – предизвикателство за всеки специалист

- В нашето съвремие инхибиторите на FVIII и FIX са най-тежките свързани с лечението усложнения на хемофилията.
- Риск и честота :
  - тежка форма на хемофилия А - 20-30%,
  - средно тежка и лека форма – 5-10 %
  - хемофилия В – по – малко от 5%.
- Средна възраст – при тежка форма - 3 години в развитите страни, по–често при нелекувани болни до 50 ЕД. При средно тежка и лека форма до 30 години.
- Резултат : влошаване качеството на живот и протичане на заболяването при пациентите и съответно рязко покачване разходите за лечение.

http://www.haemophiliacare.co.uk/what-are-inhibitors.html
https://www.wfh.org/en/page.aspx?pid=650
https://www.cdc.gov/ncbddd/hemophilia/inhibitors.html

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- **Силно реагиращите инхибитори обикновено са постоянни.**
- **Някои инхибитори с нисък титър могат да са временни и да изчезнат в рамките на 6 месеца.**
- **Пациент със слабо реагиращ "инхибитор" се определя при инхибиторно ниво < 5,0BU,със силно реагиращ "инхибитор" с ниво >5,0BU.**
- **Някои от инхибиторите с много нисък титър може да не се установят чрез Бетезда количествен анализ, а само чрез скъсен период на полуразпад /T-1/2/.**

Therapeutic Advances in Hematology, (2013) 4(1) 59–72 , Char Witmer and Guy Young

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Рискови фактори

1.Генетични – постоянни
- **фамилна история за инхибитори;**
- **тип 8/9 генна мутация : large del; nonsense; intron 22 inv. ; missens**
- **етнос;**
- **HLA клас II**
- **имунна генетика**

2. Вариабилни :
- **интензитет на терапия**
- **терапевтичен режим**
- **тип на продукта**
- **други сигнали**

American Journal of Medicine and Medical Sciences 2013, 3(6): 190-196 DOI: 10.5923/j.ajmms.20130306.12

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Практически подход

- **Цел – минимум кръвоизливи сведени до 0, липса на странични реакции, намаляване титъра на инхибитори.**
- **Индивидуализиране на лечението.**
- **Място на лечение на кръвоизливите – специализиран център.**
- **Избор на продукт – пациенти със слабо реагиращ инхибитор могат да се лекуват с високи дози от липсващият фактор.**
- **Пациенти с ниво на инхибитори над 5,0 BU :**
  - **индукция на имунен толеранс-условие за успех : ранно започване и титър на инхибитори< 10,0 BU**
  - **байпас агенти : APCC и rFVIIa ;**
  - **свръх висока доза на липсващия ф-р в продължителна инфузия;**

Therapeutic Advances in Hematology, (2013) 4(1) 59–72 , Char Witmer and Guy Young

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Рискови групи пациенти

- **Пациенти с нисък риск**
- **Пациенти със среден риск**
- **Пациенти с висок риск**
- **Изследване титър на инхибитори след всеки трети ЕД до 50 ЕД.**

http://www.haemophiliacare.co.uk/what-are-inhibitors.html
https://www.wfh.org/en/page.aspx?pid=650
https://www.cdc.gov/ncbddd/hemophilia/inhibitors.html

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РАННА ВТОРИЧНА ПРОФИЛАКТИКА С БАЙПАС ПРОДУКТИ СЛЕД НЕУСПЕХ НА ITI

- **Режими на приложение :**
- **FEIBA 50-100 UI/kg;**
  - **1x или 2x дневно; през ден; 3x седмично.**
- **Оценка на ефикасност:**
  - **63.9% редукция на хеморагичните епизоди;**
  - **78% редукция в годишната честота на хемартрозите**
- **Оценка на безопасност:**
  - **5/25 – анамнестичен отговор;**
  - **1/25 - алергични реакции;**
  - **0/25 – тромботични усложнения;**
  - **Не се съобщава за вирусна трансмисия и други странични ефекти.**

Escuriola, Kreuz. Haemophilia 2010
Valentino Haemophilia 2010
Leissinger C. NEJM 2011

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Въведение и цели

- Употребата на байпас фактори значително повлия на лечението и профилактиката на епизодите на кървене при пациенти с хемофилия с инхибитори[1]
- ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) е лекарствен продукт, наличен на пазара от 1975 година.[2]
- ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) съдържа активиран фактор VII и факторите II, IX и X, предимно в неактивирана форма[2]

References: 1. Hilgartner MW, Knatterud GL. 2.FEIBA Study Group.Blood 1983; 61: 36–40.
2. FEIBA® ((Factor VIII Inhibitor Bypassing Activity) BG Summary of Product Characteristics. November 2016

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- Началното разрешение за употреба на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) е дадено през 1978 г. на базата на 3 рандомизирани клинични проучвания за ефикасност и безопасност и едно ретроспективно мултицентрово проучване. От тогава досега са проведени клинични изпитвания, които доказват терапевтичната ефективност, ефикасност и безопасност на продукта. Редица постмаркетингови проучвания и мета-анализи доказват ползите и предимствата на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) при индикациите, за които продуктът е разрешен[1].

Референции: 1.„FEIBA® ((Factor VIII Inhibitor Bypassing Activity) BG Summary of Product Characteristics. November 2016
2.Haemophilia. 2014 Jan; 20(1): 65–72., Antunes SV et al;
3.Blood. 2007 Jan 15;109(2):546-51. Epub 2006 Sep 21, Astermark J et al;

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ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)-индикации[1]

- Лечение на кръвоизливи при пациенти с хемофилия А с инхибитори
- Лечение на кръвоизливи при пациенти с хемофилия В с инхибитори, ако не се предлага друго специфично лечение
- Лечение на кръвоизливи при пациенти без хемофилия с придобити инхибитори срещу фактор VIII
- **Профилактика на кръвоизливи при пациенти с хемофилия А с инхибитори**, които са имали значително кървене или са високо рискови за значителен кръвоизлив
- ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) **се прилага и периоперативно при пациенти с хемофилия А, на които предстои хирургическа интервенция**

Reference: FEIBA® ((Factor VIII Inhibitor Bypassing Activity) BG Summary of Product Characteristics. November 2016

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Материали и методи

- Тази презентация обобщава всички спонтанни и описани в медицинската литература случаи на тромбоемболични усложнения, възникнали при прилагането на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- при пациенти с вродена хемофилия, документирани в глобалната база данни на Shire за безопасност/ Shire's global safety base/.
- Прегледани са всички съобщения за нежелани лекарствени реакции, възникнали при прилагането на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII), получени от 1975г. до юли 2016г.
- Взети са под внимание и демографските показатели на пациентите, дозовите режими и рисковите фактори за развитието на тромбоемболични усложнения

References: 1.http://www.bloodjournal.org/content/128/22/5031; Roberto Crea et al. Blood 2016 128:5031
2. FEIBA® ((Factor VIII Inhibitor Bypassing Activity) BG Summary of Product Characteristics. November 2016

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Приложение[1]

- Дозата и продължителността на заместителната терапия зависят от тежестта на дефицита на фактор VIII, локализацията и степента на кръвоизлива, както и от клиничното състояние на пациента.
- Като общо правило се препоръчва доза от 50-100 U ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) на килограм телесно тегло, без обаче да се надвишава еднократната доза от 100 U/kg т.т. и максимална дневна доза от 200 U/kg т.т.
- При прилагането на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- трябва да се отчитат предупрежданеията и предпазните мерки за употреба. Подобно на други протеинови продукти за интравенозно приложение са възможни алергичен тип реакции на свръхчувствителност

Reference: FEIBA® (Factor VIII Inhibitor Bypassing Activity) BG Summary of Product Characteristics. November 2016

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Проучвания доказващи клиничния ефект

- Клиничните проучвания на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) са насочени в две основни направления: **прилагането на препарата при необходимост (on demand, при нужда) за спонтанни кръвоизливи** и неговото **профилактично приложение**.

Haemophilia. 2014 Jan; 20(1): 65–72., Antunes SV et al
Blood. 2007 Jan 15;109(2):546-51. Epub 2006 Sep 21, Astermark J et al
FEIBA® ((Factor VIII Inhibitor Bypassing Activity) BG Summary of Product Characteristics. November 2016

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Приложение[1]

- За да не повлияе ефективността и съвместимостта на разтвора, ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) като всички концентрати с коагулиращ фактор, не трябва да се смесва с други лекарствени продукти преди приложение.

Reference: FEIBA® ((Factor VIII Inhibitor Bypassing Activity) BG Summary of Product Characteristics. November 2016

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Основни клинични изпитвания за терапевтична ефективност и ефикасност

- **Jan Astermark**, Sharyne M. Donfield, Donna M. DiMichele, Alessandro Gringeri, Steven A. Gilbert, Jennifer Waters, and Erik Berntorp: A randomized comparison of bypassing agents in hemophilia complicated by an inhibitor: the FEIBA NovoSeven Comparative (FENOC) Study. Anti-Inhibitor Coagulant Complex Prophylaxis in Hemophilia with Inhibitors. **Blood**, 2007, 109: 546-551. [Публикация по проучването **FENOC**.]

Reference: Blood. 2007 Jan 15;109(2):546-51. Epub 2006 Sep 21, Astermark J et al

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- FENOC е първото сравнително проучване, което прави оценка на препаратите, които заобикалят инхибиторите на FVIII. По тази причина това проучване е от особена важност за специалистите, които лекуват пациенти с хемофилия А и инхибитори.

Reference: Blood. 2007 Jan 15;109(2):546-51. Epub 2006 Sep 21, Astermark J et al

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- Резултатите от проучването показват, че ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) е еднакво безопасен при епизодично лечение и профилактичен режим на лечение
- Отличен профил на безопасност е установен при лечението както на пациентни с хемофилия А, така и на пациенти с хемофилия В.

Haemophilia. 2014 Jan; 20(1): 65–72., Antunes SV et al

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- **Cindy Leissinger, M.D**., Alessandro Gringeri, M.D., Bulent Antmen, M.D., Erik Berntorp, M.D., Chiara Biasoli, M.D., Shannon Carpenter, M.D., Paolo Cortesi, M.Sc., Hyejin Jo, M.S., Kaan Kavakli, M.D., Riitta Lassila, M.D., Massimo Morfini, M.D., Claude Négrier, M.D., Angiola Rocino, M.D., Wolfgang Schramm, M.D., Margit Serban, M.D., Marusia Valentina Uscatescu, M.D., Jerzy Windyga, M.D., Bülent Zülfikar, M.D., and Lorenzo Mantovani, D.Sc.: Anti-Inhibitor Coagulant Complex Prophylaxis in Hemophilia with Inhibitors. **The New England Journal of Medicine**, 2011, 365:1684-92. [Публикация по проучването **pro-FEIBA**.]

N Engl J Med. 2011 Nov 3;365(18):1684-92

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Подробен анализ на данните за безопасността на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) е направен в постмаркетинговото проучване на Ehrlich et al., където са обобщени данните от десетгодишен клиничен опит (**Ehrlich HJ**, Henzl MJ, Gomperts ED. Safety of factor VIII inhibitor bypass activity (FEIBA®): 10-year compilation of thrombotic adverse events. **Haemophilia**. 2002;8:83-90).

- При това проучване на подробен анализ са подложени 55 нежелани събития, сред които 16 тромботични (29 %), свързани с различни нарушения.
- Установен е само един случай с фатален изход, като общата честота на тромботични нежелани събития е приблизително 4 на 100 000 вливания.
- При 81 % от тромботичните събития е имало познати рискови фактори.
- Резултатите от проучването показват, че рискът от тромботични усложнения при пациенти, които приемат ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII), е относително нисък.

Reference: Haemophilia. 2002;8:83-90, Ehrlich HJ et al

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Проучвания, насочени към безопасността на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)

- Безопасността на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) е била конкретен обект на три начални клинични проучвания (1 интервенционално и 2 постмаркетингови проучвания на безопасността). Освен това факторите на безопасността са отчитани и при всички клинични изпитвания за терапевтична ефективност на продукта.

Reference:FEIBA® ((Factor VIII Inhibitor Bypassing Activity) BG Summary of Product Characteristics. November 2016
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C-APROMBG/0020;DOP:09/2017

Резултати

- Повече от **7 милиарда** международни единици ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII) (повече **2 милиона** инфузии) са били дистрибутирани през разглеждания период.
- Общо **108** тромбоемболични усложнения са били докладвани при пациенти с различни индикации (вродена хемофилия, придобита хемофилия, anticoagulation reversal, etc.).

Reference: http://www.bloodjournal.org/content/128/22/5031; Roberto Crea et al. Blood 2016 128:5031

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- Phase 3 Study: FEIBA NF (Активност, заобикаляща инхибитора на фактор VIII): проспективно, open-label, рандомизирано, паралелно проучване за отценка на ефикасността и безопасността на профилактиката спрямо лечението при нужда при пациенти с хемофилия А и Б и висок титър на инхибиторите.

Haemophilia. 2014 Jan; 20(1): 65–72., Antunes SV et al

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Резултати при пациенти с хемофилия

85 съобщения, включващи едно или повече тромбоемболични събития, са получени за пациенти с хемофилия/ възраст: 0-73 години/

От тези 85 тромбоемболични събития, при 31 събития има **комбинираната употреба** с **rFVIIa** (7 дълбока венозна тромбоза и/или белодробна тромбоемболия, 9 миокарден инфаркт/инсулт, 9 ДИК, 6 други).

Reference: http://www.bloodjournal.org/content/128/22/5031; Roberto Crea et al. Blood 2016 128:5031

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Заключение

- Докладваната честота на тромбоемболични усложнения е сравнима с публикуваните до момента данни и потвърждава профила на безопасност на ФЕЙБА (Активност, заобикаляща инхибитора на фактор VIII)- в дългосрочен аспект.
- Повечето от тромбоемболичните събития са възникнали при наличието на допълнителни придружаващи рискови фактори като съпътстващо заболяване и комбинирано лечение.
- Този преглед на всички тромбоемболични усложнения е ценен източник за лечението и превенцията на кръвоизливи при пациентите с хемофилия с инхибитори.

Reference: http://www.bloodjournal.org/content/128/22/5031; Roberto Crea et al. Blood 2016 128:5031

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ФЕЙБА NF 500 U прах и разтворител за инжекционен разтвор (Активност, заобикаляща инхибитора на Фактор VIII)
Съкратена кратка характеристика на продукта[1]

ПРЕДПАЗНИ МЕРКИ Тромботични и тромбоемболични усложнения При следните ситуации, ФЕЙБА трябва да се прилага само, ако не може да се очаква реакция към лечението с подходящи концентрати на фактор на кръвосъсирването, като например в случай на висок инхибиторен титър и животозастрашаващ кръвоизлив или риск от кървене (например посттравматично или постоперативно): Дисеминирана вътресъдова коагулация (ДИК): лабораторни данни и/или клинични симптоми. Чернодробно увреждане: поради забавен клирънс на активирани фактори на коагулация, пациентите с увредена чернодробна функция са с повишен риск за развитие на ДИК. Коронарна болест на сърцето, остра тромбоза и/или емболия. Пациентите, които получават ФЕЙБА, трябва да бъдат наблюдавани за развитието на ДИК, остра коронарна исхемия и за признаци и симптоми на други тромботични или тромбоемболични събития. При първите признаци или симптоми на тромботични и тромбоемболични събития, инфузията трябва да се прекрати незабавно и да се предприемат съответни диагностични и терапевтични мерки. Анамнестични отговори Приложението на ФЕЙБА при пациенти с инхибитори може да доведе до първоначално „анамнестично" повишаване на нивата на инхибитора. При продължително приложение на ФЕЙБА, нивата на инхибиторите могат да се понижат с времето. Клинични и публикувани данни показват, че ефективността на ФЕЙБА не се понижава. Педиатрична употреба Отделни съобщения и ограничените клинични данни посочват, че ФЕЙБА може да се използва при деца по-малки от 6-годишна възраст. Същият режим на дозиране, както при възрастните трябва да бъде адаптиран към клиничното състояние на детето. **4.6 Фертилитет, бременност и кърмене** Няма адекватни данни за употребата на ФЕЙБА при бременни и кърмещи жени. Лекарите следва да балансират потенциалните рискове и да предписват ФЕЙБА само, ако е категорично необходимо, като вземат предвид, че бременността и периодът след раждането са с повишен риск от тромбоемболични събития и редица усложнения на бременността, които са свързани с повишен риск от ДИК. Не са провеждани проучвания с ФЕЙБА при животни за репродуктивна токсичност, а ефектите на ФЕЙБА върху фертилитета не са установени в контролирани клинични изпитвания. За информация относно инфекция с парвовирус B19 вижте точка 4.4. **4.8 Нежелани лекарствени реакции** ФЕЙБА може да предизвика реакции на свръхчувствителност от алергичен тип, които включват уртикария, ангиоедем, стомашно-чревни прояви, бронхоспазъм и рязко понижаване на артериалното налягане, тези реакции могат да бъдат тежки и системни (като например анафилаксия с уртикария и ангиоедем, бронхоспазъм и циркулаторен шок). Вижте също точка 4.4 Реакции на свръхчувствителност. Чести нежелани реакции (≥ 1/100 до <1/10): Свръхчувствителност, главоболие, замаяност, хипотония, обрив, положителен хепатит B повърхностен антиген. НПР с неизвестна честота (от наличните данни не може да бъде направена оценка): ДИК, повишен титър на инхибитора (анамнестичен отговор), уртикария, анафилактична реакция, парастезия, хипоастезия, тромботичен удар, емболичен удар, сънливост, нарушение на вкуса, тромбоза (венозна , артериална), емболия, хипертония, зачервяване, белодробна емболия, бронхоспазъм, хрипове, кашлица, диспнея, повръщане, диария, коремен дискомфорт, гадене, чувствителност и изтръпване на лицето, ангиоедем, уртикария, пруритус, болка на мястото на инжектиране, неразположение, чувство за парене, втрисане, пирексия, болка и дискомфорт в гърдите, рязко понижаване на артериалното налягане. Реакции на кожата Други симптоми на реакции на свръхчувствителност към получени от плазма продукти включват летаргия и безпокойство.

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FEIBA NF 500 U, 20 ml powder and solvent for solution for injection
(Factor VIII Inhibitor Bypassing Activity)

- **Лекарственият продукт се отпуска по лекарско предписание**
- **Последно актуализирана КХП[1] е на разположение в заседателната зала или може да бъде намерена на: http://www.bda.bg/images/stories/documents/bdias/2016-11-23-6716.pdf**
- **Дата на последно одобрение: 03/11/2016**
- **Притежател на разрешението за употреба: Baxter AG, Vienna, Австрия**
- **Адрес, на който медицинските специалисти могат да получат пълна информация:** Баксалта България, ул. «Съборна» 2А, ет.2, 1000 София, тел.: 02/926 43 48, факс: 02/926 43 50
- **Съобщаването на подозирани нежелани реакции след разрешаване на употреба на лекарственият продукт е важно. Това позволява да продължи наблюдението на съотношението полза/риск за лекарственият продукт. От медицинските специалисти се изисква да съобщават всяка подозирана нежелана реакция.**
- **Моля, съобщете всяка информация за подозирана нежелана реакция на Изпълнителна агенция по лекарствата, София, ул. Дамиан Грубев 8, тел. +359 2 8903417 Е-мейл :** pharmacovig@bda.bg, www.bda.bg **и на притежателя на разрешението за употреба на Е-мейл : drugsafety@shire.com.**
- **Преди да изпишете продукта, моля прочетете последната одобрена Кратка Характеристика на продукта.**

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ФЕЙБА NF 500 U прах и разтворител за инжекционен разтвор (Активност, заобикаляща инхибитора на Фактор VIII)
Съкратена кратка характеристика на продукта[1]

4.9 Предозиране При употребата на високи дози ФЕЙБА може да се увеличи риска от тромботични или тромбоемболични събития (включително ДИК, миокарден инфаркт, венозна тромбоза и белодробна емболия). Някои от съобщените тромбоемболични събития са настъпили при дози надвишаващи 200 U/kg или при пациенти с други рискови фактори за тромбоемболични събития. Ако се наблюдават признаци или симптоми на тромботично и тромбоемболично събитие, инфузията трябва да се спре незабавно и да се предприемат подходящи диагностични и терапевтични мерки. Вижте точка 4.4. **5.1 Фармакодинамични свойства** Фармакотерапевтична група: фактори на кръвосъсирването, **АТС код:** B02BD03. Въпреки че ФЕЙБА е разработен в началото на 70те и неговата активност, заобикаляща инхибитора на Фактор VIII е доказана in vitro и in vivo, активната му съставка е все още обект на научен дебат. ФЕЙБА, както се установява от анализа на активността, се състои от протромбинов комплекс цимогени, които са едновременно проколагенази (протромбин FVII, FIX, FX) и антикоагуланти (протеин C) в относително еднакви количества спрямо единица за мощност на ФЕЙБА, но неговото прокоагулантно еквимно съдържание е относително ниско. Така ФЕЙБА съдържа проензимите на факторите на протромбиновия комплекс, но само много малко количество от техните активационни продукти, като съдържанието на FVIIa е най-високо. Въпреки това, най-новите научни разработки показват ролята на специфичните компоненти от активирания протромбинов комплекс - zимогени протромбин (FII) и активирания фактор X (FXa) комплекс, в начина на действие на ФЕЙБА. ФЕЙБА контролира кървенето чрез индукция и улесняване на тромбиновото генериране, процес, при който формирането на протромбиназен комплекс е от съществено значение. Няколко in vitro и in vivo проучвания показват, че FXa и протромбинът играят критична роля в активността на ФЕЙБА. Установено е, че протромбиназният комплекс е основно целево място за ФЕЙБА. Освен протромбин и FXa, ФЕЙБА съдържа и други протеини на протромбиновия комплекс, които също могат да упенят хемостазата при пациенти с хемофилия с инхибитори. **5.2 Фармакокинетични свойства**Тъй като механизмът на действие на ФЕЙБА все още се обсъжда, не е възможно да се даде окончателно становище по отношение на фармакокинетичните свойства на ФЕЙБА. **6.2 Несъвместимости** Този лекарствен продукт не трябва да се смесва с други лекарствени продукти с изключение на разтворителя посочен в точка 6.6. Както при всички продукти за кръвосъсирване, ефикасността и поносимостта на лекарствения продукт може да бъде нарушена, като се смесва с други лекарствени продукти. Препоръчва се да се изплаче общият венозен достъп с подходящ разтвор, напр. изотоничен разтвор на натриев хлорид, преди и след приложението на ФЕЙБА. Факторите на кръвосъсирване, получени от човешка плазма, е възможно да се адсорбират от вътрешните повърхности на някои видове изделия за инжектиране/инфузия. Ако това се случи, то може да доведе до провал на лечението. Ето защо, трябва да се използват само одобрени пластмасови изделия за инфузия с ФЕЙБА. 6.3 **Срок на годност** Две години. Данните за физико-химичната стабилност потвърждават, че готовият разтвор е стабилен за 3 часа при стайна температура (до 25ºC). От микробиологична гледна точка, освен ако методът на разтваряне изключва риска от микробно замърсяване (контролирани и валидирани асептични условия), продуктът трябва да се използва незабавно. Ако готовият за употреба разтвор не се използва незабавно, потребителят е отговорен за условията и времето на съхранението му. Готовият за употреба разтвор не трябва да се замразява. **6.4 Специални условия за съхранение** Да се съхранява под 25ºC. Да не се замразява. Да се съхранява в оригиналната картонена опаковка, за да се предпази от светлина. **6.6 Специални предпазни мерки при изхвърляне и работа** ФЕЙБА трябва да се разтвори непосредствено преди употреба, след което разтворът трябва да се използва веднага (разтворът не съдържа консерванти). Разклатете внимателно докато целият материал се разтвори. Уверете се, че ФЕЙБА е разтворен напълно, в противен случай, по-малко количество единици от продукта ще преминат през филтъра. След разтваряне продуктът трябва да се провери за наличие на неразтворени частици и за промяна на цвета. Да не се използват разтвори, които са мътни или имат утайки. Само за еднократна употреба. Продуктът да не се използва, ако системата за стерилност е нарушена или опаковката е повредени, или показва признаци на замърсяване.

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ФЕЙБА NF 500 U прах и разтворител за инжекционен разтвор (Активност, заобикаляща инхибитора на Фактор VIII)
Съкратена кратка характеристика на продукта[1]

Активно вещество: Активност, заобикаляща инхибитора на Фактор VIII. Активното вещество, съдържащо се в 1 ml ФЕЙБА е 25 U. Опаковка с 500 U съдържа 500 U активност, заобикаляща инхибитора на Фактор VIII в 200-600 mg човешки плазмен протеин. ФЕЙБА съдържа също и факторите II, IX и X, предимно в неактивирана форма, както и активиран фактор VII. Кръвосъсиряващият антиген на фактор VIII (F VIII C:Ag) присъства в концентрация до 0,1 U/1 U ФЕЙБА. Факторите на каликреин-кининовата система присъстват само в следи или изобщо не се съдържат. 1U ФЕЙБА съкява активираното парциално тромбопластиново време (аРТТ) на плазма, съдържаща инхибитор на фактор VIII до 50% от изходната стойност (бланкетна стойност). **4.1 Терапевтични показания** Лечение на кръвоизливи при пациенти с хемофилия А с инхибитори, Лечение на кръвоизливи при пациенти с хемофилия В с инхибитори, ако не се предлага друго специфично лечение (вж. точка 5.1). Лечение на кръвоизливи при пациенти без хемофилия с придобити инхибитори срещу фактор VIII. Профилактика на кръвоизливи при пациенти с хемофилия А с инхибитори, които са имали значително кървене или са високо рискови за значителен кръвоизлив. **4.2 Дозировка и начин на приложение** Дозировка Дозировката и продължителността на лечението зависят от тежестта на хемостатичното нарушение, локализацията и степента на кръвоизлива и клиничното състояние на пациента. Дозировката и честотата на приложение трябва винаги да се определят според клиничната ефикасност във всеки отделен случай. Като общо правило се препоръчва доза от 50-100 U ФЕЙБА на килограм телесно тегло (т.т.). Еднократна доза от 100 U/kg т.т. и максимална дневна доза от 200 U/kg т.т. не трябва да се превишават, освен ако тежестта на кръвоизлива не дава основание и не налага използването на по-високи дози. Вижте точка 4.4. *Педиатрична употреба (деца)* Опитът при деца на възраст под 6 години е ограничен; същата дозировка като при възрастни трябва да се адаптира към клиничното състояние на детето. **1) Спонтанен кръвоизлив Ставни, мускулни и мекотъканни кръвоизливи** При леки до умерено силни кръвоизливи се препоръчва доза от 50-75 U/kg т.т., приложени през 12-часови интервали. Лечението трябва да продължи до появата на явни признаци за клинично подобрение като отзвучаване на болката, намаляване на отока или раздвижване на ставата. При големи мускулни и мекотъканни кръвоизливи, като например ретроперитонеален кръвоизлив, се препоръчва доза от 100 U/kg т.т. през 12-часови интервали. **Кръвоизливи от лигавици** Препоръчва се доза от 50 U/kg т.т., която се прилага на всеки 6 часа при внимателно мониториране на пациента (визуален контрол на кръвоизлива, повторно определяне на хематокрита). Ако кръвоизливът не се овладява, дозата може да бъде увеличена до 100 U/kg т.т., без да се надвишава максималната дневна доза от 200 U/kg т.т. **Други тежки кръвоизливи** При тежки кръвоизливи, като кръвоизлив в ЦНС, се препоръчва доза от 100 U/kg т.т., приложена през 12-часови интервали. В отделни случаи ФЕЙБА може да се приложи и през интервали от 6 часа до постигане на ясно изразено клинично подобрение. (Да не се надвишава максималната дневна доза от 200 U/kg т.т.!). **2) Хирургически операции** При хирургически интервенции може да се приложи начална доза от 100 U/kg телесно тегло предоперативно, а последваща доза от 50-100 U/kg телесно тегло може да се приложи след 6-12 часа. Като постоперативна поддържаща доза, 50-100 U/kg телесно тегло може да се прилага през интервали от 6-12 часа; дозировката, интервалите на дозиране и продължителността на терапия се определят от хирургическата интервенция, общото състояние на пациента и клиничната ефикасност при всеки отделен случай. (Не трябва да се надвишава максимална дневна доза от 200 U/kg телесно тегло). **3) Профилактика при пациенти с хемофилия А с инхибитори Профилактика на кръвоизливи при пациенти с висок инхибиторен титър и чести кръвоизливи, при които ITI (терапия за имунен толеранс) е неуспешна или не е обсъждана:** препоръчва се доза от 70-100 U/kg т.т. през ден. Ако е необходимо, тази доза може са бъде увеличена до ежедневна доза от 100 U/kg т.т., ако пациента продължава да кърви, или постепенно да бъде намалена. **Профилактика на кървене при пациенти с висок инхибиторен титър, подлежащи на ITI (терапия за имунен толеранс):** ФЕЙБА може да се прилага едновременно с фактор VIII концентрати, в дози от 50-100 U/kg т.т., двукратно дневно, докато инхибитора на фактор VIII бъде редуциран до <2 B.U. **4) Употреба на ФЕЙБА при специални групи пациенти** Вижте точка 5.1 за информация относно пациенти с хемофилия В с инхибитор на фактор IX В комбинация с концентрат на фактор VIII, ФЕЙБА е използвана и за продължително лечение за постигане на пълно и постоянно отстраняване на инхибитора на фактор VIII.

FEIBA® ((Factor VIII Inhibitor Bypassing Activity) BG Summary of Product Characteristics. November 2016

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Благодаря за вниманието!

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ФЕЙБА NF 500 U прах и разтворител за инжекционен разтвор (Активност, заобикаляща инхибитора на Фактор VIII)
Съкратена кратка характеристика на продукта[1]

Начин на приложение Пригответе продукта както е описано в точка 6.6 и приложете като бавна интравенозна инфузия. Не трябва да се надвишава скорост на инфузия от 2 U/kg т.т. на минута. **4.3 Противопоказания** В зависимост от лечебните алтернативи, ФЕЙБА не трябва да се използва в следните случаи: Свръхчувствителност към активното вещество или към някое от помощните вещества. Дисеминирана интраваскуларна коагулация (ДИК). Остра тромбоза или емболия (включително миокарден инфаркт). ПРЕДУПРЕЖДЕНИЯ Реакции на свръхчувствителност ФЕЙБА може да предизвика реакции на свръхчувствителност от алергичен тип, които включват уртикария, ангиоедем, стомашно-чревни прояви, бронхоспазъм и хипотония. Тези реакции могат да бъдат тежки и системни (като например анафилаксия с уртикария и ангиоедем, бронхоспазъм и циркулаторен шок). Има съобщения и за други инфузионни реакции като втрисане, пирексия и хипертония. При първата проява или симптом на инфузионна реакция/реакция на свръхчувствителност, приложението на ФЕЙБА трябва да се спре и да се предприемат съответните медицински мерки. Когато се обсъжда повторно приложение на ФЕЙБА при пациенти със съмнение за свръхчувствителност към продукта или към някои от неговите съставки, трябва да бъдат внимателно преценени очакваната полза и риска от повторна експозиция, като се отчитат известните или подозирани типове (алергични или неалергични) свръхчувствителност на пациента, включително и на потенциални лечебни и/или профилактични терапии или алтернативни лекарствени продукти. Тромботични и тромбоемболични събития Тромботични и тромбоемболични събития, включително дисеминирана интраваскуларна коагулация (ДИК), венозна тромбоза, белодробен емболизъм, инфаркт на миокарда и инсулт, са възниквали в хода на лечението с ФЕЙБА. Някои от тези събития възникват при дози над 200 U/kg/ден или при пациенти с други рискови фактори (включително ДИК, напреднало атеросклеротично заболяване, травма със смазване или асептицемия) за тромбоемболични събития. Съпровождащото лечение с рекомбинантен Фактор VIIa може да повиши риска от развитие на тромбоемболично събитие. Рискът от тромботични и тромбоемболични събития може да се увеличи при високи дози ФЕЙБА. ФЕЙБА трябва да се прилага с особено внимание и само при липсата на терапевтични алтернативи при пациенти с повишен риск за тромбоемболични усложнения. Това включва, но не се ограничава до пациенти с анамнеза за коронарна болест на сърцето, чернодробно заболяване, ДИК, артериална или венозна тромбоза, следоперативно обездвижване, пациенти в напреднала възраст и новородени. При първите прояви или симптоми на тромбоемболични събития, инфузията трябва да бъде преустановена незабавно и да се предприемат необходимите диагностични и терапевтични мерки. Единична доза от 100 U/kg телесно тегло и дневна доза от 200 U/kg телесно тегло не трябва да се надвишават, освен ако тежестта на кръвенето не го налага и обосновава използването на по-високи дози. Значение на броя на тромбоцитите В случай на неадекватен отговор към лечението с ФЕЙБА се препоръчва да бъде изследван броя на тромбоцитите, тъй като за ефективното действие на ФЕЙБА са необходими достатъчно висок брой функционално интактни тромбоцити.

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SESSION 4

Moderators: Vily Stoyanova, Dimitrina Konstantinova

- **Application of NGS in the diagnostic work-up of rare genetic diseases**
  R. Vazharova
- **Pre-implantational genetic testing for monogenic and chromosomal disorders: the experience in Bulgaria**
  S. Hadjidekova

Oral presentations:

- **Preimplantation genetic diagnosis (PGD) in a family with Waardenburg syndrome**
  M. Atanasoska
- **Application of next-generation sequencing for balanced tranlocations in pre-implantation embryos**
  M. Rizov
- **Stem cell transplantation – present and future**
  T. Chervenkov
- **Ancient mtDNA studies on Thracian and Proto-Bulgarian samples: new perspectives on the origin of contemporary Bulgarians**
  D. Nesheva

APPLICATION OF NGS IN THE DIAGNOSTIC WORK-UP OF RARE GENETIC DISEASES

R. Vazharova, L. Balabanski, S. Andonova, S. Bichev, M. Ivanova, I. Sinigerska, M. Atanasoska, A. Savov, D. Toncheva

Genetic testing

- There is no a single universal genetic test for all rare genetic diseases…
- CNV detection: karyotyping, FISH, MLPA, qPCR, aCGH, aSNP
- Gene mutation detection: DNA fragment analysis, DNA sequencing

Rare genetic diseases

- Rare genetic diseases affect at least 1 in 50 individuals
- The total number of these diseases is estimated to be 6000–8000
- and while each is individually rare, together these genetic conditions contribute significantly to morbidity, mortality, and healthcare costs
- Estimates suggest that up to 50% of patients with a rare genetic disease never receive a diagnosis

DNA sequencing…

…is the process of determining the precise order of nucleotides within a DNA molecule

- Since 1977 – Sanger sequencing (is still the golden standard!)
- Since 2005 – massive parallel sequencing (NGS)

Patients facing a rare genetic disease…

- Patients without a diagnosis often embark on a diagnostic odyssey that includes multiple specialist consultations, imaging studies, invasive investigations and other laboratory and genetic tests.
- The diagnostic odyssey is by definition a slow, costly venture, and for many is ultimately disappointing when a diagnosis is not reached.
- A survey of patients with rare diseases demonstrated that for 25% of participants the time to diagnosis was extensive (from 5 to 30 years), and during that time 40% received an incorrect diagnosis.
- As many as a third of these patients with rare diseases incurred inappropriate care for their eventual diagnosis.

Goal

To evaluate the application of massive parallel sequencing of large gene panels to detect pathogenic mutations in rare diseases in a clinical setting

Genetic testing

- Providing a molecularly confirmed diagnosis, in a timely manner:
  - shortens the diagnostic odyssey,
  - improves disease management, including targeted treatments and surveillance for later-onset comorbidities for a subset of patients, and
  - informs genetic counseling with respect to recurrence risks and prenatal diagnosis options for families
- Recent advances in sequencing, in particular whole-exome sequencing (WES), are identifying the genetic basis of disease for 25–40% of patients.
- Key reasons for diagnostic failure:
  - **genetic heterogeneity** associated with a clinical diagnosis and
  - **atypical presentation** of known, clinically recognized diseases

Individuals included

Total number: 58

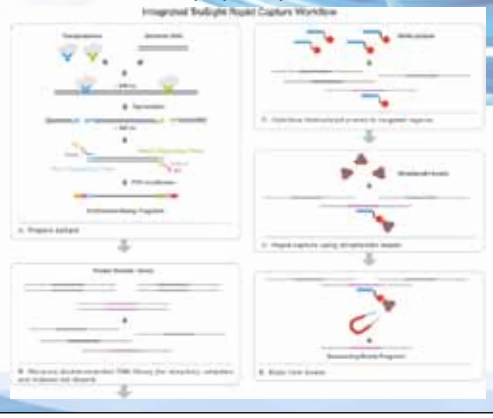
- **control individuals**: 10 (7 males, 3 females)
  - healthy adults
  - Bulgarian descent
  - unrelated
  - median age: 36 y 6 m (25 – 56)
- **patients**: 43
  - 17 with recognizable disease specific phenotype
  - 26 with unknown diagnosis / suspective locus heterogeneity
- **healthy first degree relatives of patients**: 5

With recognizable disease specific phenotype

- without locus, but with prominent allelic heterogeneity
- locus heterogeneity, but small number of loci
- results from laboratory, imaging and other tests supporting the working clinical diagnosis
- pedigree pattern matches the expected mode of inheritance

#	Clinical diagnosis	#	Clinical diagnosis
2	Cystic fibrosis	1	Propionic acidemia
2	DMD / BMD	1	3-methylglutaconic aciduria
1	Ataxia telangiectasia	1	Niemann-Pick type C
1	Epidermolysis bullosa	1	Leucinosis
1	Aortic dissection	1	Zellweger spectrum
1	Osteogenesis imperfecta	1	Mitochondrial encephalopathy, complexes I, III and IV deficiency
3	Glycogenosis		

Library preparation

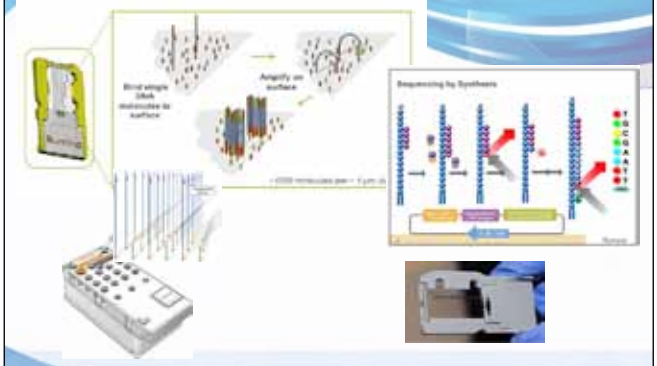


With unknown diagnosis / suspective locus heterogeneity

- phenotype of monogenic disease, but the data are incomplete / contradictory
- laboratory tests conform partially / not match the working diagnosis
- excluded initial clinical diagnosis

#	Clinical diagnosis
13	Neurological / neuromuscular diseases (SMA, epilepsy, spastic quadriparesis, MR, autism)
5	Metabolic disorders (Krabbe, Pompe, tyrosinemia type 1, unspecified)
4	Multiple congenital anomalies
2	Obs. Osteogenesis imperfecta
2	Others (Tuberous sclerosis, Growth retardation and aniridia)

Massive parallel sequencing



Healthy first degree relatives of patients

- The proband's phenotype is specific
  - sister of a patient with MPSIIIB disease (Sanfilippo B),
  - parents of a child with Progressive intrahepatic cholestasis
  - parents of a child with Herlitz bullous epidermolysis

Massive parallel sequencing

- Real time information:
  - run info, cluster density, quality scores by cycle,
  - expected yield of sequenced DNA

Methods

Data analysis

- Mapping the fragments relative to the reference genome (**alignment**) and **variants calling**
  - *BWA Enrichment 2.1.0.0*
  - *Isis 2.5.41.27*
  - *SAMtools 0.1.19-isis-1.0.3*
  - *BWA (Aligner) 0.7.7-isis-1.0.0*
  - *Picard 1.79(1282)*
  - *GATK (Variant Caller) v1.6-23-gf0210b3*
- **Annotation** and **filtering** of variants
  - *IAS (Annotation Service) v3*
  - *Variant Studio v 2.1.46*
- Data visualization
  - *GenomeBrowse 2.0.7.*

Criteria for variants assessment

Sequencing quality:

- Base quality: > 30
- Sequencing depth: > 20
- Frequency of the alternative allele: > 30%

Clinical interpretation:

- Presence in:
  dbSNP, EVS, ClinVar, HGMD, OMIM, ExAC, Cosmic, Invitae etc.
- MetaSVM Prediction
  - 2 categories: "T(olerated)" or "D(amaging)". The rankscore cutoff between "D" and "T" is 0.83357. MetaSVM is a SVM based ensemble prediction score, which incorporated 10 scores (SIFT, PolyPhen-2 HDIV, PolyPhen-2 HVAR, GERP++, MutationTaster, Mutation Assessor, FATHMM, LRT, SiPhy, PhyloP) and the maximum frequency observed in the 1000 genomes populations.
- Human Splicing Finder (HSF3.0)

Pathogenic variants in control subjects

A total of **45** different pathological variants

- **15** variants associated with a **dominant phenotype** *(Hypertrophic cardiomyopathy, Familial atrial fibrillation, Diabetes mellitus type 2, Familial combined hyperlipidemia, Bronchiectasis, Nephrolithiasis, Macular degeneration, age-related, Mononeuropathy of the median nerve, Neuropathy, small fiber, Central core disease)*
- **1** associated with a X-recessive disease (*COL4A5, p.Arg1416Cys*)
- **26** associated with autosomal recessive phenotypes
- **3** associated with dominant and recessive phenotypes

5 variants present with MAF ≥ 0,01:
AMPD1:*p.Gln45Ter;* ***CPN1***:*p.Gly178Asp;* ***CYP11B2***: *p.Val386Ala;* ***HPD*** *p.Ala33Thr,* ***ADA***:*p.Asp8Asn;*

Gene panels

Gene panel	TruSight Cancer	TruSight Inherited	TruSight One
# targeted genes	94	552	4 813
# targeted exons	1 700	8 801	62 000
Total target region size	255 Kb	2.25 Mb	12 Mb
# individuals	3	29	16

Pathogenic variants in patients with recognizable phenotype

- ✓ pathogenic variants associated with the clinical phenotype were found in 13 of the investigated patients
- ✓ pathogenic variants not directly related to the clinical phenotype were found in 14 of the investigated

Sex	Diagnosis	Result	Compliance with the phenotype
F	CF	*CFTR*: p.Phe508del, heterozygous and ?	? only one allele
M	CF	*CFTR*: p.Phe508del и c.4046G>A, p.Gly1349Asp, compound heterozygote	yes
M	DMD	***DMD*: g.31986568C>A, c.6502G>T, p.Glu2168Ter**, hemizygote	yes
M	DMD	*DMD*: p.His2921Arg, hemizygote	? yes / no (BMD?)
F	MSUD	*BCKDHA*: c.979G>A, p.Glu327Lys, homozygote	yes

continues

Results

Distribution of variants in the study of **control subjects** with a panel comprising **4,813 genes**

- Average overall number of variants ~7360 (6165 - 8533)
- Rare (MAF <1%) - 290 (3.98%)
- Not listed in dbSNP - 72 (0.98%)
- Known pathogenic - 4 (0.05%)
- Probably pathogenic - 10 (0.14%)
- Known pharmacogenetic defects - 3-4 (0.05%)

Pathogenic variants in patients with recognizable phenotype

Sex	Diagnosis	Result	Compliance with the phenotype
M	Ataxia telangiectasia	*ATM*: c.6412_6413delAG, p.Glu2139IlefsTer6, homozygote	yes
F	Propionic acidemia	*PCCA*: g.100953860A>G c.1209+3A>G, homozygote	yes
M	Zellweger spectrum	*HSD17B4*: c.-133G>A, c.46G>A, p.Gly16Ser, rs137853096, homozygote	yes
F	Niemann–Pick type C	***NPC1*: c.3127A>G, p.Thr1043Ala** / c.2972_2973delAG, p.Gln991Argfs, compound heterozygote	yes
F	Glycogenosis	***PHKG2***: p.Asp153Val / **p.Leu160del**	yes
M	Aortic dissection	*ACTA2*: c.772C>T, p.Arg258Cys, heterozygote	yes
F	Osteogenesis imperfecta	***COL1A1*: c.2775delT, p.Gly926ValfsTer182** , heterozygote	yes
F	Epidermolysis bullosa	*COL7A1*: c.2527C>T, p.Arg843Ter / c.425A>G, p.Lys142Arg, compound heterozygote	yes

Results

Distribution of the variants in the study of **patients** with a panel comprising **4,813 genes**

- Average overall number of variants ~7829 (3794 - 8625)
- Rare (MAF <1%) - 383 (4.89%)
- Not listed in dbSNP - 89 (1.14%)
- Known pathogenic - 4 (0.06%)
- Probably pathogenic - 12 (0.16%)
- Known pharmacogenetic defects - 4-5 (0.06%)

Pathogenic / probably pathogenic variants in patients with unknown diagnosis

- ✓ pathogenic variants partially or fully explaining the clinical phenotype were found in 12 of the 26 patients studied
- ✓ pathogenic variants not directly related to the clinical phenotype were found in 23 of the patients

Sex	Working diagnosis	Result gene / variant	Associated phenotype
M	Osteogenesis imperfecta	*PHEX*: p.Pro401Leu, rs145778165, hemizygote	Hypophosphatemic rickets, X-linked dominant
M	Osteogenesis imperfecta	*ASCC1*: c.626+1G>A, homozygote	? SMA with congenital bone fractures 2
F	Obs. Pompe disease	*HADHA*: p.Glu510Gln / p.Arg291Ter, compound heterozygote	Mitochondrial trifunctional protein deficiency (LCHAD)
		AMPD1: p.Gln45Ter, homozygote	Muscle AMP deaminase deficiency
		HBB: p.Gln40Ter, heterozygote	Thalassemia, beta-, carrier

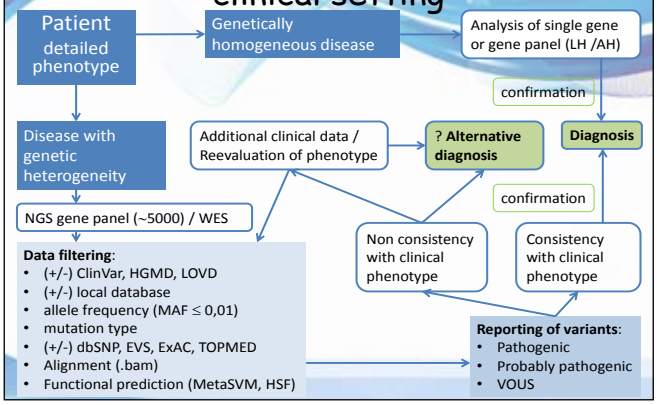
continues

Pathogenic / probably pathogenic variants in patients with unknown diagnosis

Sex	Working diagnosis	Result gene / variant	Associated phenotype
F	Epilepsy, Developmental disorder of motor function Obs. Rett s-me	*CLCN1*: c.1436_1449delTACCCTGCGGAGGC, p.Pro480HisfsTer24, heterozygote	? Myotonia congenita, AD Myotonia congenita, AR
F	GEFS+	*SCN8A*: c.5615G>A, p.Arg1872Gln, heterozygote	Epileptic encephalopathy, early infantile, 13, AD
M	Spastic quadriparesis	*RNASEH2B*: c.529G>A, p.Ala177Thr, homozygote	Aicardi-Goutieres type 2
M	Spastic quadriparesis	***AP1S2*: c.258T>A, p.Tyr86Ter**; hemizygote	Mental retardation, X-linked syndromic 5
M	Spastic quadriparesis	***IDS*: c.1222C>T, p.Pro408Ser**, hemizygote	? Mucopolysaccharidosis II

continues

Algorithm for application of NGS in a clinical setting



Pathogenic / probably pathogenic variants in patients with unknown diagnosis

Sex	Working diagnosis	Result gene / variant	Associated phenotype
M	Polymalformative syndrome – bilateral radial and thumb aplasia, unilateral renal agenesis	***FANCD2*: p.Leu700del**, homozygote	Fanconi anemia, complementation group D2
M	Growth retardation, Partial aniridia	*GHR*: p.Arg179Cys, rs121909362, heterozygote	Growth hormone insensitivity, partial ?
F	Obs. Tuberous sclerosis	*FANCA*: p.Ser858Arg / p.Ser1088Phe, compound heterozygote	Fanconi anemia, complementation group A
M	Polymalformative syndrome, MR	***MKS1*: p.Ser372del**, homozygote	Bardet-Biedl syndrome 13, Meckel syndrome 1
M	MR, Obs. WBS	***RPS6KA3*: c.913C>T, p.Arg305Ter**, hemizygote	Coffin-Lowry syndrome

Conclusions

- We found that when tested with a panel of 4,813 genes healthy individuals carry on average 3-4 known pathogenic variants.
- Applying massive parallel sequencing using a 4,813 gene panel has the potential to detect a genetic defect associated with the phenotype in about 48% of patients with rare monogenic diseases.
- The greatest chance of receiving a genetic diagnosis have patients with recognizable specific phenotype of a disease without / with limited locus heterogeneity.

Pathogenic variants in healthy 1st degree relatives of patients

	Diagnosis	Result gene / variant
1a	Sister of patient with MPS-III B (Sanfilippo B)	***NAGLU*: g. 40695196A>G, c.1172A>G, p.Tyr391Cys**, heterozygote
2a	Parent of a child with Progressive intrahepatic cholestasis	? suspected to carry a heterozygous deletion of *ABCB11* gene based on the presence of homozygosity for all SNPs
3a	Parent of a child with Progressive intrahepatic cholestasis	***ABCB11*: g.169787209A>T, c.3377T>A, p.Leu1126Ter**, heterozygote
4a	Parent of a child with Epidermolysis bullosa	***LAMB3*: c.1133-22G>A**, heterozygote
5a	Parent of a child with Epidermolysis bullosa	***LAMB3*: c.1133-22G>A**, heterozygote

Thank you for your attention

PRE-IMPLANTATIONAL GENETIC TESTING FOR MONOGENIC AND CHROMOSOMAL DISORDERS: THE EXPERIENCE IN BULGARIA

Savina Hadjidekova Rada Staneva, Silvia Andonova, Alexey Savov, Stoyan Bitchev, Slaviyana Yaneva, Maria Pancheva, Maria Serafimova, Draga Toncheva, Georgi Stamenov

"NADEZHDA" Women's Health Hospital
www.nadezhda.bg

PGS Indications

- Women of advanced maternal age
- Couples with history of recurrent pregnancy loss
- Couples with repeated IVF failure
- Male partner with severe male factor infertility

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Pre-implantational Genetic Testing (PGT)

Preimplantation genetic testing is technique for identifying genetic defects in embryos created through in vitro fertilization (IVF) before pregnancy.

- Only unaffected embryos are transferred

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PGD Alternatives

- Natural conception- prenatal diagnosis
- ART- Preimplantation Genetic Diagnosis
- Donor gametes
- Adoption
- No children

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Pre-implantational Genetic Testing (PGT)

- PGD - preimplantation genetic diagnosis - one or both genetic parents has a known genetic abnormality
- PGS - preimplantation genetic screening - chromosomally normal genetic parents

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PGT – Tips

The best clinic for PGT is the clinic with the best IVF success rates

Extensive genetic counseling before the procedure

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PGD Indications

- Couples with chromosome abnormalities
- Carriers of single gene defects
  - autosomal recessive diseases
  - autosomal dominant diseases
    - *Late onset diseases
    - *HLA matching
- Parents with a family history of X-linked recessive disorders

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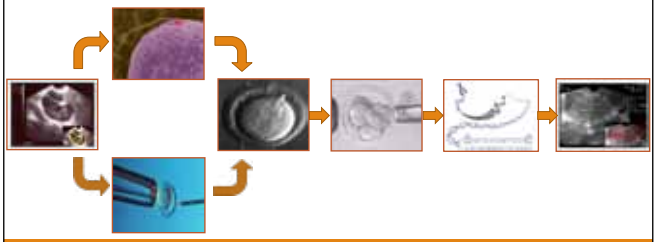
How is PGT Performed?



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Biopsy Techniques

Polar body biopsy *Cleavage-stage embryo biopsy* *Blastocyst biopsy* *Non-invasive approach*

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PGT by NGS Analysis

PGS performed using NGS - targeted sequencing

- Highly sensitive method for screening embryos
- Requires as little as 1 ng of amplified DNA from a blastomere or a trophectodermal biopsy
- Results in 12 hours

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Genetic Testing

- Fluorescence in situ hybridization (FISH)
- Polymerase chain reaction (PCR)
- Array-based comparative genomic hybridization (array CGH)
- DNA analysis (real time PCR, Sanger sequencing etc.)
- Next generation sequencing (NGS)

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Our Experience

- **PGD for complex chromosomal translocations**
  - Case 1: female carrier - 46,XX,t(3;6;7)
  - Case 2: male carrier - [46,XY,t(1;8;11)
- **PGD for monogenic disorders**
  - Duchenne dystrophy
  - Hemophilia A
  - Myotonic dystrophy type 1
  - Huntington's disease
  - Fragile X syndrome
  - Epidermolysis bullosa
  - Beta-thalassemia

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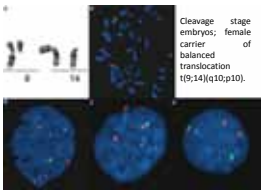
PGT by FISH Analysis

FISH for parental translocations



Cleavage stage embryos; female carrier of balanced translocation t(9;14)(q10;p10).

FISH for aneuploidy screening

Disadvantages:
- Limited No of investigated chromosomes
- Non-specific hybridization – signal interpretation
- PGS FISH day 3: not improving pregnancy rate

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PGD for Complex Chromosomal Rearrangements (CCR)

Risks for the carriers of balanced translocations

- Fertility problems
- Recurrent pregnancy loss
- Unbalanced prenatal results
- Unbalanced liveborn offspring

Characteristics

- At least three breakpoints affecting two or more chromosomes.
- Very rare
- Formed hexavalent meiotic configuration
- Four patterns of segregation: 3:3, 4:2, 5:1 and 6:0

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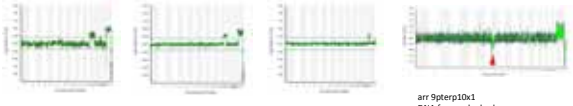
PGT by Array CGH Analysis

- First accurate and fast technology for PGS
- 24 chromosome analysis
- Provides result in 12-24 (fresh transfer is possible)



arr (15,16)x3,(X)x2 arr (16)x3,(X)x2 arr (22)x3,(XY)x2

arr 9pterp10x1
DNA from polar body
Woman, carrier of t(9;14)(t(9;14)(q10;p10)

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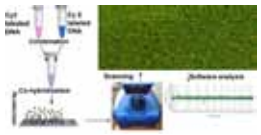
Our Approach for CCR - PGD

- Stimulated and Natural-Cycle-IVF
- ICSI fertilization
- Embryo biopsy on Day 5, collecting 5-7 trophectoderm cells
- Whole genome amplification through SurePlex
- Array CGH by 24sure+ microarrays (Illumina)
- Analysis by BlueFuseMulti software.
- Fresh or frozen embryo transfer



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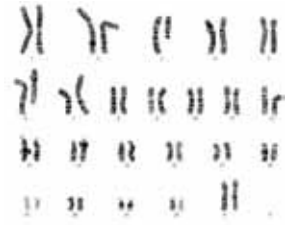
Case 1: Woman with Familial Complex Translocation - 46,XX,t(3;6;7)



Medical history

- Non-consanguineous couple (a 35-years old female and a 39-years old male)
- Two early pregnancy losses
- Karyotype: female carrier - 46,XX,t(3;6;7)(3pter→3q13.2::7p22→7pter;6pter→6q21::3q13.2→3qter;7qter→7p22::6q21→6qter)
- First cousin with the same translocation

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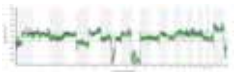
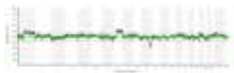
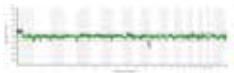
Case 2: Male with Familial Complex Translocation - 46,XY,t(1;8;11)

- In total of 10 ova were retrieved
- Five survived to blastomere stage
- None was normal/balanced







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Case 1: Woman with Familial Complex Translocation - 46,XX,t(3;6;7)

In total 36 ova were retrieved and two ICSI–PGD procedures were performed

First cycle
- 9 ova were aspirated
- 4 embryos were biopsied
- None was normal/balanced.

Second ICSI-PGD procedure
- 27 eggs
- 11 embryos were biopsied
- 3 normal/balanced embryos
- Two embryos were transferred on Day 6 after fertilization (the third normal/balanced embryo was frozen).

The women conceived with one embryo.

She refused prenatal diagnosis but after birth the cytogenetic analysis confirmed normal male karyotype 46,XY.

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PGD for Duchenne dystrophy

Prevalence: 1:3500 males - most common form of muscular dystrophy

Gene: *DMD gene* : Xq28

Mutation:
- Deletion/duplications
- Point Mutation
- Small duplications

Inheritance: X-linked recessive

Characteristics:
- Weakness
- Swollen Calve Muscles
- Strange Gait
- Muscle Wasting
- Loss of Ambulation
- Respiratory Insufficiency
- Scoliosis
- Cardiomyopathy (Some Cases)

Poor prognosis

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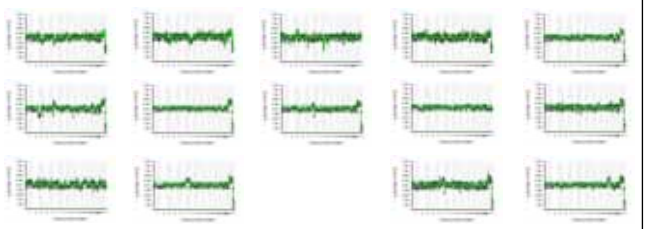
Case 1: Woman with Familial Complex Translocation - 46,XX,t(3;6;7)



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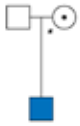
PGD for Duchenne Dystrophy - Sexing



Medical history

- Non-consanguineous couple (a 38-years old female and a 39-years old male) with infertility
- Female partner - carrier of mutation in *DMD* gene
- Her son – muscular dystrophy Duchenne
- IVF + PGS cycle for sex selection

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Case 2: Male with Familial Complex Translocation - 46,XY,t(1;8;11)

Medical history

- Non-consanguineous couple (a 27-years old female and a 28-years old male)
- 4 unsuccessful IVF cycles
- Karyotype: male carrier - 46,XY,t(1;8;11)(1qter→1p22::8q11.1→8qter)(8pter→8q11.1::11q22→11qter)(11pter→11q22::1p22→1pter)
- Two first cousins with the same translocation

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PGD for Duchenne Dystrophy - Sexing

Embryo №	result	Interpretation
1	arr (1-22)x2,(X)x1	nonbalanced
2	arr (1-22,X)x2	balanced/normal
3	arr mos (19)x1,(3-9)x3,(X)x2	nonbalanced
4	arr (1-22,X)x2	balanced/normal

- Four embryos were biopsied
- Results: four female: two normal, two unbalanced
- One embryo was transferred
- The women conceived
- Prenatal diagnosis confirmed the PGD results
- The woman delivered healthy baby girl

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PGD for Hemophilia A

Bleeding disorder caused by a deficiency in the activity of coagulation factor VIII

Prevalence: 1 in 5000 to 1 in 10,000 males

Gene: factor 8 gene: Xq28

Mutation:
- Majority of the mutations are point mutations
- Gene inversions account for approximately 45% of the F8 pathogenic variants
- Benign variants

Inheritance: X-linked recessive

Characteristics:
- Joints hemorrhage
- Muscle hemorrhage
- Hematuria
- Bleeding from tongue or lip is persistent.
- Prolonged bleeding from wounds.

Prognosis: good, treatment available - intravenous infusion of factor VIII concentrate

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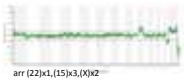
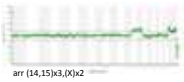
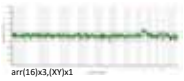
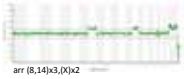
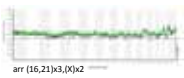
PGD for Hemophilia A


arr (22)x1,(15)x3,(X)x2


arr (14,15)x3,(X)x2

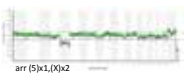

arr(16)x3,(XY)x1

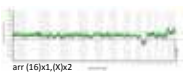

arr (8,14)x3,(X)x2


arr (16,21)x3,(X)x2


arr chaotic mosaic


arr (1-22)x2,(XY)x1 carrier of c.5405A>G, F8) gene


arr (5)x1,(X)x2


arr (16)x1,(X)x2

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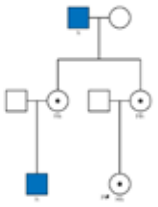
PGD for Hemophilia A



Medical history

- Non-consanguineous couple (a 42-years old female and a 45-years old male) with infertility
- Female partner - carrier of mutant allele c.5405A>G, p.(Tyr1783Cys or Tyr1802Cys) in exon 16 of Factor VIII (F8) gene
- Proband's mother - carrier of mutant allele c.5405A>G, p.(Tyr1783Cys or Tyr1802Cys) in exon 16 of Factor VIII (F8) gene
- Proband's aunt- carrier of mutant allele c.5405A>G, p.(Tyr1783Cys or Tyr1802Cys) in exon 16 of Factor VIII (F8) gene
- Proband's nephew– presented with symptoms of the disease
- Proband's grandfather – presented with symptoms of the disease
- IVF + PGD

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PGD for Myotonic Dystrophy type 1

Myotonic dystrophy is characterized by progressive muscle wasting and weakness.

The mean age of onset is 30 to 40 years.

Prevalence: 1 in 100,000

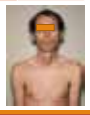
Genetics:
- **Gene**. *DMPK*. Locus 19q13.32

Gene defect: a trinucleotide CTG repeat expansion:
- normal: 5-34 CTG repeats
- premutation: 27-35 CTG repeats
- full penetrance: >50 CTG repeats

Type of inheritance: autosomal dominant, anticipation

Poor prognosis: No specific treatment exists

Phenotype	Clinical Signs	CTG Repeat
Mutable normal (premutation)	None	35-49
Mild	Cataracts, Mild myotonia	50-~150
Classic	Weakness, Myotonia, Cataracts, Balding, Cardiac arrhythmia, Others	~100-~1000
Congenital	Infantile hypotonia, Respiratory deficits, Intellectual disability, Classic signs present in adults	>1000



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PGD for Hemophilia A

Methods:

- Throphectoderm biopsy, blastocyst stage at day 5
- In total 9 embryos were biopsied
- DNA amplification with **SurePlex** amplification system
- Array CGH for PGS
- Sanger sequencing for c.5405A>G, p.(Tyr1783Cys or Tyr1802Cys) in exon 16 of Factor VIII (F8) gene

First our successful combined PGT for monogenic disease and aneuploidy screening

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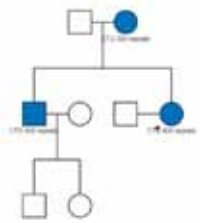
PGD for Myotonic Dystrophy type 1



Medical history

- Non-consanguineous couple (a 36-years old female and a 39-years old male) with infertility
- Female partner - carrier of mutant allele with expansion $(CTG)_{900}$ in *DMPK* gene
- Brother - carrier of mutant allele with expansion $(CTG)_{400}$ in *DMPK* gene. Two healthy children after PGD-IVF cycle
- Proband's mother - carrier of mutant allele with expansion $(CTG)_{300}$ in *DMPK* gene
- IVF + PGD

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PGD for Hemophilia A

Results

1	arr (5)x1,(X)x2	nonbalanced
2	arr (16)x3,(XY)x1	nonbalanced
3	arr (16,21)x3,(X)x2	nonbalanced
4	arr (8,14)x3,(X)x2	nonbalanced
5	arr (16)x1,(X)x2	nonbalanced
6	arr chaotic mosaic	nonbalanced
7	arr (1-22)x2,(XY)x1	balanced/normal
8	arr (14,15)x3,(X)x2	nonbalanced
9	arr (22)x1,(15)x3,(X)x2	nonbalanced

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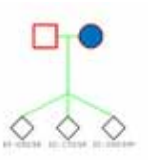
PGD for Myotonic Dystrophy type 1

Methods:
- 3 embryos were biopsied at blastocyst stage on day 5
- DNA amplification with RepliG amplification system
- DNA fragment analysis by triplet repeat primed PCR with FastDM1™ DMPK Sizing Kit



Embryo №	Allele 1	Allele 2
1	$(CTG)_n$ – normal repeats number	$(CTG)_n$ – normal repeats number
3	$(CTG)_n$ – normal repeats number	$(CTG)_{11}$- normal repeats number
2	$(CTG)_n$ – normal repeats number	$(CTG)_n$ – normal repeats number

- Two embryos were transferred
- The women conceived with one embryo
- Prenatal diagnosis confirmed the PGD results
- The woman delivered normal baby girl

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PGD for Huntington's Disease

Progressive degenerative disorder of motor, cognitive and psychiatric disturbances.

The mean age of onset is 35 to 44 years and the median survival time is 15 to 18 years after onset.

Prevalence: 3-7 in 100,000 people of European origin

Genetics:

- **Gene**. *HTT*. Locus 4p16.3
- **Gene defect:** a trinucleotide CAG repeat expansion:
  - normal: 26 or fewer CAG repeats
  - intermediate: 27-35 CAG repeats
  - HD-causing: 36 or more CAG repeats
    - reduced-penetrance HD-causing alleles 36-39 CAG repeats
    - full-penetrance HD-causing alleles, 40 or more CAG repeats
- **Type of inheritance: autosomal dominant, anticipation**

Poor prognosis: there is no cure

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PGD for Fragile X Syndrome

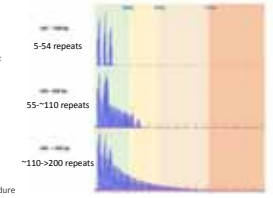
Methods:

- Two embryos were biopsied at blastocyst stage on day 5
- DNA amplification with RepliG amplification system
- DNA fragment analysis by triplet repeat primed PCR with FastFraX™ FMR1 Sizing Kit

Results:

Embryo№	Allele 1	Allele 2
1	$(CGG)_{30}$ - normal repeats number	(CGG) – expanded number of repeats
2	$(CGG)_{30}$- normal repeats number	$(CGG)_{77}$- premutation

- Embryo 2 was transferred.
- No pregnancy was realized
- At the moment the woman is in ovarian stimulation for second IVF-PGD procedure



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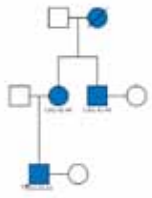
PGD for Huntington's Disease



Medical history

- Non-consanguineous couple (a 28-years old female and a 31-years old male)
- Male partner - carrier of mutant allele with expansion $(CAG)_{43-44}$ in *HTT* gene
- Proband's mother - carrier of mutant allele with expansion *HTT* gene $(CAG)_{43-44}$
- Proband's uncle - carrier of mutant allele with expansion $(CAG)_{43-44}$ in *HTT* gene.
- Proband's grandmother – presented with symptoms of the disease.
- IVF + PGD

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PGD for Epidermolysis Bullosa

Genetically transmitted skin disorders characterized by spontaneous blistering or blistering caused by minor trauma

Prevalence: fewer than 1 per million people

Genes: *LAMA3*, *LAMB3*, *LAMC2*, and *COL17A1*

Mutation:

- *LAMA3* - nonsense, missense, splicing, and insertion/deletion
- *LAMB3* - nonsense, missense, splicing, and insertion/deletion
- *LAMC2* - nonsense, missense, splicing, and insertion/deletion
- *COL17A1* - premature termination codon, nonsense, insertion/deletion, splice junction, and missense variants

Inheritance: autosomal recessive

Characteristics:

- fragility of the skin and mucous membranes, manifest by blistering with little or no trauma
- aplasia cutis congenita, milia, nail dystrophy, scarring alopecia, hypotrichosis, pseudosyndactyly, and other contractures

Prognosis: Depends on the clinical form.

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PGD for Huntington's Disease

Methods:

- 10 embryos survived to blastocyst stage and biopsied on day 5
- DNA amplification with RepliG amplification system
- DNA fragment analysis by triplet repeat primed PCR

Embryo №	Allele 1	Allele 2
1	$(CAG)_{16}$ - normal repeats number	$(CAG)_{22}$ - normal repeats number
2	$(CAG)_{16}$- normal repeats number	$(CAG)_{27}$ -nnormal repeats number
3	$(CAG)_{16}$ –неколкократно увеличен брой повтори	$(CAG)_{18}$ - normal repeats number
4	$(CAG)_{16}$ -normal repeats number	$(CAG)_{22}$ - normal repeats number
5	$(CAG)_{16}$ -normal repeats number	$(CAG)_{22}$ - normal repeats number
6	$(CAG)_{17}$ –repeat expansion	$(CAG)_{22}$ - normal repeats number
7	$(CAG)_{16}$ –repeat expansion	$(CAG)_{22}$ - normal repeats number
II-3	$(CAG)_{16}$ - normal repeats number	$(CAG)_{22}$ - normal repeats number
II-4	$(CAG)_{16}$ - normal repeats number	$(CAG)_{22}$ - normal repeats number
II-7	$(CAG)_{16}$ - normal repeats number	$(CAG)_{22}$ - normal repeats number

- 7 embryos were with normal repeats number
- 3 embryos were with expanded repeats number
- Two embryos were transferred
- The women conceived with one embryo
- Prenatal diagnosis confirmed the PGD results
- The woman delivered normal baby girl

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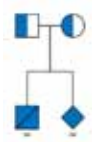
PGD for Epidermolysis Bullosa



Medical history

- Mother - carrier of: *LAMB3*: c.1133-22G>A
- Father - carrier of: *LAMB3:* c.1133-22G>A
- First child– homozygous for the mutation
- Second pregnancy – fetus homozygous for the mutation – termination of the pregnancy
- IVF + PGD

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PGD for Fragile X Syndrome

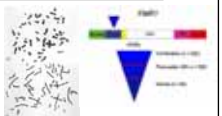
Developmental problems including learning disabilities and cognitive impairment

Prevalence: 1 in 4,000 males and 1 in 8,000 females

Genetics:

- **Gene**. *FMR1*. Locus Xq27.3
- **Gene defect:** a trinucleotide CGG repeat expansion:
  - normal: 5-44 CGG repeats
  - Intermediate : 45-54 CAG repeats - "grey zone"
  - Premutation allele: 55-200 CGG repeats (intermediate and distinct syndrome, fragile X tremor/ataxia)
  - HD-causing full mutation: > 200 CGG repeats
- **Type of inheritance:** X-linked, anticipation

Prognosis: there is no cure



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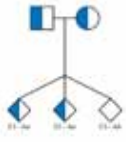
PGD for Epidermolysis Bullosa

Methods

- 3 embryos were biopsied at blastocyst stage on day 5
- DNA amplification with RepliG amplification system
- Allelic discrimination analysis by real time PCR with FRET (Fluorescence resonance energy transfer)



Results

- E1 – heterozygous LAMB3: c.1133-22G>A
- E2 – heterozygous LAMB3: c.1133-22G>A
- E3 – normal G/G

Fresh transfer

- Two embryos were transferred,
- No pregnancy was achieved

Frozen transfer

- One embryo was transferred
- The women conceived
- Prenatal diagnosis confirmed the PGD results
- The woman is still pregnant

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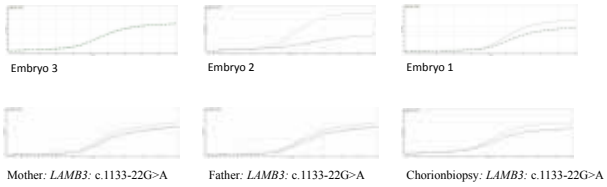
PGD for Epidermolysis Bullosa

- Allelic discrimination analysis by real time PCR with FRET (Fluorescence resonance energy transfer)



Mother: *LAMB3:* c.1133-22G>A Father: *LAMB3:* c.1133-22G>A Chorionbiopsy: *LAMB3:* c.1133-22G>A

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Challenges of Preimplantation Genetic Testing

- IVF is needed
- Misdiagnosis – technical limitations or embryonic mosaicism, "self-correction" of aneuploidies
- PGT does not guaranteed pregnancy after transfer
- Damage to the embryo (below 1%)
- Time-consuming and expensive
- No or few healthy embryos may be found for transfer

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PGD for Beta-Thalassemia

Reduced synthesis of the hemoglobin subunit that results in microcytic hypochromic anemia

Prevalence: The highest incidences are reported in Cyprus (14%), Sardinia (12%), and Southeast Asia

Gene: ***HBB***

Mutation: more than 200 different *HBB* pathogenic variants

Inheritance: autosomal recessive

Characteristics:

- *Thalassemia major (Cooley's anemia)* - severe hemolytic anemia, compensatory bone marrow hyperplasia, failure to thrive, fatigue, jaundice, hepatosplenomegaly, pathological fractures, characteristic facial features.
- *Thalassemia intermedia*. Hemoglobin levels are 70g/l, anemia is moderately severe, splenomegaly is not that prominent.
- *Thalassemia minor.* Heterozygous individuals have no symptoms, but present with mild microcytic hypochromic anemia that can be easily mistaken for iron deficiency anemia..

Prognosis:

- *Thalassemia major - in the past was bleak because of the complications resulting from iron overload due to constant blood transfusions.*

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PGT Advantages

- May help eliminate some genetic diseases in the future
- Eliminates the dilemma of pregnancy termination following unfavorable prenatal diagnosis
- Gives the opportunity of carriers of genetic diseases to have a child free of their particular disease
- To date, there are no reports of increased fetal malformation rates or other identifiable problems

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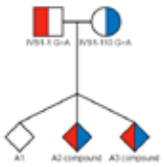
PGD for Beta-Thalassemia

Medical history

- Mother - carrier of: IVS1-110 G>A in *HBB* gene
- Father - carrier of: IVS1-1 G>A in *HBB* gene
- Three natural pregnancies
  - pregnancy loss
  - compound heterozygous affected fetus – termination of the pregnancy
  - compound heterozygous affected fetus – termination of the pregnancy
- IVF + PGD



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Future Directions

Late-onset diseases with genetic predisposition

- Cancers
- Diabetes
- Cardiovascular diseases etc.

Gene-editing in human embryos

Emmanuelle Charpentier, Jennifer Doudna

Shoukhrat Mitalipov

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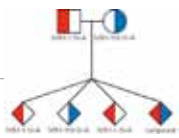
PGD for Beta-Thalassemia

Methods:

- Throphectoderm biopsy, blastocyst stage at day 5
- DNA amplification with RepliG amplification system
- Allelic discrimination analysis by real time PCR with FRET (Fluorescence resonance energy transfer)

Results

Embryo №	result
1	Heterozygous - IVS1-1 G>A
2	Heterozygous - IVS1-110 G>A
3	Heterozygous - IVS1-110 G>A
4	"Compound" heterozygous IVS1-110 G>A/IVS1-1 G>A



Fresh transfer

- One embryo was transferred,
- No pregnancy was achieved

Frozen transfer

- One embryo was transferred,
- No pregnancy was achieved

Frozen transfer

- One embryo was transferred,
- No pregnancy was achieved

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Douglas Adams - 3 Rules That Describe Our Reactions To Technologies

1. Anything that is in the world when you're born is normal and ordinary and is just a natural part of the way the world works.

2. Anything that's invented between when you're fifteen and thirty-five is new and exciting and revolutionary and you can probably get a career in it.

3. Anything invented after you're thirty-five is against the natural order of things.

PREIMPLANTATION GENETIC DIAGNOSIS (PGD) IN A FAMILY WITH WAARDENBURG SYNDROME

Maya Atanasoska

Initial diagnosis

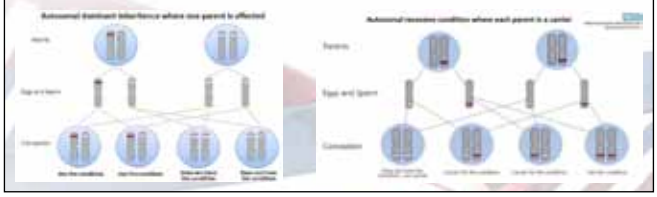
- Next-generation sequencing performed using an Illumina TruSight One gene panel (4813 disease-associated genes)
- Alignment → Variant calling → Variant annotation → Variant filtering
- Nonsense mutation in *PAX3* detected:

*Chr 2: g.223160283T>A, NM_181459.3:**c.415A>T**, NP_852124.1:**p.Lys139Ter** (rs876661317)*

- Novel mutation not reported in the literature
- Genetic diagnosis: Waardenburg syndrome, type 1 or 3

Preimplantation genetic diagnosis (PGD)

- Embryo profiling prior to implantation
- PGD = In-vitro fertilization + Genetic testing
- Prevention of inheritance of pathogenic mutations in the offspring
- First X-linked diseases (1990), now monogenic disorders



Initial diagnosis

Our case: Waardenburg syndrome

- Rare disease
- Four types, several subtypes
- In total, 9 genes associated: *EDN3, EDNRB, MITF, PAX3, SNAI2, SOX10, TYR, WS2B, WS2C*
- Mostly autosomal dominant inheritance
- Variability in penetrance and severity
- Symptoms include:
  - Deafness / Progressive hearing loss
  - Pigmentation changes (e.g. forelock of white hair)
  - Facial malformations (e.g. dystopia canthorum)
  - Abnormalities of the upper limbs
  - Mental retardation (rare)

Co-segregation analysis

- Patient's brother also diagnosed with the disease
- Phenotype more severe:
  - Congenital bilateral deafness
  - Pigmentation changes
  - Blue eyes
  - Hydrocephaly (corrected)
  - Slight intellectual disability
- Targeted NGS sequencing for *PAX3* (exon 3)
- Detection of the mutation in a heterozygous state

Initial diagnosis

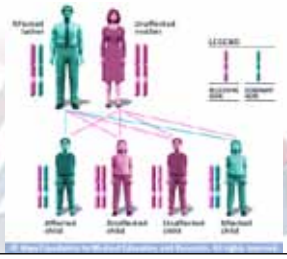
- Patient referred with a clinical diagnosis of Waardenburg syndrome:
  - Unilateral hearing loss
  - Pigmentation changes
  - Facial malformations
  - No intellectual disability

Co-segregation analysis

Several months later…

Genetic counselling in regard to reproduction and disease inheritance

Waardenburg syndrome inheritance pattern



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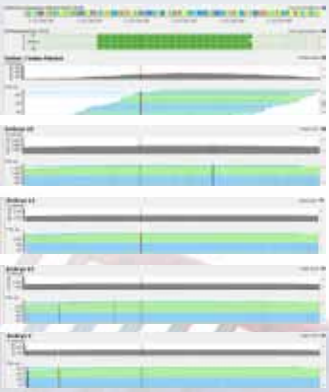
PGD results



Father – **carrier**

Embryo 10 – **healthy**

Embryo 11 - **carrier**

Embryo 15 - **healthy**

Embryo 7 – **unimformative (allele dropout)**

→ no mutation detected, but presence of homozygous alleles not present in the father

Preimplantation genetic diagnosis

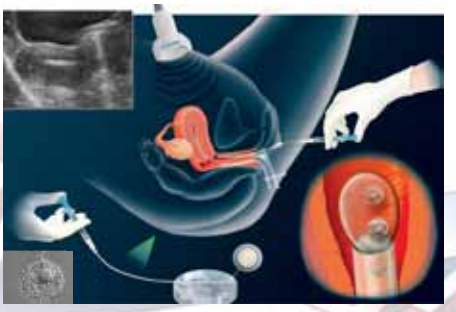
PDG offered to the family
→They agreed!

Design: only test for father's mutation in embryos

We did not test the mother for mutations in WS genes:
- no symptoms
- even if recessive carrier – inheritance in embryos wouldn't be a reason not to transfer

Transfer

One of the healthy embryos was transferred



PGD procedure

Ovarian hyperstimulation
↓
In Vitro Fertilisation / IMSI
↓
Biopsy (day 3) / Vitrification (day 5)
↓
Whole-genome amplification
↓
Amplification of target region
↓
NGS deep sequencing
↓
Analysis → **DIAGNOSIS**

BUT…

Unfortunately, no successful pregnancy occurred.

However, second transfer of second healthy embryo coming soon!

Fingers crossed!!

PGD Procedure

- 17 MII eggs after stimulation
- 12 eggs fertilised
- 9 embryos developed to Day 3
- 7 embryos biopsied on Day 3
- 4 of them developed to Day 5 - frozen
- Target regions (PAX3 exon 3) successfully amplified for all 4 embryos
- Sequencing of 4 target regions

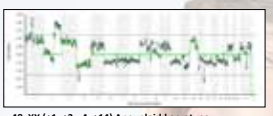
Conclusion

- Preimplantation genetic diagnosis = chance for healthy children
- The genetic defect needs to be elucidated in advance
- Still some limitations – invasive, expensive, not high success rate, freezing of embryos → optimisation required
- Potential to significantly reduce the number of inherited diseases in the future generations

APPLICATION OF NEXT-GENERATION SEQUENCING FOR BALANCED TRANSLOCATIONS IN PREIMPLANTATION EMBRYOS

Momchil Rizov

Results



48, XX (+1; +3; -4; +14) Aneuploid karyotype

48, XXX (+8; -11; +15; +X) Aneuploid male karyotype

44, XX (+8; -14; -16; -17) Aneuploid female karyotype
(sub chromosomal aberrations of chromosomes 2 and 15).

46, XY (+2; -15) Aneuploid male karyotype

Application of next-generation sequencing for balanced translocations in preimplantation embryos

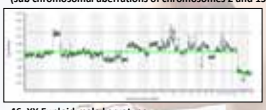
Momchil Rizov

Office for Technology Transfer in the area of Molecular Genetics and Diagnostics (OTTMGD), Medical Centre "ReproBioMed" Ltd. ,Sofia, Bulgaria

Results

46, XX Euploid female karyotype
(sub chromosomal aberrations of chromosomes 2 and 15)

46, XY Eeuploid male karyotype.



46, XY Euploid male karyotype
(sub chromosomal aberrations of chromosome 2)

46, XY Eeuploid male karyotype

Statement of purpose:

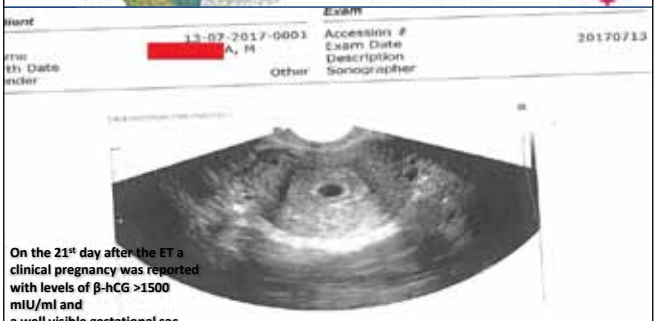
A case which show the importance of next-generation sequencing-preiplatation screening (NGS-PGS) for a couple, where one of the partners is a carrier of balanced translocation.

The Case:

An infertile couple with a series of recurrent implantation failures (RIF) and miscarriages

What was performed:

- Karyotyping by G-banded using standard cytogenetic technique – The woman was reported as a **46, XX, t(2;15)(q2;qter)** carrier;
- a laser-assisted biopsy was performed on trophectoderm *(*day 5);
- Whole genome amplification (WGA) and Next- Generation Sequencing (NGS).



On the 21st day after the ET a clinical pregnancy was reported with levels of β-hCG >1500 mIU/ml and a well visible gestational sac.

The advantages of NGS for PGS:

- NGS can aid the detection of segmental imbalances in embryos as translocations;
- the high-throughput;
- high-resolution;
- as well as higher levels of implantation and pregnancy rates.

Conclusions

- Application of NGS allows screening of all 24 chromosomes. It is objective, ensuring a high level of confidence in the results and subsequent selection of euploid embryos for transfer.

- NGS is one of the most reliable methods for detection of balanced translocations in preimplantation embryos.

STEM CELL TRANSPLANTATION – PRESENT AND FUTURE

T. Chervenkov, L. Angelova

HSCT Future Directions

Target problem: effective treatment of neoplasia

Key considerations

- Neoplastic disease heterogeneity => necessitates targeted approach
- Neoplastic evolution (at the cellular level) => "chase" the neoplasm
- Evolvability is a function of tumor mass => the earlier the better
- Cancer/Leukemia Stem Cells (CSCs/LSCs) (not universal)

HSCT Present

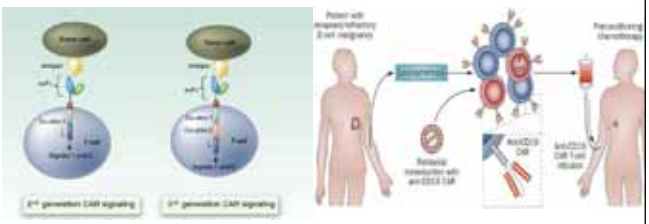
Today's standard of care for

- hematologic malignancies
- congenital or acquired disorders of the hematopoietic system
- therapeutic option in some of the solid tumors

Novel indications such as

- autoimmune disorders

HSCT (Near) Future Directions



Chimeric Antigen Receptor – T cells (CAR-T)

HSCT Present

Two major types:

- Autologous (self)

- Allogeneic (healthy donor)
  - matched related donor
  - haploidentical (partially matched) related donor
  - matched unrelated donor
  - cord blood unit

HSCT Future Directions

Personalized cancer vaccines/immunotherapy

- Against common tumor-associated antigens
- Against patient-specific tumor-associated antigens
  - technological challenge (sensitivity)

HSCT Present

- Autologous HSCT is not by itself therapeutic but allows the administration of high-dose chemotherapy without prolonged bone marrow aplasia

- Allogeneic HSCT has additional therapeutic effect against neoplasia
  - Graft-versus-Leukemia (GvL)/Graft-versus-Tumor (GvT) effects, inseparable from
  - Graft-versus-Host Effect/Disease (GvHE, GvHD)

HSCT Future Directions

Complex logic devices to target cellular function (similarity to oncolytic viruses)

- ssRNA-based logic?
- vehicle?

```
If
(CANCER_STEM_CELL)
Then
    unleash_hell();
Else
    keep_it_quiet();
End If
```

LETTER

Complex cellular logic computation using ribocomputing devices

HSCT – Our Experience

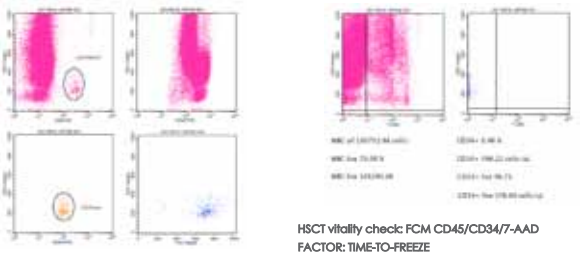
- 70 cryopreservations of peripheral blood stem cells
- 42 patients (age 6 - 68 years)
  - 39 autologous
  - 1 matched related donor (allogeneic)

Cryopreservation protocol
- 10% DMSO (MHH Hannover CTC, Dr Lyubomir Arseniev)
- 5% DMSO (Liseth et al., Cytotherapy (2005) v7, n4, 328-333)

Conclusions

- HSCT is well established procedure
- HSCT is still and will be applicable
- HSCT will change: Cancer vaccines,
  Advanced (Cellular) Therapeutics...

HSCT – Our Experience



HSCT vitality check: FCM CD45/CD34/7-AAD
FACTOR: TIME-TO-FREEZE

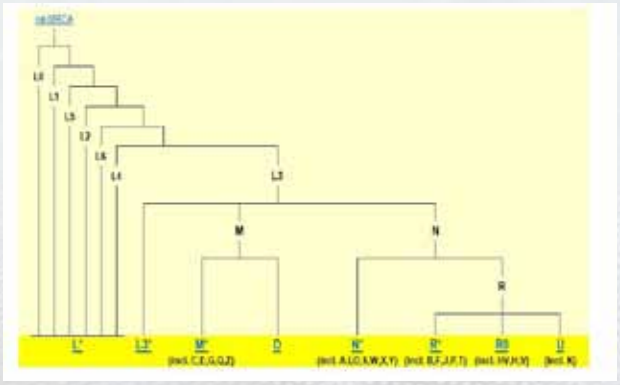
Thank you!

e-mail: tuckata@gmail.com

ANCIENT MTDNA STUDIES ON THRACIAN AND PROTO- BULGARIAN SAMPLES: NEW PERSPECTIVES ON THE ORIGIN OF CONTEMPORARY BULGARIANS

Nesheva D., Modi A., Vai S., Karachanak-Yankova S., Lari M., Galabov A., Caramelli D., Toncheva D.

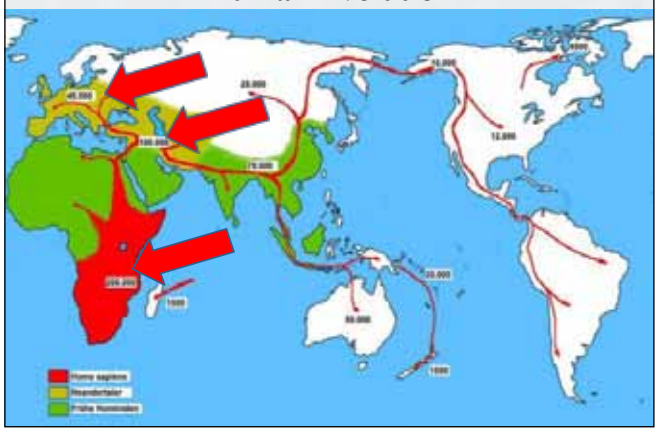
Globally phylogenetic tree of mitochondrial DNA haplogroups



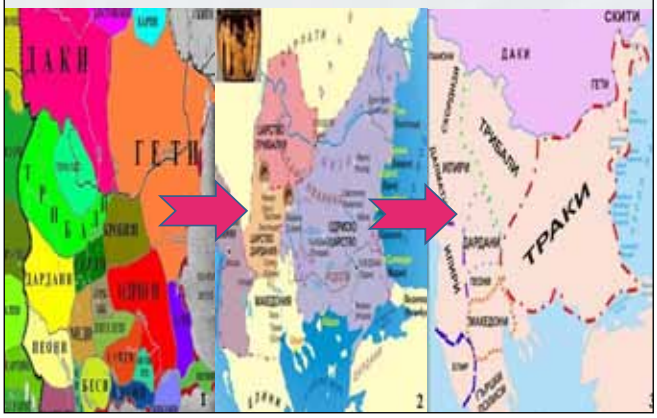
- The Bulgarian past is one of the most discussed topics
- The most effective approach for understanding the past is the study of certain variants in ancient mitochondrial DNA (aDNA)
- Till now no anthropogenetic analysis of ancient samples from the population of the Bulgarian territories has been made
- The study of ancient DNA of Proto-Bulgarians and Thracians is the first of its kind in Bulgaria and will help to reveal their genetic roots

Regions of origin and distribution of mitochondrial DNA haplogroups

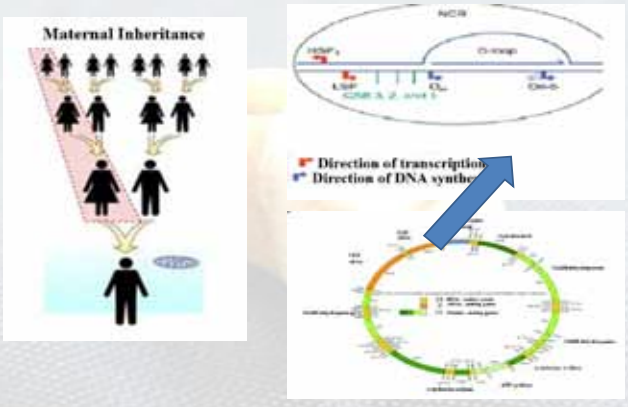
Human Evolution



Thracian populations



Mitochondrial DNA



Proto- Bulgarians

Main goals

Characterization and comparative analysis of mitochondrial DNA profiles of populations on Bulgarian lands from the Thracian and early medieval period.

1. Isolation of ancient mitochondrial DNA from bone remains from different regions of the country dating back to the Thracian and Early Middle Ages.

2. Determination of the sequence of hypervariable segment I (HVS I) in ancient mitochondrial DNA by classical methods of analysis and sequencing.

3. Characterization of whole mitochondrial DNA of ancient samples from the Thracian period by next generation sequencing (NGS - platform).

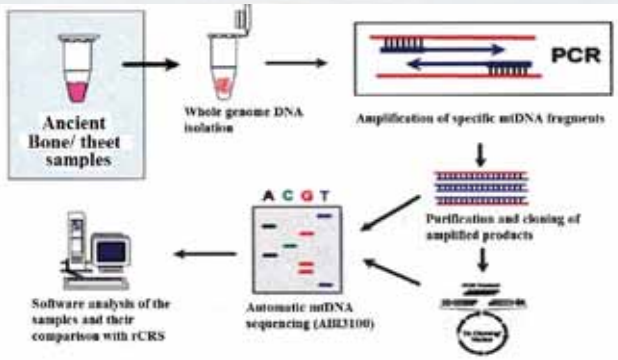
4. Determination and comparison of mtDNA haplogroups of Thracian and Proto-Bulgarian populations.

5. Interpreting the genetic profiles from a historical perspective.

6. Create a database with unique genetic material from ancient samples.

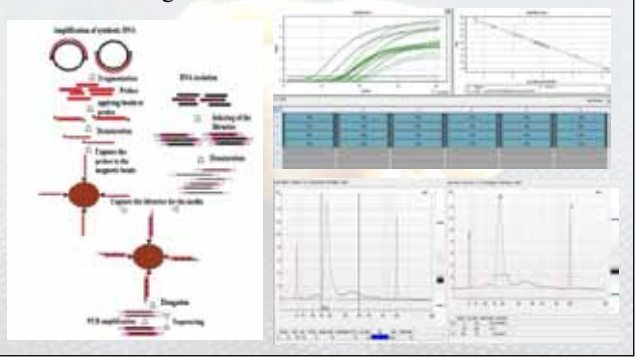
The Classical method

The classical method for analysis of HVS I in ancient samples using Sanger sequencing



Next generation sequencing

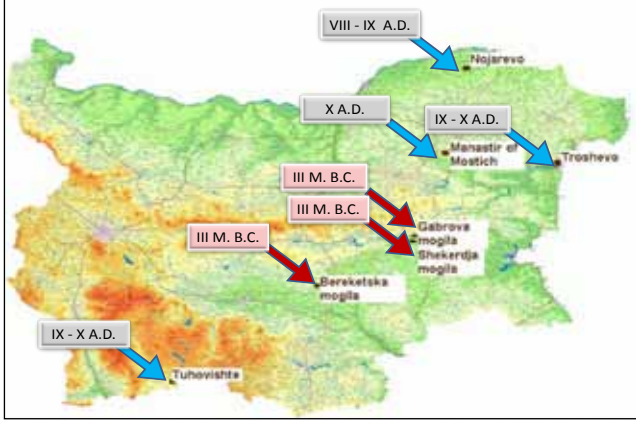
NGS with Illumina MiSeq platform for whole mitochondrial genome.



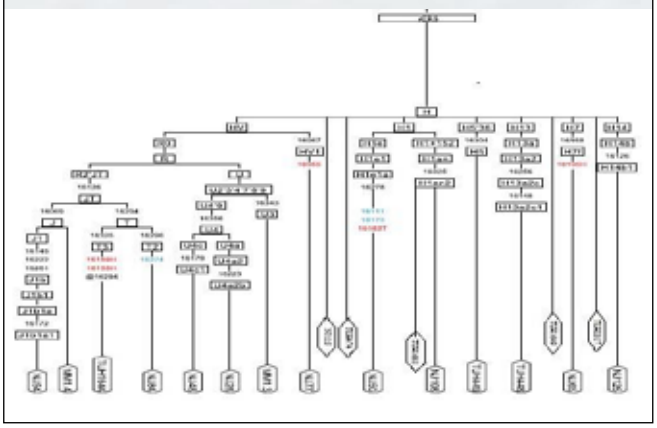
Materials

	Samples	Size	Hg	Quality	Haplotype
1	MM1.2	16024-16383	H11a1	60%	16092C 16142T 16266T 16278T
2	MM1.3	16225-16383	U3	100%	16343G
3	MM1.4	16024-16281	J	100%	16069T 16126C
4	NJ28	16024-16281	U4a2b	100%	16223T
5	NJ48	16024-16386	U4c1	100%	16179T 16356C
6	NJ50	16024-16383	H1	95%	16111T 16173T 16183T 16278T
7	NJ54	16024-16383	J1b1a1	100%	16069T 16126C 16145A 16172C 16222T 16261T
8	NJ77	16024-16383	HV1	100%	16055T 16067T
9	NJ84	16024-16383	T2	91%	16126C 16274A 16294T 16296T
10	NJ108	16225-16383	H1an2	100%	16325C
11	NJ125	16024-16281	H	100%	CRS
12	NJ126	16083-16281	H14b1	100%	16126C
13	TUH448	16024-16281	H13a2c1	100%	16148T 16256T
14	TUH449	16024-16383	H5	100%	16304C
15	TUH474	16024-16281	H	100%	CRS
16	TUH1644	16024-16383	T3	85%	16126C 16189H 16193H 16325C
17	TUH1649	16024-16383	H	100%	CRS
18	TUH1652	16024-16383	T	100%	16126C 16294T
19	TUH1665	16024-16383	H1an2	70%	16219G 16325C
20	TUH2327	16024-16383	H	100%	CRS

Materials

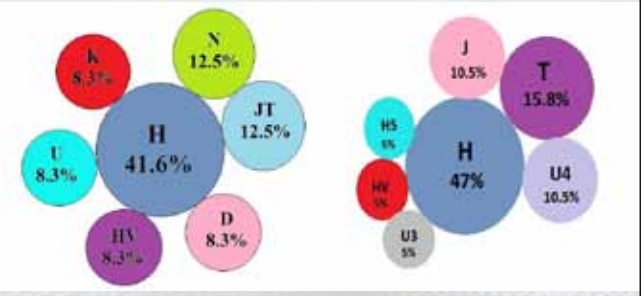


Phylogenetic tree of the Proto- Bulgarians



Samples	Coverage	Coverage	Average	Average
	3-fold	3-fold	coverage	length
BM36	100.00%	100.00%	693.183	55.2
BM40	100.00%	100.00%	147.96	54.27
BM44	99.97%	99.96%	110.14	50.47
BM68	100.00%	100.00%	450.591	52.01
BM69	99.95%	99.93%	174.065	49.45
BM6	100.00%	100.00%	617.925	53.73
BM73	100.00%	100.00%	301.007	43.71
SM24.2	97.96%	96.04%	22.749	45.12
SM8	98.12%	95.55%	32.437	46.46
BM15	100.00%	100.00%	181.217	65.12
BM3	100.00%	100.00%	339.807	68.12
BM59A	100.00%	100.00%	285.411	55.88
BM5	100.00%	100.00%	163.073	65.8
BM76	100.00%	100.00%	207.095	51.84
BM9	99.99%	99.99%	107.772	63.26
BMAG	100.00%	100.00%	187.072	56.21
GM30.2	95.98%	92.28%	14.6	54.28

Percentage distribution of major haplogroups in populations



Thracians **Proto- Bulgarians**

Samples	mapCoverage		ContamMix	Samples	mapCoverage		ContamMix
	6-fold	CAT	MAP authentic	BM15	0.32	0.3	1
BM36	0.34	0.31	0.94	BM3	0.24	0.22	0.98
BM40	0.36	0.32	0.97	BM59A	0.34	0.31	0.95
BM44	0.38	0.37	0.99	BM5	0.24	0.22	0.97
BM68	0.35	0.32	0.97	BM76	0.31	0.29	0.99
BM69	0.35	0.33	1	BM9	0.34	0.32	0.99
BM6	0.32	0.3	0.98	BMAG	0.22	0.2	0.98
BM73	0.43	0.4	0.96	GM30.2	0.37	0.34	0.96
SM24.2	0.43	0.4	0.95				
SM8	0.5	0.46	0.95				

Conclusion

•For the first time, a mtDNA genetic structure of Proto-Bulgarians and Thracians was determined.

•It has been established the genetic position of Proto-Bulgarians and Thracians among other populations. It was found that the Thracian and Proto- Bulgarian mitochondrial gene pool is diverse and belongs to Eurasian groups.

•Thracians are genetically most close to modern Slovaks, Germans and Swiss. A large genetic distance exists between Thracians and contemporary Greek population. Proto-Bulgarians are very close to contemporary Bulgarians, Hungarians, Central Italians and Slovaks.

Samples	HaploFind results	Samples	HaploFind results
	Haplogroups		Haplogroups
BM36	N1a1a1a	BM15	T2b
BM40	T2e2a	BM3	H3ak
BM44	HV0	BM59A	T2b
BM68	K1c1	BM5	H5a1a
BM69	H	BM76	H4a1
BM6	H7a1a	BM9	H7
BM73	H	BMAG	U5b2a1a1
SM24.2	HV1a'b'c	GM30.2	K1c1
SM8	U5a1		

Conclusion

•The theory of common genetic origin of Proto-Bulgarians and contemporary Bulgarians with Altaic and Turkic populations was rejected.

•The presence of polymorphisms (T6126C, C16223T, C16270T and T16362C) associated with high or low risk of development of different diseases was proved in Thracians and Proto-Bulgarians.

•For the first time is optimized a method for the determination of mtDNA haplogroups by optimal sequencing of mitochondrial DNA with a next generation sequencer.

•We created a database of unique genetic material from ancient samples belonging to two major and essential for the Bulgarian history periods.

PCA chart with a comparison of proto-Bulgarians, Thracians , contemporary Bulgarians, populations from Europe and Asia and ancient populations from different periods in the past

Thank You

PARALLEL SYMPOSIUM 4
In collaboration with BACI

Moderators: Mariana Murdjeva, Marta Baleva

- **Rare immune-mediated diseases in Bulgaria – the challenge continues [in Bulgarian]**
  M. Murdjeva, M. Baleva

- **Molecular diagnostics and genetic screening of primary immune deficiencies [in Bulgarian]**
  S. Mihaylova

- **Study of the signaling pathways of T- and B-cell activation in common variable immunodeficiency - sight to the pathogenesis of disease [in Bulgarian]**
  N. Gesheva

Oral presentations:

- **Firazyr (icatibant) for treatment of hereditary angioedema (supported by Shire) [in Bulgarian]**
  T. Beleva-Popova

- **Ataxia telangiectasia – case report [in Bulgarian]**
  P. Yankova

- **Homozygous MyD88 deficiency – case report and literature review [in Bulgarian]**
  N. Spasov

РЕДКИТЕ ИМУНО-МЕДИИРАНИ БОЛЕСТИ В БЪЛГАРИЯ – ПРЕДИЗВИКАТЕЛСТВОТО ПРОДЪЛЖАВА

Мариана Мурджева, Марта Балева

Регистрира се първият в България Експертен център за редки болести - ПИД

- през 2016 г. с решение на Комисия за редки болести (23.2.2016 г.) при определени условия и процедури за регистрация на редките болести, експертни центрове и референтни мрежи за редки болести
- Ръководител: Проф. д-р Е. Наумова
- Локализиран в Клиниката по клинична имунология при УМБАЛ „Александровска" – София

- Стартира първият Национален регистър за пациенти с ПИД:
  - Събира данни за честотата, епидемиологията, клиниката и лечението на пациенти с ПИД в България (> 180)

Поглед към началото...

- Преди повече от 10 години БАКИ прие за един от водещите приоритети в дейността си:
  - диагностиката,
  - лечението и
  - регистрацията на ПИД като редки имуно-медиирани болести
- Първите инициативи на БАКИ в тази област са свързани с:
  - диагностиката и лечението на ПИД
  - обучението на пациенти, лекари и обществото за ПИД

Постижения на БАКИ, свързани с обучението за ПИД

- Цел на обучителните програми
  - Да повишат информираността на студенти, лекари, мед. специалисти и пациенти върху ПИД

Проведени обучения:
- Летни училища за пациенти с ПИД – в Пловдив (2014 - HAE), Цигов чарк (2016 – възрастни , 2017 - деца)
- Семинари за ОПЛ и мед. специалисти – в София и Плевен (април 2017)

Фокус върху информираността на обществото

– чрез медии – ТВ, радио, интернет, публикации

- всяка последна седмица на м. април в рамките на Световната седмица за първичните имунни дефицити и около Световния ден на имунологията (29.04) - безплатни прегледи на пациенти

Настоящи постижения на БАКИ в диагностиката и лечението на ПИД

- Консенсус за диагностика и лечение на ПИД:
  - предложен от Национална работна група за ПИД през 2010 г.
  - основа на КП „Лечение на пациенти с доказани ПИД"
- Установени имунологични диагностични тестове за ПИД, включени в НРД – НОЗК
- Установена и реимбурсирана заместителна терапия:
  - IVIG и SCIG
  - C1- естеразен инхибитор (Ruconest и Berinert)

Научни изяви и работни срещи с фокус ПИД

- 4 работни срещи, 1 кръгла маса и 1 симпозиум са организирани в рамките на Националните конференции по редки болести в Пловдив (2008, 2010, 2012, 2013, 2014, 2017 г.).

- Национална конференция ПИД и автоимунитет – 2012 г. , Цигов чарк, с участие на чуждестранни експерти – prof. L. Marodi, Dr. C.Picard, Dr. K. Warnatz

- Сесия „ПИД" в рамките на Юбилейната Национална Конференция на БАКИ– 2016 г. В София с участие на prof. Cant (президент на ESID)

Създадоха се два Центъра за ПИД в България

- В УМБАЛ „Александровска" – София и УМБАЛ „Св. Георги"-Пловдив
- В тях работят имунолози, педиатри, микробиолози на функционален принцип, обслужвайки пациенти по КП, както и амбулаторно болни

- Има специализирани комисии за насочване към извън-болнично лечение на пациенти със заместителна терапия с IVIG, SCIG и C1-INH.

Сътрудничество с чуждестранни партньори

- Участие в HAENETWORK project (2005-2008):
  - Координиран от проф.Dr. H. Farkas от Hungarian Angioedema Center в Semmelweiss Institute,Будапеща
  - За подобряване на диагностиката и лечението на пациенти с HAE

- Участие в J Project:
  - България е присъединена от 2005 г.
  - Координиран от Prof. L. Marodi
  - За подобряване на диагностиката и лечението на болни с ПИД и повишаване на ангажираността на обществото върху ПИД
- Участие на представители на БАКИ в ESID и HAE научни форуми и експертни срещи в чужбина
- Колаборация с Jeffry-Model Centers; открит JMC в Клиника по клинична имунология на УМБАЛ „Александровска"-София, 2015 г.
  - Цел: да разпространява актуална информация за ПИД сред пациентите и обществото

Партньорства на национално ниво

- **Педиатрична асоциация**
- **МЗ, НЗОК***
- **Пациентски асоциации***
- **Институт по редки болести**
- **Фармацевтични компании**

ЗАКЛЮЧЕНИЕ

- През последната декада БАКИ постигна редица **успехи** в диагностиката, лечението и обучението на ПИД:
  - Реимбурсация на лечението с IVIG, SCIG,C1 INH от 2013г. чрез НЗОК
  - Включване на КП „Лечение на пациенти с доказани ПИД“ от 2013 г.
  - Клинична процедура за обучение на пациенти, прилагащи субкутанни препарати (от 2016 г.)
- Създадоха се **експертни центрове за ПИД**, част сме от програмата J PROJECT, както и от международната мрежа за ПИД центрове – Jeffry Model.
- **Основните предизвикателства** в бъдещата дейност са свързани с въвеждане на генетичните тестове за скриниране на ПИД, достъп до генетична диагноза и точната регистрация на пациентите.

Бъдещи предизвикателства, свързани с ПИД в България

- **Въвеждане на генетичен скрининг за ПИД на новородени**
- **Затруднения в потвърждаване на генетичната диагноза**
- **Регистриране на пациентите с HAE**
- **Регистриране на други препарати за заместителна терапия на ПИД**

Ето защо програмата на днешната сесия е фокусирана върху

- Нови възможности за молекулярна диагностика и генетичен скрининг на ПИД
- Собствени проучвания и резултати върху патогенезата и лечението на някои ПИД - CVID, атаксия-телеангиектазия, MyD88 дефицит
- Icatibant - за лечение на HAE

МОЛЕКУЛЯРНА ДИАГНОСТИКА И ГЕНЕТИЧЕН СКРИНИНГ НА ПЪРВИЧНИ ИМУННИ ДЕФИЦИТИ

Снежина Михайлова, Елисавета Наумова

Подобряване диагностиката на ПИД

- Стандартизиране алгоритмите за диагностика и терапия на ПИД
- Да се осигури достъпна адекватна диагноза за всички потенциални пациенти с ПИД в България
- Внедряване на нови диагностични подходи като:

функционални тестове антитялов отговор, DHR test, Phago Test, T клетъчна активация (CD3/CD28) и др.

Протеинна експресия

Молекулярни тестове: RT-PCR, химеризъм, HLA типизиране чрез NGS, секвениране на единични мутации и гени

Разширени генетични тестове на ПИД чрез новогенерационните технологии на секвениране

Скрининг на новородени чрез TREC/KREG технологии

Функционален високоефективен център за обучение, развитие и подобряване на диагностиката, лечението и грижите за пациенти с първични имунодефицитни заболявания

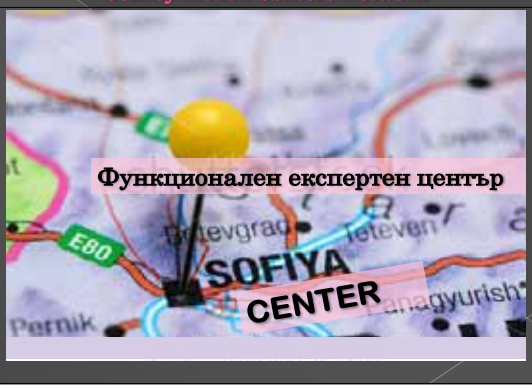
Определяне на ПИД експертни центрове и вписването им в Регистъра на МЗ чрез Комисията за регистриране на редките болести, съгласно наредба No 16/2014г., ще позволи включването им в европейските референтни мрежи за диагностика, консултация и терапия.

Национален регистър на пациентите с ПИД

	2011	2012	2013	2014	Август 2017
# пациенти, идентифицирани с ПИД	106	120	127	146	201
# брой пациенти, идентифицирани с HAE	76	76	76	77	78
# брой пациенти, лекувани с C1 инхибитор	-	-	17	17	40
# брой пациенти, лекувани с IG	13*	3*	8	19	24
# IVIG	-	-	5	5	1
# SCIG	-	-	3	14	23
# брой пациенти, лекувани с имуномодулация **	-	-	-	6	8

- Спорадични инфузии
- ** Чрез протокол

Jeffrey Modell Centers Network



Човешки генетични вариации и методи за откриване

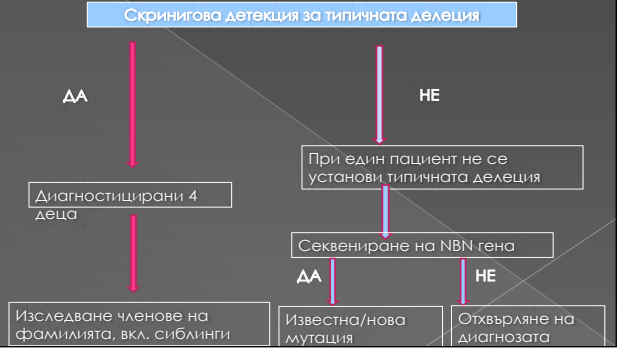
14(10): 468

Основни цели

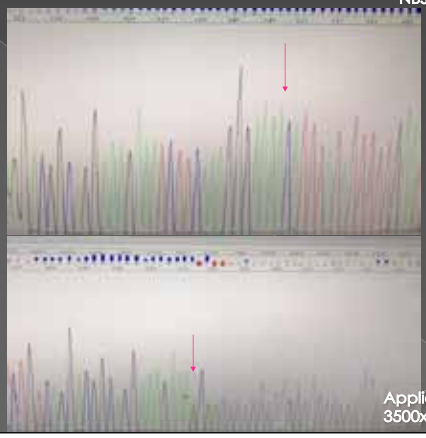
1. Образование и насърчаване
2. Повишаване на информираността за ПИД в българското общество
3. Подобряване на ПИД диагностиката
4. Грижа за пациента
5. Регистър на пациентите
6. Взаимодействие и сътрудничество с Пациентската организация

Молекулярна диагностика на единични гени/мутации по Sanger

Nijmegen breakage syndrome NBS/NBN ген, 657del5 (exon 6)



NBS/NBN 657del5



Applied Byosystems 3500xL Dx

Защо NGS при ПИД

- Изключителна хетерогеност и голям брой нозологични единици.
- Липса на корелация генотип-фенотип.
- Генетична плейотропност и генетична хетерогенност.
- Хетерогенен модел на унаследяване.
- Динамика в броя на новооткритите генетични варианти в гени, асоциирани с ПИД или в нови гени.
- Вариабилна експресия и непълна пенетрантност.

Известни гени, свързани с ПИД

NGS

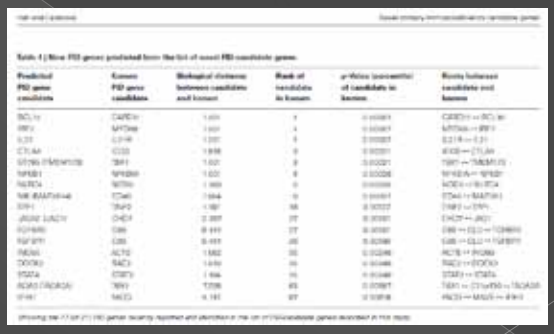
- Таргетен панел

290+17 нови гена за ПИД

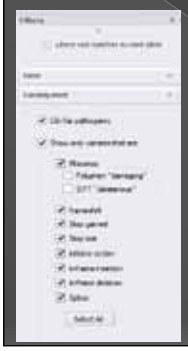
С изключение на:
IKAROS, FLH (NOTO), CLEC6A, CIAS1, LRBA, REXAP, STAT5A, CARD11, RPP30, RHOH, LCK, MALT1, IKBKB, RTEL1, POLE1, TTC7A, TCF3, TWEAK, NFKB2, PRKCD, TNFRSF6B, ISG15

TruSight One Sequencing Panel, Illumina

Нови кандидат-гени, свързани с ПИД



Стратегия за филтриране на биоинформационните данни при ПИД



http://exac.broadinstitute.org

https://mseqdr.org/hpo_browser/ Human pnenotype ontology

dbSNP database

- Идентифицирани са нарастващ брой нови генетични дефекти, лежащи в основата на първични имунодефицити (ПИД) увеличавайки броя им до повече от 250 добре дефинирани синдрома.
- Технологиите на секвенирането от следващо поколение (NGS) и подходящите стратегии за филтриране допринесоха за тази бърза еволюция, осигурявайки възможност за бързо и едновременно анализиране на голям брой гени или целия екзом.

Резултати: Т клетъчен имунен дефицит

- Момиче на 13 г.
- На двудневна възраст е установена сърдечна малформация- транспозиция на големите съдове, междукамерен дефект и коарктация на аортата, корегирани оперативно. По време на сърдечната операция в оперативния протокол е описано извършване на тимектомия.
- На три годишна възраст е диагностицирана бронхиална астма, за която провежда системно лечение. От същата възраст започва изява на чести инфекции, свързани основно с възпаление на ГДП, като се съобщава за прекарана пневмовния.
- През 2011 г.-установен намален слух, за което подлежи на протезиране. С оптично корегиран астигматизъм.

Абс. брой Ly 0.803 x10³ кл./µl

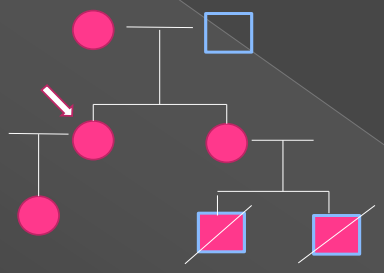
Клетъчна популация	Резултат		Референтни граници	
	%	кл./µl	%	кл./µl
Общи Т лимфоцити (CD3+)	54	434	66 ÷ 76	1430 ÷ 2630
Активирани Т лимфоцити (CD3+DR+)	2	16	9.5 ÷ 17	15 ÷ 129
Хелперно-индуцерни Т лимфоцити (CD3+CD4+)	39	313	33 ÷ 41	730 ÷ 1630
Супресорно-цитотоксични Т лимфоцити (CD3+CD8+)	13	104	27 ÷ 35	491 ÷ 1079
CD4/CD8 индекс	3.0		1.0 ÷ 2.4	
В- лимфоцити (CD19+)	32	257	12 ÷ 22	60 ÷ 320
NK клетки (CD3-CD16&56+)	10	80	9 ÷ 16	200 ÷ 300
NKT клетки (CD3+CD16&56+)	3	24		
Т н/кл NK (CD57+CD8-) клетки	25	201		64 ÷ 352
Т н/кл NK (CD57+CD8+) клетки	3	24	12 ÷ 24	70 ÷ 277

Заключение: Перистира лимфоцитоза с понижение на абсолютния брой на почти всички изследвани клетъчни популации. Понижени общи Т лимфоцити основно за сметка на супресорно-цитотоксичната им субпопулация. Компенсаторно повишен процент В-лимфоцити. НК клетки в референтни граници по процент.

ZAP70 в CD3+ лимфоцитите- 94.7%

Резултати: хронична грануломатозна болест

- Жена на 38 години



Клетъчна популация	Резултат
	%
Неактивирана цяла кръв CD3+CD69+ от CD3 клетки	0.56
Активирана с PHA цяла кръв (18ч) CD3+CD69+ от CD3 клетки	65
Неактивирани PBMC CD3+CD69+ от CD3 клетки	0.2
Активирани PBMC с antiCD3/ CD28 dynabeads CD3+CD69+ от CD3 клетки	30.3

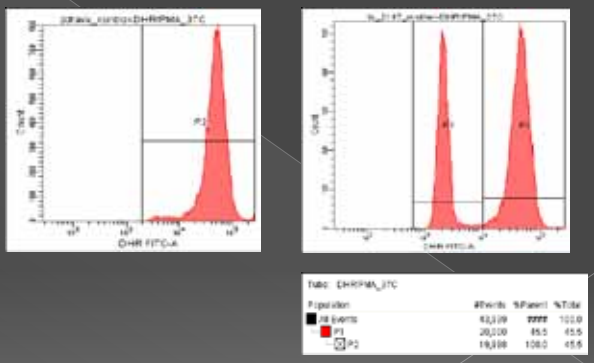
Заключение: Нормална експресия на CD69 върху Т лимфоцити при стимулация с PHA. Понижена експресия на CD69 върху Т лимфоцити при стимулация с anti CD3/CD28 dynabeads магнитни сфери.

Не се установяват микроделеционни синдроми /изследване в Национална генетична лаборатория

От хуморалния имунитет бяха наблюдавани слабо повишени стойности на ИгМ на фона на нормални серумни нива на ИгГ, ИгА и ИгЕ, но при леко понижена стойност на ИгГ1.

Нормален титър на антитела, образувани в отговор на прилаган в миналото тетанус-антитоксин

DHR тест

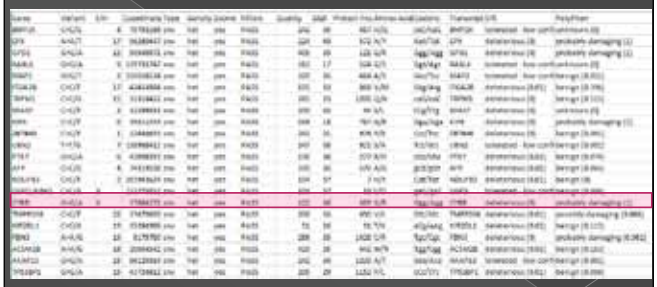


? TruSight One Sequencing Panel, Illumina

WES, WGS ? или вторичен Т клетъчен ИД?

Illumina VariantStudio 3.0

TruSight One Sequencing Panel, Illumina



Illumina VariantStudio 3.0

Неописани варианти, в гени, свързани с ПИД

- AP3B1, c.77425028, missense/ имунна дисрег.
- CTSC, c.88068256, deletion, splice reg./фагоц. дефект
- PMS2, c.6037057, deletion, splice reg./синдроми с ИД
- MRE11A, c.94212930, insertion, splice reg./синдроми с ИД

Недостатъци NGS технологиите при ПИД

- В ДД отношение от значение е познаването на ПИД гени, свързани с мутации, причиняващи заболяване, които не са добре откриваеми от NGS. В такива случаи се препоръчва консултация с клиничен и/или молекулярен генетик, за преценка на най-подходящ тест.
- Молекулярната диагноза предшества разбирането на патофизиологията, водеща до клиничната изява при някои пациенти.
- При установяване на потенциални нови гени или фенотипове, са необходими функционални изследвания и модели, за да се докаже биологичният и патогенен ефект на варианта (ите).
- Методи за измерване на въздействието на епигенетичните фактори като ДНК метилиране и хистонова модификация или епистаза все още не са налични за използване при диагностични оценки.

Пилотен проект за скрининг на вродени Т клетъчни имунни дефицити

Синдроми с променливо засегнат клетъчен имунитет, включително тежко протичащи

- 22q11.2 deletion syndrome
- Синдром на CHARGE
- Синдром на Jacobsen
- Trisomy 21
- RAC2 доминантна интерфериращa мутация
- Хипер-IgE синдром, дължащ се на DOCK8 дефицит
- Cartilage hair hypoplasia

Живородени общо за страната по години

2013	2014	2015	2016
Живородени	Живородени	Живородени	Живородени
66 578	**67 585**	**65 950**	**59 633**

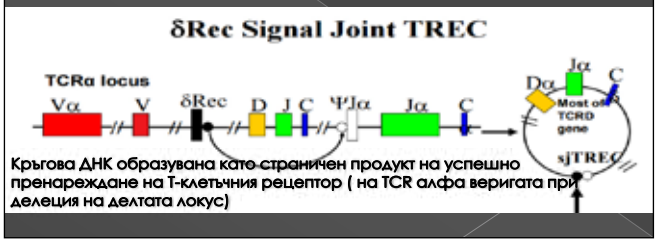
Очаквана честота на SCID/CID : 1/50,000-1/100,000
Di George : 1/ 4 000

Ниски Т-клетки, като последица от други причини

- Неонатална сърдечна хирургия
- Неонатална левкемия
- Гастроинтестинални малформации
- Изключителна незрялост (възстановяване с времето)
- Вътреутеринно изоставане в растежа
- Инфекции при майката (HIV)

T-cell receptor excision circles (TRECs)

- TRECs са малки кръгови ДНК молекули, генерирани в Т-клетките по време на зреенето им в тимуса, следствие реаранжирането на гените за ТКР. Тяхното присъствие показва съзряването на Т-клетките.
- TRECs са намалени, при имунни дефицити, засягащи Т клетките



Кръгова ДНК образувана като страничен продукт на успешно пренареждане на Т-клетъчния рецептор (на TCR алфа веригата при делеция на делтата локус)

Заболявания, с нормални TRECs, но с нарушена функция на Т клетките

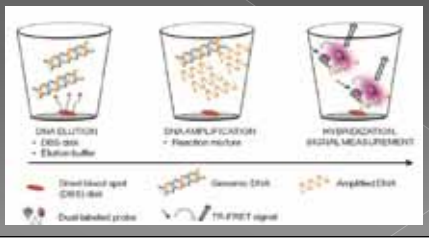
- Zap70 дефицит
- MHC Class II дефицит
- Дефицит на NF-kappa-b модулатора (NEMO)
- ADA дефицит с късна изява

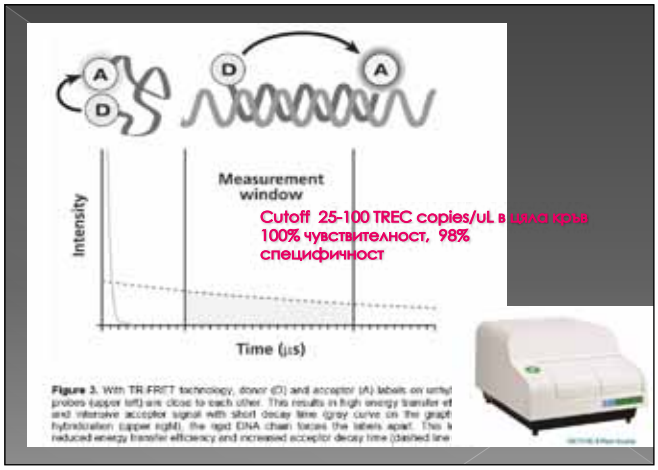
ИД, които се откриват посредством ниски или липсващи TRECs

- Типични SCID, поради дефект в IL2RG (X-linked), ADA, IL7R, JAK3, RAG1, RAG2, DCLRE1C (Artemis), TCRD, TCRE, TCRZ, CD45
- Leaky SCID или синдром на Omenn дължащи се на мутации в SCID гените, но без напълно увредена функционалност на гена
- Варианти на SCID (CID), с персистиращи ниски Т-клетки, но без дефект в познатите гени за SCID
- Неуточнена Т клетъчна лимфопения

EnLite™ Neonatal TREC assay
EnLite Neonatal TREC

- EnLite Neonatal TREC е комбинация от PCR базирана амплификация на нуклеинови киселини и детекция в реално време посредством фруоресцентно резонансен енергиен трансфер (TR-FRET). Тестът открива две цели: TREC, маркера на SCID и бета-актин, който се използва като вътрешен контрол във всеки отделен тест.

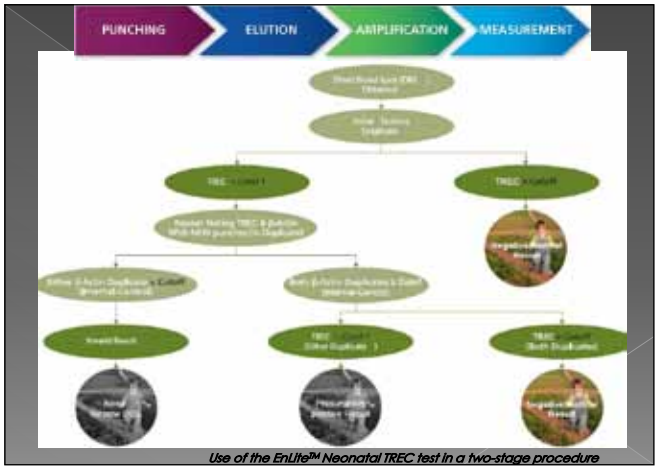




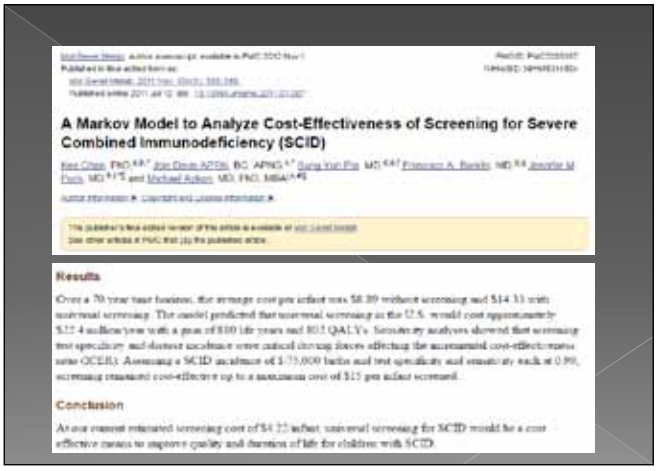
АЛЕКСАНДРОВСКА

Проспективно пилотно проучване

- Валидиране на методиката
- Ретроспективна детекция на TRECs от проби на пациенти с доказан ПИД или с неуточнена Т клетъчна лимфопения
- Тестване на прибл. 1000 проби на новородени
- Анализ на данните
- Оценка на ефикасността
- Тясна колаборация със съществуващите скринингови програми
- Съгласуване с нормативната база
- Финансово обезпечаване на програмата



Use of the EnLite™ Neonatal TREC test in a two-stage procedure

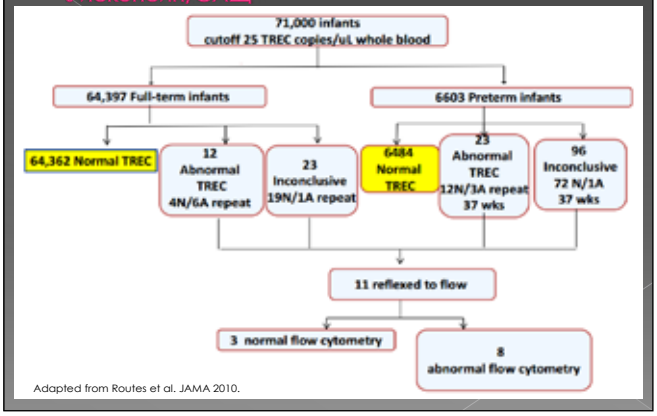


A Markov Model to Analyze Cost-Effectiveness of Screening for Severe Combined Immunodeficiency (SCID)

Results

Conclusion

Резултати от пилотно проучване Уисконсин/САЩ



Adapted from Routes et al. JAMA 2010.

АЛЕКСАНДРОВСКА

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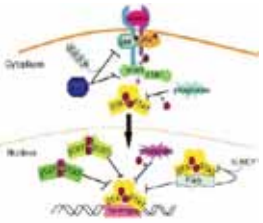
ИЗСЛЕДВАНЕ НА СИГНАЛНИ ПЪТИЩА НА Т- И В-КЛЕТЪЧНА АКТИВАЦИЯ ПРИ ПАЦИЕНТИ С ОБЩ ВАРИАБИЛЕН ИМУНЕН ДЕФИЦИТ ПОГЛЕД КЪМ ПАТОГЕНЕЗАТА НА ЗАБОЛЯВАНЕТО

Н. Гешева, С. Михайлова, С. Лесичкова, А. Михайлова, Е. Наумова

STAT (Signal transducer and activator of transcription) протеините са седем членна фамилия от цитоплазмени транскрипционни фактори, които осъществяват предаването на сигнали от цитокини, хормони и растежни фактори до ядрото на клетката. STAT протеините контролират основни процеси, включващи клетъчната пролиферация, диференциация, функция и оцеляване.



Цел на проучването:

Да се проучат различни лимфоцитни вътреклетъчни сигнални пътища, включващи JAK/STAT сигналната каскада и митоген активираните протеин кинази (MAPK) при пациенти с CVID

Общ вариабилен имунен дефицит

Общият вариабилен имунен дефицит (Common variable immunodeficiency – CVID) включва хетерогенна група заболявания, характеризиращи се с:

•Значително понижено серумно ниво на ИгГ в комбинация с ниско ниво на ИгА и/или ИгМ, нарушен или липсващ антителен отговор към инфекции или при имунизация, както и изключване на друг дефиниран имунен дефицит

•тежки, рецидивиращи инфекции

•склонност към автоимунни прояви и грануломатоза

•склонност към онкологични заболявания

Той е най-честият хуморален имунен дефицит изискващ провеждане на имунозаместителна терапия.

Материали и методи:

Изследваха се кръвни проби от 10 пациента (4 мъже и 6 жени, на средна възраст – 43.7 години) с диагноза общ вариабилен имунен дефицит и 10 клинично здрави неродствени индивида

Изследването на вътреклетъчните сигнални пътища (MAPK и JAK/Stat) на Т и В-клетъчна активация се извърши с BD Phosflow T-cell activation kit

Чрез използване на многоцветен флоуцитометричен анализ се оцени базалната и постстимулационната вътреклетъчна експресия на различни фосфорилирани сигнално-трансдюсерни молекули (STAT3, STAT5, STAT6) и протеин кинази (p38MAPK, Erk1,2).

Патогенеза на общия вариабилен имунен дефицит ?

Клиничната хетерогенност на заболяването предполага множество имунорегулаторни дефекти, резултиращи в нарушена имуноглобулинова синтеза

В-клетъчни промени

TLR7 и TLR9

Понижение на паметови В-лимфоцити

Относителна или пълна загуба на плазматични клетки в КМ

Цитокини – IL 21 е ключов цитокин за създаване, поддържане и регулиране на качеството на антителния отговор

Нарушена В-клетъчна диференциация с нарушена имуноглобулинова продукция

Материали и методи:

Активатор на клетъчния отговор	Крайна концентрация на активатора	Таргет на действие на активатора
PMA	2 µg/ml	ERK1/2, p38MAPK
IL6	100 ng/ml	Stat3
IL2	100 ng/ml	Stat5
IL4	100 ng/ml	Stat6

- Третиране на клетки със специфични активатори
- Пермеабилизиране и фиксиране на клетките
- Маркиране на клетките с моноклонални антитела
- Флуоцитометричен анализ на изработените проби

Патогенеза на общия вариабилен имунен дефицит ?

Т-клетъчни промени

Понижен CD4/CD8 индекс, поради понижаване на CD4+ клетките или поради увеличаване на CD8+Т лимфоцитите

Понижени Treg

Понижено количества TRECs (T cell receptor excision circles)

Понежени Аг праймирани Т лимфоцити

Понижена Т лимфоцитна пролиферация след стимулация с митогени и антигени
Понижена експресия на CD40L от активирани Т лимфоцити

Нарушена цитокинова продукция от Т лимфоцитите

Нарушена Т клетъчна сигнална трансдукция

Флуоцитометричен анализ на изработените пробите

Флуоцитометричният анализ на пробите от пациентите и здравите контроли се извърши на FACS Canto II с помощта на FACS Diva софтуер. Оформиха се електронни прозорци (gates) на субпопулациите от лимфоцити въз основа на линейно-специфичните антигени на диференциация
CD3+CD4+ (хелперно-индусерни Т лимфоцити)
CD3+CD8+ (супресорно-цитотоксични Т лимфоцити)
CD19+ (В-лимфоцити)

Експресията на вътреклетъчните фосфорилирани протеини в резултат на активиране на сигналните пътища се определи чрез хистограма на Alexa Fluor 647 анти-фосфопротеиново антитяло. За всяка от гейтираните популации се анализираха процентът и геометричният среден интензитет на флуоресценция (geometric mean fluorescence intensity - geo MFI). Допълнително бе изчислен индекс на геометричния среден интензитет на флуоресценция (geo MFI index) като съотношение: geo MFI на стимулирани клетки/geo MFI на нестимулирани клетки.

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Резултати

STAT3 в Т лимфоцити

- Диференциация на наивните CD4+Т лимфоцити в Th17 клетки и фоликуларни Th
- STAT3 във взаимодействие със STAT6 подпомага Th2 диференциацията като се свързва към Th2-асоциирани генетични локуси
- IL6-STAT3 активацията води до IL21 продукция

STAT3 в В лимфоцити

- STAT3 сигнализацията в В лимфоцити е необходима при формиране на герминативните центрове
- Ключова роля в генерирането на ефекторни В лимфоцити от наивни прекурсори
- Потенциален регулатор на В клетъчната диференциация

Инактивиращи STAT3 мутации намаляват значително броя на функционалните Аг специфични паметови В лимфоцити и води до невъзможност за диференциране на наивните В лимфоцити в плазматични клетки в отговор на IL 21 стимули

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Резултати

STAT6 в Т лимфоцити

- Критична роля в диференциацията на Th2 и IL4 стимулираните пролиферативни отговори (STAT6 регулира експресията на GATA3)
- Роля в развитието на IL9 продуциращите Т лимфоцити
- В CD8 лимфоцитите STAT6 е необходим за Tc2 диференциране

STAT6 в В лимфоцити

- Води до превключване на класа към синтез на ИгЕ и ИгГ1
- Води до експресия на повърхностни молекули отговорни за Аг презентация от В лимфоцитите (MHC II, CD80, CD86, както и експресия на CD23 и IL-4Rα)

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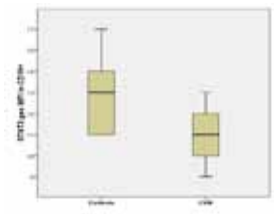
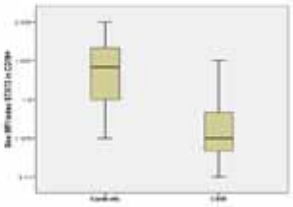
Резултати

Понижена IL6 индуцирана STAT3 активация, както и понижен STAT3 активационен индекс в CD19+ В лимфоцити на пациентите с CVID в сравнение със здравите котроли



нива (geo MFI, p=0.024) на STAT3 фосфорилиране при здрави контроли и пациенти с CVID



индекс (geo MFI index, p=0.01) на STAT3 фосфорилиране при здрави контроли и пациенти с CVID.

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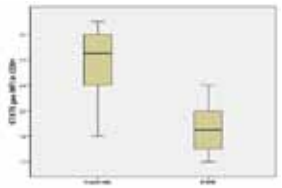
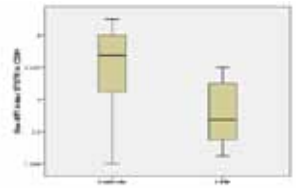
Резултати

Понижена индуцирана STAT6 активация на CD8+ Т клетки и понижен STAT6 активационен индекс на CD8+ Т-клетъчна популация



STAT6 сигнализиране в CD8+ Т клетки. Нива (geo MFI) на STAT3 фосфорилиране при здрави контроли и пациенти с CVID (p<0.001)



STAT6 сигнализиране в CD8+ Т клетки. Индекс (geo MFI index) на STAT6 фосфорилиране при здрави контроли и пациенти с CVID (p=0.045)

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Резултати

STAT5 в Т лимфоцити

- Ключова роля в развитието на γδ Т клетките, регулирайки TCRγ генното реаранжиране
- IL7/STAT5 сигналния път води до CD8 Т-клетъчна диференциация в тимуса
- IL2/STAT5 води до генериране на ефекторни CD8+Т клетки, както и диференциране в паметови клетки
- Роля в развитието на Th1, Th2, Th9, Treg
- Блокира развитието на Th17 и Tfh

STAT5 в В лимфоцити

- IL7/STAT5 сигнализацията контролира В лимфопоезата (IL7 играе роля в пролиферацията, оцеляването и диференциацията на В клетъчните прогенитори и регулира реаранжирането на гените за IgH веригата)
- Пролиферация и оцеляване на про-В клетките

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Резултати

ERK (extracellular signal-regulated kinases) в Т лимфоцити

- ERK2 благоприятства диференциацията в Th1
- ERK2 потиска диференциацията на CD4+ наивните клетки в Th2 и Treg

ERK (extracellular signal-regulated kinases) в В лимфоцити

- ERK сигналният път е необходим за pre-BCR-индуцираната диференциация на pro-B клетките в pre-BII клетки
- ERK играе ключова роля в диференциацията на В клетките в антитяло продуциращи плазматични клетки

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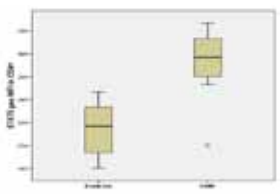
Резултати

Повишена IL-2 индуцирана STAT5 активация и повишен STAT5 активационен индекс в CD4+ клетки в съчетание с по-ниската спонтанна STAT5 активация на CD8+ лимфоцитите, но повишен STAT5 IL-2 зависим активационен индекс в CD8+Т клетките



STAT5 сигнализиране в CD4+ Т клетки. Нива на STAT5 фосфорилиране при здрави контроли и пациенти с CVID (p=0.001)

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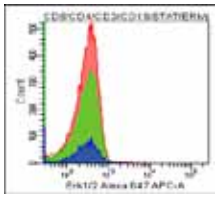
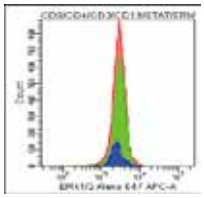
Резултати

Повишена спонтанна (базална) Erk активация на CD19+ В клетки и на CD4+ хелперно-индусерни Т лимфоцити





Хистограма на базална Erk1/2 активация (нестимулирани лимфоцити) и PMA индуцирано Erk1/2 фосфорилиране
Червена хистограма – лимфоцити; зелена хистограма - Т клетки; синя хистограма – В-клетки

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Дискусия

1. Пониженатa индуцирана с IL6 STAT3 активация в В лимфоцитите, е възможно да бъде отговорна за нарушено формиране на герминативни центрове и съответно В клетъчна функция

2. Повишената IL-2/STAT5 индуцирана активация и повишен STAT5 активационен индекс в CD4+ клетките е възможно да потиска диференциацията на наивните Th в Tfh, имащи отношение към В клетъчната диференциация и функции

3. Пониженатa индуцирана STAT6 активация на CD8+ Т клетки и понижения STAT6 активационен индекс на CD8+ Т-клетъчна популация е възможно да е свързана с намален капацитет на цитотоксичните Т лимфоцити на пациентите към Tc2 поляризация

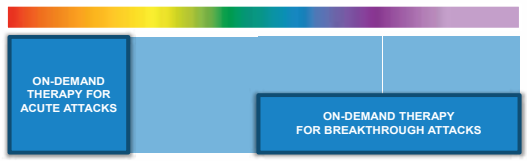
4. Повишената спонтанна (базална) Erk активация на CD19+ клетките би могла да е отговорна за развитие на анергия от В лимфоцитите

5. Повишената базова Erk сигнализация в CD4+ Т лимфоцитите би могла да е една от причините за поляризация на имунния отговор при пациентите с CVID към Th1, както и за нарушения в диференциацията и функцията на Treg

FIRAZYR® (ICATIBANT) FOR ON-DEMAND TREATMENT OF ACUTE HAE ATTACKS

Todorka Beleva-Popova

Firazyr (icatibant) for on-demand treatment of HAE attacks[1]



Firazyr (icatibant) is indicated for symptomatic treatment of acute attacks of hereditary angioedema (HAE) in adults (with C1-esterase-inhibitor deficiency)[1].

Firazyr (icatibant) is not indicated for prophylaxis of HAE attacks

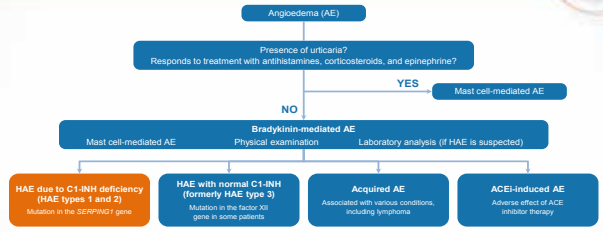
1. Firazyr (icatibant) Summary of Product Characteristics. Marxh 2017.

Disclosure

The presenter is employee of Baxalta, now part of Shire.

The presentation has been developed by Shire, for promotional purposes.

Diagnostic algorithm for HAE in the ER[1-3]



ACE = Angiotensin-converting enzyme ACEi = ACE inhibitor

Adapted from Bas et al. 2007,[1] Craig et al. 2012,[2] and Jaiganesh et al. 2013.[3]

Angioedema is termed idiopathic or spontaneous if, even after a thorough assessment, a trigger or a cause cannot be identified. It may occur with or without accompanying urticaria, and it may involve histamine or other mediators, such as bradykinin.[3]

Icatibant is only indicated for HAE type 1 and type 2[4]

1. Bas M, et al. Allergy. 2007; 62(8): 842-56. 2. Craig T, et al. World Allergy Organ J. 2012;5(12):182-99.
3. Jaiganesh T, et al. Eur J Emerg Med. 2013;20(1):10-7. 4. Firazyr (icatibant) Summary of Product Characteristics. June 2014.

The burden of HAE[1,2]

In the HAE Burden of Illness Study in Europe[1,2]

Daily activities

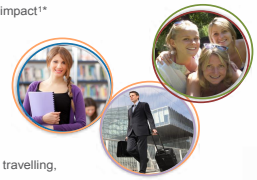
- HAE had an impact on patients' ability to perform daily activities during attacks[1*]
- HAE also impacted patients between attacks[1*]
- Higher attack pain severity was associated with a greater impact[1*]

Education / career

- Patients were estimated to miss a mean of 20 days of work / school per year due to HAE[2†]
- 51% of patients reported that HAE had hindered their educational and / or career advancement[2*]

Emotional well-being

- Patients reported substantial anxiety about future attacks, travelling, and passing HAE to their children[1*]
- Higher attack pain severity was predictive of higher anxiety[1*]



*Of the 186 participants in the HAE Burden of Illness Study in Europe, 164 (88%) had experienced an attack in the past 6 months and formed the analysis sample.[1,2]
†This estimate was based on patients who were employed and / or in school, and provided absenteeism data (n=72).[2]

1. Caballero T, et al. Allergy Asthma Proc. 2014;35(1):47-53.
2. Aygören-Pürsün E, et al. Orphanet J Rare Dis. 2014;9:99.

Icatibant– The *first and only* subcutaneous treatment for acute HAE attacks in adults, licensed for self-administration[1*]

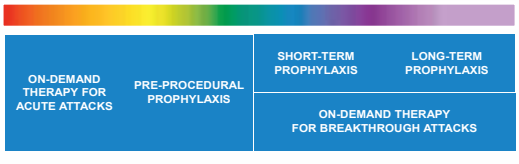
FIRST & ONLY SUBCUTANEOUS TREATMENT

Icatibant is indicated for symptomatic treatment of acute attacks of hereditary angioedema (HAE) in adults (with C1-esterase-inhibitor deficiency)[1].

1. Firazyr (icatibant) Summary of Product Characteristics. March 2017.

A spectrum of treatment plans is available[1]

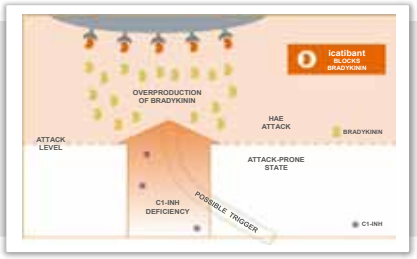
- The WAO 2012 Guideline for the Management of HAE describes the spectrum of treatment plans available to HAE patients[1]
- Individualised treatment is central to effective patient care and different treatment approaches should be tailored to best meet the needs of the individual patient[1]



1. Craig T, et al. World Allergy Organ J. 2012;5(12):182-99.

Icatibant is a selective competitive bradykinin B2 receptor antagonist[1]

- Icatibant binds to the bradykinin B2 receptor on endothelial cells, preventing the binding of bradykinin and the bradykinin-induced increase in vascular permeability which leads to angioedema[2,3]



1. Firazyr (icatibant) Summary of Product Characteristics. March 2017.
2. Craig T, et al. World Allergy Organ J. 2012;5(12):182-99.
3. Tso K, Zuraw BL. Cleve Clin J Med. 2013;80(5):297-308.

Icatibant Phase III trials[1,2]

- The efficacy and safety of icatibant for the treatment of acute HAE attacks was evaluated in three multicentre, double-blind, randomised, controlled Phase III trials: FAST-1, FAST-2 and FAST-3[1,2]

Primary endpoints[1,2]

- **FAST-1:** Median time to clinically significant relief of the index symptom (cutaneous swelling, cutaneous pain, or abdominal pain)[1]
  - **Not significant** (p=0.14)[1]
- **FAST-2:** Median time to clinically significant relief of the index symptom (cutaneous swelling, cutaneous pain, or abdominal pain)[1]
  - **Highly significant** (p<0.001)[1]
- **FAST-3:** Time to 50% reduction in symptom severity (for cutaneous and/or abdominal attacks) assessed by the subject[2]
  - **Highly significant** (p<0.001)[2]

1. Cicardi M, et al. N Engl J Med. 2010;363(6):532-41.
2. Lumry WR, et al. Ann Allergy Asthma Immunol. 2011;107(6):529-37.

The Icatibant Outcome Survey[1]

- The Icatibant Outcome Survey (IOS) is a Shire-sponsored, international, prospective, ongoing observational study in patients with HAE due to C1-INH deficiency who are treated with icatibant[1]
- Data on timings and outcomes of icatibant treatment for 426 angioedema attacks in 136 patients were collected in a patient registry between July 2009 and February 2012[1]
- Following enrollment, data were collected from the patient during routine visits at 6-month intervals including: a physical exam, details relating to icatibant treatment (i.e. treatment of attacks over the previous months), concomitant medications, and adverse events[1]

Study limitations[1]

- The study was a post-hoc analysis of real-world data from a patient registry
- Data were collected at 6-monthly visits, not immediately after each attack
- Statistical analyses included both HCP-treated and self-treated attacks

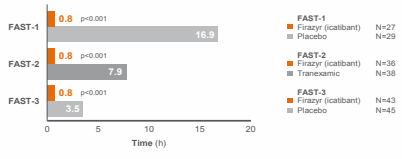
1. Maurer M, et al. PLoS One. 2013;8(2):e53773.

Icatibant started working within an hour to relieve painful HAE attacks[1,2]

- Icatibant resulted in rapid first symptom improvement* in cutaneous and abdominal attacks, as assessed by the patient:[1,2]

FAST-1: 48 minutes **FAST-2: 48 minutes** **FAST-3: 48 minutes**

Median time to first symptom improvement according to patients in FAST-1, FAST-2 and FAST-3[1,2]



Adapted from Cicardi et al. 2010[1] and Lumry et al. 2011.[2]

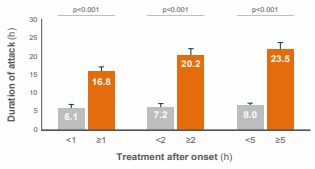
*In all three trials, median time to first symptom improvement was a secondary endpoint.[1,2] FAST-1 did not meet the primary endpoint (p=0.14).[1]

1. Cicardi M, et al. N Engl J Med. 2010;363(6):532-41.
2. Lumry WR, et al. Ann Allergy Asthma Immunol. 2011;107(6):529-37.

Early treatment with icatibant reduced the duration of HAE attacks[1]

- A post-hoc analysis of IOS data demonstrated that the earlier patients received icatibant, the shorter the attack duration (p<0.001); this was irrespective of attack location (cutaneous or abdominal) and severity (p<0.01)[1]
- Mean duration of attacks was between 2.7 and 2.9-fold shorter when attacks were treated with icatibant before vs after each time point for all time points studied (p<0.001)[1]

Mean duration of attacks when attacks were treated with icatibant before or after each time point after onset[1]

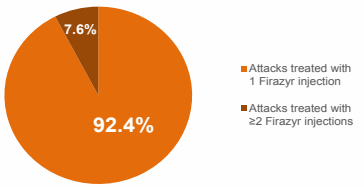


Adapted from Maurer et al. 2013.[1]
Mean duration of attack was defined as the mean time from the start of the attack to the complete resolution of symptoms. N = 207 attacks (<1 hour, n = 80; ≥1 hour, n = 127; <2 hours, n = 120; ≥2 hours, n = 87; <5 hours, n = 145; ≥5 hours, n = 62); all attacks are included at each time point.[1]

1. Maurer M, et al. PLoS One. 2013;8(2):e53773.

Icatibant one-dose efficacy brings symptom relief in 9 out of 10 patients[1]

Proportion of attacks treated with one or more icatibant injections across FAST-1, FAST-2 and FAST-3[1]



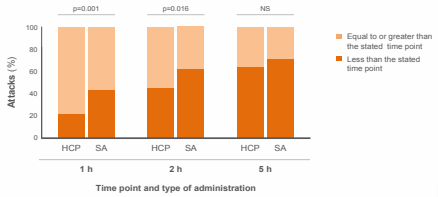
Adapted from Firazyr (icatibant) Summary of Product Characteristics, April 2017.[1]
Based on an assessment of the first 15 Firazyr (icatibant)-treated attacks (1114 doses for 1030 attacks) in patients with repeated attacks.[1]

1. Firazyr (icatibant) Summary of Product Characteristics. March 2017.

Self-administration of icatibant resulted in earlier treatment than HCP-administration[1]

- Of 426 attacks in 136 patients, 233 attacks were self-treated and 174 attacks were treated by a HCP (data on who administered icatibant are missing for 19 attacks)[1]
- Twice as many attacks were treated within 1 hour when icatibant was self-administered vs administered by an HCP (p=0.001)[1]

Proportion of attacks treated before and after each time point according to type of administration (HCP-administered or self-administered)[1]



Adapted from Maurer et al. 2013.[1]
n = 72 for attacks treated by HCP-administration; n = 158 for attacks treated by self-administration; all attacks are included at each time point; HCP, healthcare professional; NS, not significant; SA, self-administration.[1]

1. Maurer M, et al. PLoS One. 2013;8(2):e53773.

Icatibant was well-tolerated[1-4]

- Injection site reactions occurred in almost all icatibant-treated subjects;[1-4] however, these reactions were generally mild to moderate in severity, transient, and resolved without further intervention[1,3,4]
- No icatibant-related serious adverse events were reported in any study[1,2,4]
- No icatibant-treated subject discontinued any study because of an adverse event[2,4]

Adverse reactions reported with icatibant[1]

Very common, ≥1/10	Common, ≥1/100 to <1/10
• Injection site reactions	• Dizziness • Headache • Nausea • Rash • Erythema • Pruritus • Pyrexia • Transaminase increased

Adapted from Firazyr (icatibant) Summary of Product Characteristics, March 2017.[1]
Description of selected adverse reactions:
Immunogenicity
Across repeated treatment in the controlled phase III trials, transient positivity to anti-icatibant antibodies was observed in rare cases. All patients maintained efficacy. One Firazyr (icatibant)-treated patient tested positive for anti-icatibant antibodies before and after treatment with Firazyr (icatibant). This patient was followed for 5 months and further samples were negative for anti-icatibant antibodies. No hypersensitivity or anaphylactic reactions were reported with Firazyr (icatibant).

1. Firazyr (icatibant) Summary of Product Characteristics. March 2017.
2. Cicardi M, et al. N Engl J Med. 2010;363(6):532-41.
3. Cicardi M, et al. N Engl J Med. 2010;363(6):532- 41; Supplementary Appendix.
4. Lumry WR, et al. Ann Allergy Asthma Immunol. 2011;107(6):529-37.

icatibant – The *first and only* *subcutaneous treatment* for acute HAE attacks in adults, licensed for self-administration[1]

FIRST & ONLY SUBCUTANEOUS TREATMENT

firazyr® icatibant

- icatibant effectively blocks bradykinin – the critical mediator of HAE attacks[1,2]
- icatibant one-dose efficacy brings symptom relief in 9 out of 10 patients[1]
- icatibant is well-tolerated[1,3,4]
  - Almost all subjects who were treated with subcutaneous icatibant clinical trials developed reactions at the site of injection. These reactions were generally mild to moderate in severity, transient, and resolved without further intervention[1]
- icatibant is licensed for self-administration[1]
  - icatibant may be self-administered or administered by a caregiver only after training in subcutaneous injection technique by a healthcare professional[1]
  - Self-administration enables early, on-demand treatment of HAE attacks[5]

1. Firazyr (icatibant) Summary of Product Characteristics. March 2017 2. Craig T, et al. World Allergy Organ J. 2012;5(12):182-99.
3. Cicardi M, et al. N Engl J Med. 2010;363(6):532-41. 4. Lumry WR, et al. Ann Allergy Asthma Immunol. 2011;107(6):529-37.
5. Maurer M, et al. PLoS One. 2013;8(2):e53773.

Dosing and administration[1]

- The decision on initiating self-administration should only be taken by a physician experienced in the diagnosis and treatment of HAE[1]
- icatibant is administered via a single subcutaneous injection to the abdomen[1]
- The ready-to-use pre-filled syringe can be carried by patients[1]

Ready-to-use, easy-to-carry, pre-filled syringe

1. Firazyr (icatibant) Summary of Product Characteristics. March 2017.

Firazyr (icatibant) Abbreviated Summary of Product Characteristics

Firazyr 30 mg solution for injection in pre-filled syringe : Each pre-filled syringe of 3 ml contains icatibant acetate equivalent to 30 mg icatibant. Each ml of the solution contains 10 mg of icatibant. For the full list of excipients, see section 6.1. **4.1 Therapeutic indications** Firazyr is indicated for symptomatic treatment of acute attacks of hereditary angioedema (HAE) in adults (with C1-esterase-inhibitor deficiency). **4.2 Posology and method of administration** Firazyr is intended for use under the guidance of a healthcare professional. **Posology** The recommended dose is a single subcutaneous injection of Firazyr 30 mg. In the majority of cases a single injection of Firazyr is sufficient to treat an attack. In case of insufficient relief or recurrence of symptoms, a second injection of Firazyr can be administered after 6 hours. If the second injection produces insufficient relief or a recurrence of symptoms is observed, a third injection of Firazyr can be administered after a further 6 hours. No more than 3 injections of Firazyr should be administered in a 24 hour period. In the clinical trials, not more than 8 injections of Firazyr per month have been administered. Special populations *Older people* Limited information is available on patients older than 65 years of age. Older people have been shown to have increased systemic exposure to icatibant. The relevance of this to the safety of Firazyr is unknown (see section 5.2). *Hepatic impairment* No dose adjustment is required in patients with hepatic impairment. *Renal impairment* No dose adjustment is required in patients with renal impairment. *Paediatric population* The safety and efficacy of Firazyr in children aged 0-18 years has not been established. No data are available. **Method of administration** Firazyr is intended for subcutaneous administration preferably in the abdominal area. Firazyr may be self-administered or administered by a caregiver only after training in subcutaneous injection technique by a healthcare professional. The decision on initiating self-administration of Firazyr should only be taken by a physician experienced in the diagnosis and treatment of hereditary angioedema (see section 4.4). Each Firazyr syringe is intended for single use only. Firazyr solution for injection should be injected slowly due to the volume to be administered (3 ml). **4.3 Contraindications** Hypersensitivity to the active substance or to any of the excipients listed in section 6.1. **4.4 Special warnings and precautions for use** *Laryngeal attacks* Patients with laryngeal attacks should be managed in an appropriate medical institution after injection of Firazyr until the physician considers discharge to be safe. *Ischemic heart disease* Under ischemic conditions, a deterioration of cardiac function and a decrease in coronary blood flow could theoretically arise from antagonism of bradykinin receptor type 2. Caution should therefore be observed in the administration of Firazyr to patients with acute ischemic heart disease or unstable angina pectoris (see section 5.3). *Stroke* Although there is evidence to support a beneficial effect of B2 receptor blockade immediately following a stroke, there is a theoretical possibility that icatibant may attenuate the positive late phase neuroprotective effects of bradykinin. Accordingly, caution should be observed in the administration of icatibant to patients in the weeks following a stroke. *Self-Administration* For patients who never received Firazyr previously, the first treatment should be given in a medical institution or under the guidance of a physician. In case of insufficient relief or recurrence of symptoms after self-treatment, it is recommended that the patient should seek medical advice and that subsequent doses are given in a medical institution (see section 4.2). Patients experiencing a laryngeal attack should always seek medical advice and be observed in a medical institution also after having taken the injection at home.

4.5 Interaction with other medicinal products and other forms of interaction Pharmacokinetic drug interactions involving CYP450 are not expected. **4.6 Fertility, pregnancy and lactation** Pregnancy For icatibant, no clinical data on exposed pregnancies are available. Animal studies showed effects on uterine implantation and parturition (see section 5.3), but the potential risk for humans is unknown. Firazyr should be used during pregnancy only, if the potential benefit justifies the potential risk for the foetus. (e.g for treatment of potentially life threatening laryngeal attacks). Breast-feeding Icatibant is excreted in the milk of lactating rats at concentrations similar to those in maternal blood. No effects were detected in the post-natal development of rat pups. It is unknown whether icatibant is excreted in human breast milk but it is recommended that breastfeeding women, who wish to take Firazyr, should not breastfeed for 12 hours after treatment. Fertility In both rats and dogs, repeated use of icatibant resulted in effects on reproductive organs. Icatibant had no effect on the fertility of male mice and rats (see section 5.3). In a study of 39 healthy adult men and women treated with 30 mg every 6 hours for 3 doses every 3 days for a total of 9 doses, there were no clinically significant changes from baseline in basal and GnRH-stimulated concentration of reproductive hormones in either females or males. There were no significant effects of icatibant on the concentration of luteal phase progesterone and luteal function, or on menstrual cycle length in females and there were no significant effects of icatibant on sperm count, motility and morphology in males. The dosing regimen used for this study is unlikely to be sustained in the clinical setting. **4.7 Effects on ability to drive and use machines** Firazyr has minor influence on the ability to drive and use machines. **4.8 Undesirable effects** *Summary of the safety profile* In clinical studies used for registration, a total of 999 HAE attacks have been treated with 30 mg Firazyr administered subcutaneously by a healthcare professional. Firazyr 30 mg SC has been administered by a healthcare professional to 129 healthy subjects and 236 patients with HAE. Almost all subjects who were treated with subcutaneous icatibant in clinical trials developed reactions at the site of injection (characterised by skin irritation, swelling, pain, itchiness, erythema, burning sensation). These reactions were generally mild to moderate in severity, transient, and resolved without further intervention. **Adverse reactions reported with icatibant** (Common ≥1/100 to <1/10) *Nervous system disorders:* Dizziness, Headache; *Gastrointestinal disorders:* Nausea; *General disorders and administration site conditions:* Pyrexia; *Investigations:* Transaminase increased (Very common ≥1/10) Injection site reactions Injection site bruising, Injection site haematoma, Injection site burning, Injection site erythema, Injection site hypoesthesia, Injection site irritation, Injection site numbness, Injection site edema, Injection site pain, Injection site pruritus, Injection site sensation, Injection site pruritus, Injection site swelling, Injection site urticaria, and Injection site warmth.

In case of suspicion of adverse event please contact Bulgarian Drug Agency (BDA), Damian Gruev Str. No 8, Tel: +359 2 8903417, E-mail: pharmacovig@bda.bg, www.bda.bg and Marketing Authorisation Holder Shire, E-mail: drugsafety@shire.com.

Before prescribing , please read the last approved SmPC of the product. You can find the full SmPC on: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000899/human_med_000793.jsp&mid=WC0b01ac058001d124

Special warnings and precautions for use[1]

- For patients who have never previously received Firazyr (icatibant), the first treatment should be given in a medical institution or under the guidance of a physician[1]
- In cases of insufficient relief or recurrence of symptoms, it is recommended that patients should seek medical advice and that subsequent doses are given in a medical institution[1]
- Patients with laryngeal attacks should be managed in an appropriate medical institution after injection of Firazyr (icatibant), until the physician considers discharge to be safe[1]

1. Firazyr (icatibant) Summary of Product Characteristics. March 2017.

Firazyr (icatibant) Abbreviated Summary of Product Characteristics

Description of selected adverse reactions:Immunogenicity Across repeated treatment in the controlled phase III trials, transient positivity to anti-icatibant antibodies was observed in rare cases. All patients maintained efficacy. One Firazyr-treated patient tested positive for anti-icatibant antibodies before and after treatment with Firazyr. This patient was followed for 5 months and further samples were negative for anti-icatibant antibodies. No hypersensitivity or anaphylactic reactions were reported with Firazyr. **4.9 Overdose** No clinical information on overdose is available. A dose of 3.2 mg/kg intravenously (approximately 8 times the therapeutic dose) caused transient erythema, itching or hypotension in healthy subjects. No therapeutic intervention was necessary. **5.1 Pharmacodynamic properties** ATC code: B06AC02. Mechanism of action HAE (an autosomal dominant disease) is caused by an absence or dysfunction of C1-esterase-inhibitor. HAE attacks are accompanied by an increased release of bradykinin, which is the key mediator in the development of the clinical symptoms. HAE manifests as intermittent attacks of subcutaneous and/or sub mucosall oedema involving the upper respiratory tract, the skin and the gastrointestinal tract. An attack usually lasts between 2 to 5 days. Icatibant is a selective competitive antagonist at the bradykinin type 2 (B2) receptor. It is a synthetic decapeptide with a structure similar to bradykinin, but with 5 non-proteinogenic amino acids. In HAE increased bradykinin concentrations are the key mediator in the development of the clinical symptoms. **Clinical efficacy and safety** Efficacy data were obtained from an initial open-label Phase II study and from three controlled Phase III studies. Phase III clinical studies (FAST-1 and FAST-2) were randomized, double-blind, controlled trials and had identical designs except for the comparator (one with oral tranexamic acid as the comparator and one placebo controlled). A total of 130 patients were randomized to receive either a 30 mg dose of icatibant (63 patients) or comparator (either tranexamic acid, - 38 or placebo - 29 patients). Subsequent episodes of HAE were treated in an open label extension. Patients with symptoms of laryngeal angioedema received open label treatment with icatibant. The primary efficacy endpoint was the time to onset of symptom relief using a visual analogue scale (VAS). FAST-3 was a randomized, placebo-controlled, parallel-group study of 98 adult patients with a median age of 36 years. Patients were randomized to receive either icatibant 30 mg or placebo by subcutaneous injection. A subset of patients in this study experienced acute HAE attacks while receiving androgens, antifibrinolytic agents or CI inhibitors. The primary endpoint was time to onset of symptom relief assessed using a 3-item composite visual analog score (VAS-3) consisting of assessments of skin swelling, skin pain, and abdominal pain. In these studies, patients on icatibant had a faster median time to onset of symptom relief (2.0, 2.5 and 2.0 hours, respectively) compared to tranexamic acid (12.0 hours) and placebo (4.6 and 19.8 hours). The treatment effect of icatibant was confirmed by secondary efficacy endpoints. In an integrated analysis of these controlled Phase III studies, the time to onset of symptom relief and time to onset of primary symptom relief were similar regardless of age group, sex, race, weight or whether or not the patient used androgens or antifibrinolytic agents. Response was also consistent across repeated attacks in the controlled Phase III trials. A total of 237 patients were treated with 1,386 doses of 30 mg icatibant for 1,278 attacks of acute HAE. In the first 15 Firazyr treated attacks (1,114 doses for 1,030 attacks), the median times to onset of symptom relief were similar across attacks (2.0 to 2.5 hours). 92.4% of these attacks of HAE were treated with a single dose of Firazyr. A total of 66 patients with attacks of HAE affecting the larynx were treated in these controlled Phase III clinical trials. The results were similar to patients with non-laryngeal attacks of HAE with respect to time to onset of symptom relief.

5.2 Pharmacokinetic properties *Absorption* Following subcutaneous administration, the absolute bioavailability of icatibant is 97%. The time to maximum concentration is approximately 30 minutes. *Elimination* Icatibant is mainly eliminated by metabolism with less than 10% of the dose eliminated in the urine as unchanged drug. Clearance is about 15-20 l/h and independent of dose. The terminal plasma half-life is about 1-2 hours. *Biotransformation* Icatibant is extensively metabolized by proteolytic enzymes to inactive metabolites that are primarily excreted in the urine. *In vitro* studies have confirmed that icatibant is not degraded by oxidative metabolic pathways and is not an inhibitor of major cytochrome P450 (CYP) isoenzymes (CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) and is not an inducer of CYP 1A2 and 3A4. *Special populations* Data suggest an age-related decline in clearance resulting in about 50-60% higher exposure in older people (75-80 years) compared to patients aged 40 years. Data suggests that gender and weight do not have a significant influence on icatibant pharmacokinetics. Limited data suggest that icatibant exposure is not influenced by hepatic or renal impairment. The influence of race on icatibant pharmacokinetics has not been evaluated. There are no pharmacokinetic data in children. **6.4 Special precautions for storage** Do not store above 25◦C. Do not freeze. **6.5 Nature and contents of container** 3 ml of solution in a 3 ml pre-filled syringe (type I glass) with plunger stopper (bromobutyl coated with fluorocarbon polymer). A hypodermic needle (25 G; 16 mm) is included in the pack. Pack size of one pre-filled syringe with one needle or a multipack containing three pre-filled syringes with three needles. **7. MARKETING AUTHORISATION HOLDER** Shire Orphan Therapies GmbH, Friedrichstrasse 149, D-10117 Berlin, Germany. Date of last revision of the SmPC: 23/03/2017.
Medicinal product is subject to medical prescription.

In case of suspicion of adverse event please contact Bulgarian Drug Agency (BDA), Damian Gruev Str. No 8, Tel: +359 2 8903417, E-mail: pharmacovig@bda.bg, www.bda.bg and Marketing Authorisation Holder Shire, E-mail: drugsafety@shire.com.

Before prescribing , please read the last approved SmPC of the product. You can find the full SmPC on: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000899/human_med_000793.jsp&mid=WC0b01ac058001d124

Shire in HAE

firazyr icatibant

The first and only subcutaneous treatment for acute HAE attacks in adults, licensed for self-administration[1]

CINRYZE C1 inhibitor (human)

The only C1-INH licensed in Europe for routine prevention of angioedema attacks in adults and adolescents and children (6 years old and above) with HAE[2]

Libertas PLUS

A dedicated European patient support programme from Shire

Shire offers HAE management solutions which can be tailored to your individual patient's needs

1. Firazyr (icatibant) Summary of Product Characteristics. March 2017.
2. Cinryze (C1 inhibitor [human]) Summary of Product Characteristics. January 2017.

АТАКСИЯ ТЕЛЕАНГИЕКТАЗИЯ КЛИНИЧЕН СЛУЧАЙ

Петя Янкова

Анамнеза

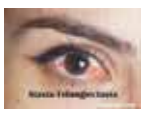
- 23 годишна жена с АТ, трудно подвижна, с тремор на тялото и със затруднения в речта, постъпва в клиниката по повод на зачестили през последните две години инфекции на ДС с протрахирано протичане.
- Проходила на 10 м., с нестабилна походка.
- На 2 г. е установен понижен мускулен тонус на долни крайници.
- На 3 г. са диагностицирани конюнктивални телеангиектазии.
- На 5 г. се поставя диагноза – АТ, която е доказана с цитогенетично изследване.

Анамнеза

- На 6 годишна възраст (2000г) по повод чести инфекции (синуити и бронхити) са проведени имунологични изследвания и е установен дисбаланс в клетъчните популации (понижени Т и В лимфоцити, CD4/CD8 индекс; повишени НК клетки) и промени в нивата на серумните имуноглобулини.
- През 2002-2003 година е провела терапия с неколкократни инфузии на Имуновенин, по схема с добро клинично повлияване, като до 2016 година не е боледувала.
- 2016-2017 трикратно инфекции на ДС, последната м. януари, наложила антибиотична терапия.
- Минали заболявания – 2001г операция на око.
- Фамилна анамнеза – не обременена. Дядо с артериална хипертония.

Обективно състояние

- Жена на видима възраст около календарната. В увредено общо състояние. Използва помощни средства за придвижване. Ориентирана за време, място и собствена личност.
- Астеничен хабитус. Ръст 150см; тегло 40 кг.
- Видими лигавици – бледи. Наличие на телеангиектазии по склерата на двете очи.
- БД – двустранно везикуларно дишане, без хрипове.
- ССС - ритмична сърдечна дейност с ясни сърдечни тонове, без шумова находка.
- ЧД и слезка не се палпират.



Клетъчна популация	%	кл/µl
Общи Т лимфоцити (CD3+)	40	1080
Активирани Т лимфоцити (CD3+DR+)	24	648
Хелперно-индусерни Т лимфоцити (CD3+CD4+)	19	513
Наивни Т клетки (CD4+CD45RA+CD62L+)	7,9	
Ефекторно-паметови Т клетки (CD4+CD45RA-CD62L-)	37,4	
Супресорно-цитотоксични Т клетки (CD3+CD8+)	18	486
Ефекторни Т клетки (CD8+CD45RA+CD62L-)	53,3	
В-лимфоцити (CD19+)	2	54
НК клетки (CD3-CD16&56+)	54	1458
Т и/или НК клетки (CD57+CD8+)	32	864

Тест (метод)	резултат	Референтни граници
ANA (IFA – Hep-2 cells)	(-) отр.	< 1:80
Immunoglobulin G g/l (nephelometry)	17,54	7,23 – 16,85
Immunoglobulin G_1	11,08	4,50 – 9,00
Immunoglobulin G_2	1,65	1,80 – 5,30
Immunoglobulin G_3	0,24	0,13 – 0,80
Immunoglobulin G_4	0,04	0,08 – 1,00
Immunoglobulin A g/l (nephelometry)	< 0,02	0,80 – 4,63
Immunoglobulin M g/l (nephelometry)	2,883	0,48 – 2,71
Immunoglobulin E U/ml (turbidimetry)	< 30	< 180
C3 комплемент g/ l (turbidimetry)	1,971	0,75 – 1,65
C4 комплемент g/ l (turbidimetry)	0,335	0,20 – 0,65

- Нормална експресия на CD69 от общи Т лимфоцити след стимулация с PHA.
- Параклинични изследвания:

Показател	промени
ПКК	PLT 536x10^9/l
Биохимия	ALT 43 U/L GGT 120 U/L
Туморни маркери	AFP 660,1 U/ml

- Ехографско изследване на коремни органи – б.о.

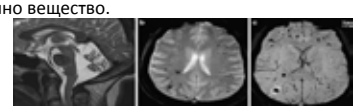
Обсъждане

- Elena Boder и Robert P. Sedgwick, през 1957 г. описват фамилен синдром , който наричат АТ.
- АТ се характеризира с прогресивна церебеларна дегенерация, телеангиектазия, имунен дефицит, повтарящи се синопулмонални инфекции, чувствителност към радиация, преждевременно стареене и предразположение към развитие на рак, особено от лимфоиден произход.
- Други аномалии включват изоставане във физическото развитие, гонадна атрофия, закъснял пубертет и инсулинва резистентност.
- АТ се проявява при 1 от 40 000 до 100 000 души по целия свят.

12th Balkan Congress of Human Genetics
8th National Conference for Rare Diseases
8-10 September 2017, Plovdiv, Bulgaria

Обсъждане

- АТ е дифузна дегенерация или атрофия на церебеларния вермис и хемисфери, включващи клетки на Purkinje (PC) и в по-малка степен гранулирани неврони.
- Проучванията с ЯМР демонстрират церебрални аномалии в бяло мозъчно вещество при по-възрастни пациенти, вкл. хемосидеринови отлагания и мозъчна телеангиектазия, както и дегенеративни промени в кортикомоторните пътища на бялото мозъчно вещество.



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Обсъждане

- Всички пациенти с АТ трябва да имат цялостна имунологична оценка, която включва броя и вида на лимфоцитите в кръвта, нивата на серумните имуноглобулини, Т-зависим и Т-независими антителен отговор.
- Тежестта на дефицита на антитела при пациенти с АТ корелира с нарушенията на хомеостазата на В- и Т-клетките, което води до намаляване на разнообразието на имунния репертоар.
- Ако функцията на антителата е нормална, трябва да се дадат всички рутинни имунизации в детска възраст, включително живи вирусни ваксини.
- Тежеста на антителния дефицит при пациенти с АТ корелира с наличието на остатъчна ATM киназна активност и с броя на наивните CD4+ клетки.

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Обсъждане

- Телангиектазията може да се появи на кожата, особено на лицето и ушите, пикочния мехур,в черния и белия дроб.
- Не влияе на зрението и зрителната острота. Контролът върху движението на очите и визуалното фиксиране често е нарушен. Страбизмът е чест.
- Неволевите движения могат да затруднят самостоятелното хранене, което се удължава.
- Дисфагията е често срещана и се появява през второто десетилетие от живота, поради неврологичните промени. Проблеми, свързани с фаринкса, могат да причинят аспирация на течност, храна и слюнка.

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Обсъждане

- Приблизително 25% от болните имат риск от развитие на рак. Лимфомите и левкемиите най-често се срещат при възраст <20-години, но възрастните са по-податливи, както на лимфоидни тумори, така и на множество солидни тумори, вкл. Са гърдата, черния дроб, езофаг.
- Все още няма начин да се предскаже кои индивиди с АТ ще развият рак, няма признати методи за осигуряване на наблюдение за лимфоми и левкемии.
- Системен мета анализ установи, че носителите на мутации на ATM имат намалена продължителност на живот поради рак (гърда и стомашно-чревен тракт) и ИБС. Според един от 2016 г. кумулативният риск от рак на гърдата в носителите е приблизително 6% на възраст до 50 и приблизително 30% на възраст до 80 г.

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Обсъждане

- Проучване на ендокринните аномалии в една израелска кохорта от пациенти с АТ показва, че изоставането в растежа е по-видно при жените, отколкото при мъжете, и че тази разлика е очевидна във възрастта, преди GTH хормони да започнат да повлияват скоростта на растеж.
- Безплодието често се описва като част от АТ, резултат от гонадална атрофия или дисгенезия, което води до забавено развитие на пубертета и ранна менопауза.
- Малка част от пациентите страдат от ИЗЗД, който обикновено се проявява късно при прогресия на заболяването. Трябва да се отбележи, че при индивиди с АТ, които нямат диабет, може да се наблюдава намалена чувствителност към инсулина.

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Образование и социализация

- Повечето деца с АТ имат затруднения в училище, поради забавяне на времето за реакция към визуални, словесни или други символи, дизартрия, окуломоторна апраксия и нарушен фин контрол на мотриката. Решението за необходимостта от специални учебни часове или допълнителна помощ в редовните уроци е силно повлияно от наличните местни ресурси.
- Проблемите с контрола в/у движението на очите затрудняват четенето, но те напълно разбират смисъла и нюансите на прочетеното.
- Животът в атаксизното тяло може да е уморителен.

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Обсъждане

- Невропсихологично проучване извършено през 2000 г., показва дефицити в преценката за продължителност, специфични увреждания в интелектуалното развитие, невербалната памет, вербалното абстрактно разсъждение и изчисление.
- От особено значение са чернодробни аномалии, като повишени нива на серумните трансаминази, стеатоза и неалкохолна цироза, включително фиброзни промени, както и повишени нива на триглицеридите и холестерола.

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ИМУНЕН ДЕФИЦИТ

- Един от най-очевидните нерешени въпроси по отношение на пациентите с класическа АТ е, защо някои страдат от ИД други не.
- Проучване (2015) на честотата на раковите заболявания в национална кохорта от френски пациенти с АТ установи, ниски нива на IgA в пациенти, които са развили ЛПЗ, в сравнение с пациенти, развили карциноми или такива без рак. Това наблюдение повдига въпроса дали ниските нива на IgA са рисков фактор или биомаркер за развитието на ЛПЗ при АТ?
- Ниски IgG и IgA в комбинация с повишени стойности на IgM, може също да бъдат рисков фактор за влошаване на цялостния ход на заболяването.

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БД усложнения

- Има много пропуски в познанията ни относно БДЗ при АТ. Съществува необходимост от: клинични методи за идентифициране на тези с повишен риск от БДЗ; оптимални протоколи за лечение на БДЗ; алтернативни техники за рутинно проследяване на БДЗ;
- Приносът на възпалението към БДЗ и прекият ефект от загубата на АТМ върху БД епител понастоящем са области на активно изследване.
- По отношение на нервно-мускулната слабост е необходима оценка на рехабилитационните ползи от упражнения, които укрепват горната част на тялото, включително: постурална намеса; вдигане на тежести; дихателна терапия; Lee Silverman Voice Therapy.

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Лечение и препоръки

- Поради зачестилите инфекции на ГДП се прецени:

1. терапия с IVIG.
2. контролно изследване на нивата на Иг Г и М, и CD3+ клетки. В зависимост от отчетените промени да се проведе съответната терапия.
3. Контролно проследяване на броя на НК клетките (% и #). Ако продължи значимо да се повишава е необходимо да се проведе разширено ИФТ и консултация с хематолог.
4. Контролно проследяване нивата на ASAT, GGT, LDH, HbA_{1c} и AFP. При нужда да се проведът допълнителни изследвания и консултации.

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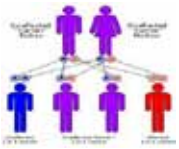
Ракови заболявания

- Критична необходимост е развитието на по-малко токсични режими за лечение на ракови заболявания.
- Стандартните указания за оценка на пациентите преди терапия и поддържащи грижи по време и след терапия също липсват. Предизвикателство е разработването на стандартни протоколи за лечение на най-често срещаните ракови заболявания при пациенти с АТ .
- Съществува също така необходимост от централизирано хистологично изследване и рутинно генотипизиране, и банкиране на туморна тъкан. Това би подпомогнало разбирането ни за биохимичните пътища, участващи в развитието на ракови заболявания в контекста на АТ и вследствие на това способността ни да развиваме целенасочени терапии.



НИЕ ПРЕПОРЪЧВАМЕ: КОМПЛЕКСЕН ПОДХОД

- Регистриране в центъра за първични имунни дефицити (ПИД) в Александровска болница.
- Проследяване и лечение.
- Проследяване на членовете на семейството с оглед предотвратяване на риска от развитие на ракови заболявания.

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Глобална платформа за данни за семейства с АТ

- За да се отговори на тази необходимост и да се съберат данни за хора с АТ, бързо достъпни за учени и лекари, платформата за глобални данни за семейства с АТ беше стартирана през юли 2016
- Родителите или настойниците на деца с АТ, или самите хора с АТ, споделят медицинската си информация и имат възможност за генетичен анализ.
- Комитетът за семеен надзор с членовете от 10 различни държави, ръководи мисията и дейностите на Платформата.
- The Global A-T Family Data Platform https://www.atfamilies.org/. Accessed 21 Nov 2016.

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Международен АТ регистър

- Паралелно с платформата, се разработва и международен регистър на пациенти на АТ.
- Финансиран от безвъзмездна финансова помощ по програма "Хоризонт 2020" на Европейската комисия и контролиран от научен съвет на клинични експерти и представители на пациенти, регистърът ще съдържа данни, предоставени от лекари и клинични центрове, които лекуват хора с АТ.
- Международната общност за застъпничество в АТ предприема стъпки, за да позволи свързването на данни между двата регистъра и потенциално други бази данни, съдържащи информация за хората с АТ.

ХОМОЗИГОТЕН MYD88 ДЕФИЦИТ – СЪОБЩЕНИЕ НА СЛУЧАЙ И ОБЗОР НА ЛИТЕРАТУРАТА

Н. Спасов, М. Спасова, М. Мурджева, А. Стоянова, И. Мумджиев

- Анамнеза

- 22-месечна възраст - плевропневмония- осъществена двукратно плеврална пункция- лимфоцитоза- овладяна с антибиотично и антимикотично лечение
- 25 месечна възраст – миелограма: изразена пролиферация на еозинофилния ред
- 33 месечна възраст - 14-дневен фебрилитет без ясен фокус, овладян с Вориконазол

- Дефицит на Миелоидния Диференциращ фактор 88 (**MyD88**):
  - АР унаследяване
  - рядка форма на ПИД,
  - засяга в NF-kB пътя на Toll-like рецептор и Интерлевкин 1-рецептор - медиирани компоненти на вродения имунитет.
- Описан - 2008г.

Von Bernuth et al. Science 2008

- Протича с:
  - рецидивиращи пиогенни инфекции,
  - причинени от ограничен спектър бактерии
  - слаб възпалителен отговор на макроорганизма.

- Анамнеза

- 36-месечна възраст - левостранен аксиларен супуративен лимфаденит → инцизия → ***S. aureus***
- 38-месечна възраст - грануломатозен менингит, енцефалит и мастоидит → кома. Овладяни с цефалоспорин трето поколение, аминоглюкозид, Вориконазол и Тейкопланин
- 50-месечна възраст - десностранна лобарна пневмония - Тейкопланин+ 4-то поколение Цефалоспорин
- 52-месечна възраст– десностранен аксиларен супуративен лимфаденит → инцизия → ***S. Aureus*** - Клиндамицин

Анамнеза

- Неусложнена перинатална анамнеза
- Роден от шеста бременност чрез цезарово сечение поради слаба родова дейност на майката
- Проведени всички редовни ваксинации
- **1-месечна възраст** (Инфекциозна клиника) - вирусна чревна инфекция
- **2-месечна възраст** (Инфекциозна Клиника) - нова чревна инфекция, довела до сепсис,чернодробна недостатъчност и ДИК синдром. От хемокултура се изолира *Enterococcus faecium*.
- **9-месечна възраст** - тежък миоперикардит (голям перикарден излив), нарушена систолна функция на лява камера и затруднено дишане - не се изолира причинител, но се повлиява от проведената антибиотична, противовъзпалителна, диуретична и инотропна терапия.

- Фамилна анамнеза

- Активен хепатит В и цироза у бабата, която отглежда детето;
  - преди 15год. е оперирана от ехинококова киста в черния дроб.

- Анамнеза

- **12-месечна възраст** - пневмония и рецидив на перикардита → лечение с НСПВС с добро повлияване.
- **18-месечна възраст** - пневмония, съпътствана с хепатомегалия, завишени трансаминази, ЛДХ и ГГТП.
  - Абдоминална ехография: уголемен черен дроб, хиперехогенен, блестящ паренхим,
  - ЕхоКГ- възстановена систолна функция на лява камера, без промени в перикарда.
  - Коагулограма- без отклонения.
  - Хепатитни маркери - отрицателни.
  - Серология за EBV - негативна.
- **19-месечна възраст** - интермитентен фебрилитет, коремна болка, безапетитие и неспокойствие.
  - Палпаторно: плътна формация в левия ЧД лоб,
  - Абдоминална ехография: хетерогенна по структура формация с неравни контури 9/6см→ тънкоиглена биопсия
  - След 1 месец - лапаротомия с биопсия (цироза с неспецифично грануломатозно възпаление и наличие на хифи) - овладяно след лечение с Вориконазол

- Обективно изследване

- Еутрофичен
- Две петна тип café-au lait по туловището.
- Без лимфадено- и спленомегалия.
- Белодробен статус- без данни за хронична пневмония.
- ССС- РСД, ясни тонове, без патологични шумове.
- Черен дроб – увеличен на 3 см под ребрената дъга.

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• Параклинични изследвания

- Левкоцитоза+ еозинофилия до 38% в хода на инфекциите, тромбоцитоза
- Лек анемичен синдром, неналагащ хемотрансфузии
- Хиперпротеинемия(96g/l) с хипоалбуминемия(24g/l)
- Хипергамаглобулинемия- ИгГ-42.9г/л и ИгЕ-над 3000Е/л.
- Умерено завишено ЛДХ-1015Е/л и фибриноген-7.2г/л
- Флоуцитометрично- нормален абсолютен брой Т- и В-лимфоцити
- Нормален NBT тест
- Нормален алфа-фетопротеин и бета- hCG
- Абдоминална ехография и КТ на черен дроб- установени множество окръглени лезии в хода на чернодробната микоза

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Генетично изследване

Prof. Kaan Boztug, Ludwig Boltzmann Institute, Medical University of Vienna, Center for Rare and Undiagnosed Diseases, c/o CeMM., Austria

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Серологични изследвания

- ELISA Candida mannan – 45 pg/ml (<62.5 pg/ml)
- РПХА и ELISA за токсоплазмоза-отрицателна
- Серология за ехинококоза - силно положителна 25.67 (>15 е позитивно)
- Серология за токсокароза-отрицателна

  Серология за фасциолоза-отрицателна
- EBV-отрицателна
- T-spot-отрицателен
- Хепатитни маркери – отр.
- Изпражнения за чревни паразити- не се откриват вегетативни форми на цисти или протозои. Не се откриват полово зрели форми или яйца на хелминти, влючително и на Фасциола хепатика.

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Диагноза

MYD88 дефицит

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Хистологичен резултат (пункционна биопсия)

- Лезия, изградена от овални и издължени клетки, оформящи солидни структури, сред които са налице много лимфоцити, **еозинофили** и неутрофили. До лезията - некротично огнище. Алфа фетопротеин (-), CD10(-) , CD34(-), PAS(-) и ван Гизон(-), като само CD34 маркира ендотела на капилярите.
- Заключение- няма морфологични данни за туморен процес. Налице е неспецифична некроза,оградена от млада гранулационна тъкан.

Хистологичен резултат (инцизионна биопсия)

- Цироза с неспецифично грануломатозно възпаление, както и наличие на хифи

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Държави, в които са съобщени случаи на 31 семейства с IRAK4 дефицит и 6 семейства с MYD88 дефицит. *Picard C, 2010*

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Патологични синдроми

1. ***Инфекциозна диатеза:***
   - Чести ГИ и респираторни инфекции;
   - Сепсис, ДИК;
   - Рецидивиращ миоперикардит.
   - Супуративен лимфаденит
   - Грануломатозен менингит+енцефалит+мастоидит
   - Плевропневмония, лобарна пневмония
2. ***Хепатомегалия***
   - чернодробна микоза
3. **Еозинофилия *с екстремно високи IgE.***
4. **Възпалително-биологичен:**
   - Левкоцитоза, тромбоцитоза, ускорена СУЕ, повишен CRP, екстремна хипергамаглобулинемия

→изключване на паразитни заболявания, ТВС, грануломатозен хепатит, по-чести форми на ПИД, чернодробен тумор

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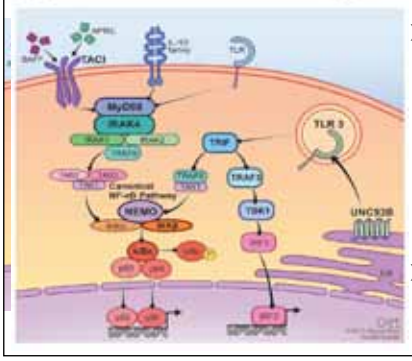
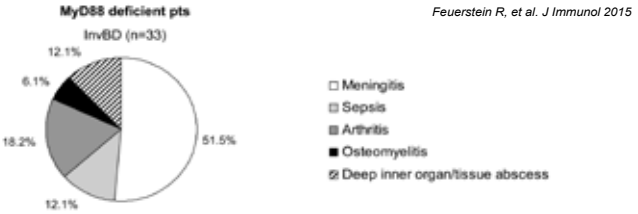


- Рядък АР ПИД - дефект в гена за първичен отговор на миелоидната диференциация 88, който участва в NF-kB пътя в Toll-like рецептора и в IL-1 рецептора.
- До 2015г. – 24 съобщени случая

Paciolla M et al. Genes Immun 2015

В 91.7% от случаите – 1º бактериална инфекция преди 6-месечна възраст:

- 50% - инвазивни;
- 66% - неинвазивни бактериални инфекции: целулит, фурункули, фоликулити, трудно овладяващи се с локална терапия. 5/10 – аденит; 2/10 – синуит; 2/10 – рецидивиращ среден отит.



Feuerstein R, et al. J Immunol 2015

Picard C, 2010

Профилактично лечение при MYD88 дефицит:

- 6/10 – перорален Penicillin или Cotrimoxazole;
- 4/10 – емпирична терапия при инфекции;
- 3/12 – анти-пневмококова ваксина;
- 8/12 – анти- Хемофилусна ваксина;
- 7/12 – анти – менингококова ваксина.

Микробиологични причинители

Бактериални инфекции: 81%

- ***S. pneumoniae: 37.5%***
- S. aureus: 31.2%
- P. aeruginosa – 12.5%
- B-хемолитичен стрептокок, Salmonella enteritidis,
- H. influenzae, Moraxella catarralis.

Picard C, et al. 2011

Други инфекции:

- Хистоплазма капсулатум, редки случаи на белодробна аспергилоза.

Coady A, et al. Infect Immun 2015

- Регистрирани са няколко токсоплазмени инфекции.

Заключение:

- Инвазивни бактериални инфекции, стартиращи в ранна детска възраст, налагат съмнение за дефект във вродения имунитет при нормални рутинни имунологични тестове.
- Генетичната диагноза е от ключово значение за определяне на точната диагноза поради дори компенсаторно стимулирания клетъчен и хуморален имунитет.

MYD88 deficiency

- Имунологични изследвания
  - Антиген-специфичният Т- и В-клетъчен отговор са нормални с рутинните имунологични тестове.
  - При 6/10- нормални CD 16 и CD56- NK клетки
- Установяват се CD3-специфични антитела
- При 4/10 деца с тази диагноза се установяват силно завишени стойности на ИгГ, ИгА , ИгМ, като при 2/10 се установят екстремно завишени ИгГ субклас 4
- При 3/10 пациента- силно завишени стойности на ИгЕ
- Увреден е IgM отговора срещу Т-независими бактериални антигени.

Maglione P. et al. 2015
Maglione P. et al. 2014

Благодаря за вниманието!

SESSION 5-I

Moderators: Ugur Ozbek, Dragomira Nikolova

- **Phenotype and genotype heterogeneity of thalassemia intermedia**
  D. Plaseska Karanfilska
- **Non-transfusion-dependent thalassaemia**
  V. Kaleva
- **Colorectal carcinoma – from genetic markers, response predictors for the treatment to personalized therapy and new genetic classification**
  G. Kurteva

Oral presentations:

- **Molecular defects determined among hemophilia patients in Republic of Macedonia**
  E. Shukarova Stefanovska
- **Molecular profiling of hereditary breast and ovarian cancer in Bulgaria**
  R. Kaneva
- **Differential expression of 12 microRNAs in breast cancer and their potential use as markers for different clinicopathologic features**
  K. Popovska-Jankovic

PHENOTYPE AND GENOTYPE HETEROGENEITY OF THALASSEMIA INTERMEDIA

Dijana Plaseska-Karanfliska

β Thalassemia phenotypes

β Thalassemia	Clinical severity
Silent carrier	Asymptomatic No hematological abnormalities
Minor / Trait	Borderline asymptomatic anemia Microcytosis and hypochromia
Intermedia	Late presentation Mild -Moderate anemia Transfusion independent Variable clinical severity
Major	Early presentation Severe anemia Transfusion dependent

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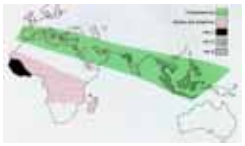
Thalassemias

- Reduced rate or complete lack of production of one or more globin chains
- Two main forms: α and β thalassemia
- Caused by mutations in the globin genes



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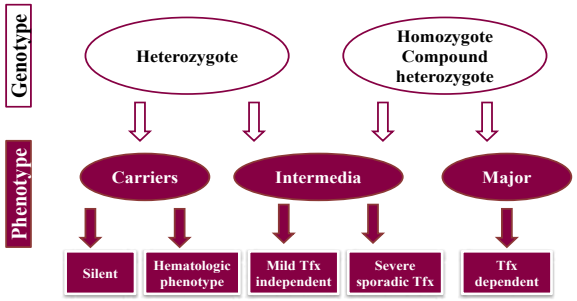
β thalassemia genotype/phenotype heterogeneity



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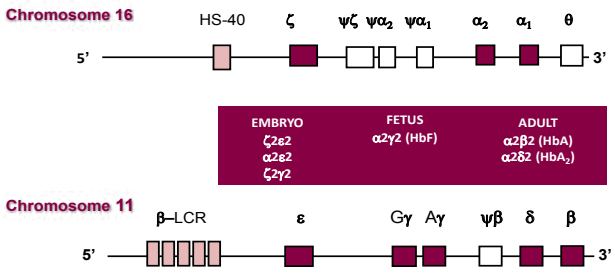
Chromosomal organization of the globin gene clusters



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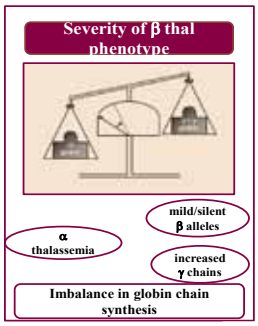
β thalassemia

- **Primary genetic modifiers**
  - ✓ broad diversity of β globin gene mutations
- **Secondary modifiers**
  - ✓ involved in modifying the degree of globin chain imbalance
- **Tertiary modifiers**
  - ✓ effect on complications of the disease



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α Thalassemia classification

α Thalassemia	Genotype	Phenotype
Silent carrier	αα / –α	Asymptomatic
α thalassemiaTrait	–α / –α αα / – –	Mild anemia
Hemoglobin H disease	–α / – –	Moderate anemia
Hb Barts/Hydrops fetalis	– – / – –	Incompatible with life

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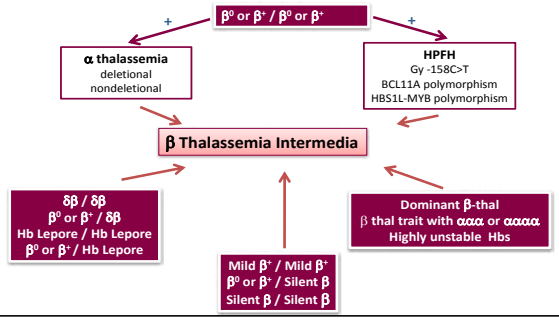
Molecular Basis of β Thalassemia Intermedia



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Thalassemia major & intermedia differential diagnosis

Clinical	Thalassaemia Major	Thalassaemia Intermedia
Presentation (years)	<2	>2
Hb levels (g/dl)	6-7	8-10
Liver/Spleen enlargement	Severe	Moderate to severe
Haematologic		
HbF (%)	>50	10-50 (up to 100)
HbA_2 (%)	<4	>4
Genetic		
Parents	Both high A_2 thalassemia carriers	One or both atypical carriers (high HbF or borderline A_2)
Molecular		
Type of mutation	Severe	Mild/Silent
HPFH	No	Yes
δβ thalassemia	No	Yes
Coinheritance of α thal	No	Yes

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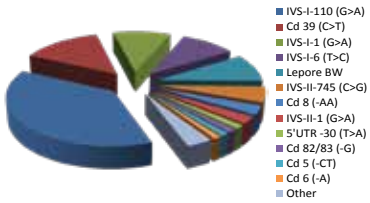
β globin gene mutations among Macedonian thalassemia patients



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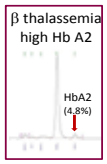
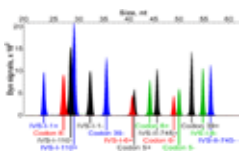
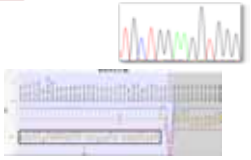
Laboratory diagnostics for thalassemias at RCGEB

Level	Methods
Hematological	Hb, MCV, MCH, blood smears
Protein (biochemical)	HPLC (Hbs, globin chains),
Molecular (genetic)	➢ Point mutations SNaPshot, DNA Sequencing ➢ Deletions gap PCR, MLPA







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Thalassemia intermedia patients diagnosed at RCGEB (2011-17)

Genotype	No
β thal double heterozygotes	**8**
$β^0/β^{+(mild)}$	1
$β^0/β^{++}$	2
$β^+/β^{++}$	4
$β^0/β^0$ + α thal + high HbF determinants	1
β thal heterozygotes + ααα	**29**
$β^0$	20
$β^{+(severe)}$	9
HbH disease	**3**
--/-α	1
$αα^{Agrinio}/αα^{Agrinio}$	2
Total	**40**

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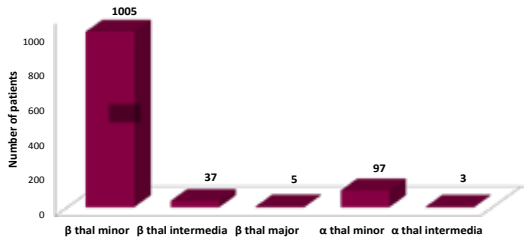
Different forms of thalassemias diagnosed at RCGEB (2011-2017)



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TI patients (double heterozygotes) diagnosed at RCGEB (2011-1017)

Lab. No.	Gender /Age	Hb g/dl	RBC 10^{12}/l	HCT %	MCV fl	MCH pg	HbA2 %	Hb F %	β Genotype	α thal	Transfusion	Complications
R6350	M/2	8,7	4,5	26,7	59,1	19,2	5,3	26,2	IVS-I-6/PolyA	No	No	No
R5284	F/7	8,4	4,0	23,1	58,2	21,2	3,7	51.6	IVS-II-848/Poly A	1	1	Hepatospleno megaly
R5284/B	M/7	8,5	4,4	24,6	55,4	19,1	5,7	32,9	IVS-II-848/Poly A	1	1	Hepatospleno megaly
R5825	M/27	8,1	3,2	24,1	76,3	25,6	3,1	54,9	Cd6/IVS-I-6	No	a few	Splenomegaly
R6353	F/30	8,6	4,8	26,4	55	17,9	5,2	1,7	Cd 6/-190	No	No	No
R5879	F/31	9,1	3,9	28,0	69,7	23,0	5,7	9,5	IVS-I-110/-101	No	No	No
R6358	F/35	8,1	4,8	25,6	53,7	17	7,5	8,3	Cd 39 /-101	No	No	No
R6618	M/6	10,8	4,8	29,8	66,8	24,2	0,8	82,4	IVS-II-1/IVS-II-1	$β^0/β^0$	$α^{-3,7}/ααα$	No	No

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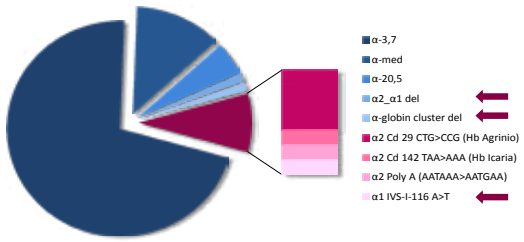
α globin gene defects among Macedonian thalassemia patients



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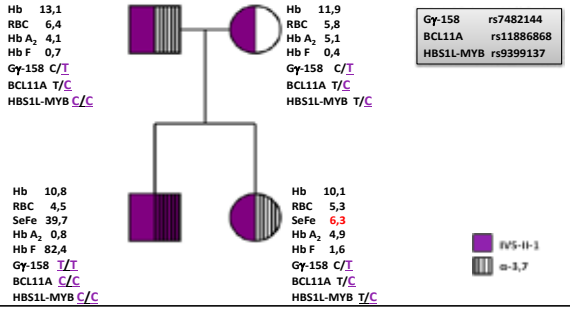
Interaction of $β^0/β^0$, α thal & HbF determinants



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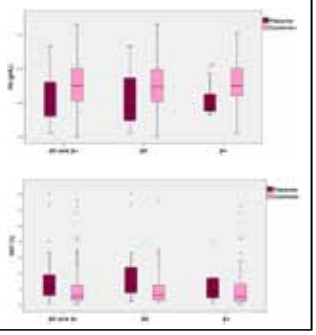
β thal heterozygotes with coinherited α triplication

	Hematological parameter	β-thal carriers + $\alpha\alpha^{anti-3.7}$	Simple β-thal carriers	P-value
All patients	No. of patients	29	145	
	Mean Age	21,3 ± 20,7	20,8 ± 19,7	
	Hb (g/dL)	10,1 ± 1,4	11,0 ± 1,3	0,0019
	RBC (10^6/μl)	5,3 ± 0,7	5,8 ± 0,7	0,0003
	HCT (%)	30,9 ± 3,7	33,8 ± 4,8	0,0021
	MCV	59,3 ± 4,2	58,9 ± 4,1	0,6083
	MCH	19,3 ± 1,6	19,1 ± 1,5	0,4353
	HbA2	4,7 ± 0,7	5,0 ± 0,7	0,0421
	HbF	3,6 ± 3,4	2,1 ± 2,8	0,0421
β°	No. of patients	20	100	
	Age	24,6 ± 20,7	22,7 ± 19,6	
	Hb (g/dL)	10,1 ± 1,6	11,0 ± 1,3	0,0130
	RBC (10^6/μl)	5,2 ± 0,8	5,7 ± 0,7	0,0035
	HCT (%)	30,4 ± 3,8	33,6 ± 5,1	0,0087
	MCV	59,2 ± 4,5	58,9 ± 3,6	0,8013
	MCH	19,4 ± 1,7	19,1 ± 1,3	0,3862
	HbA2	4,8 ± 0,7	5,1 ± 0,7	0,0679
	HbF	3,8 ± 3,8	2,1 ± 2,7	0,0219
β⁺	No. of patients	9	45	
	Age	14,1 ± 20,0	14,4 ± 18,7	
	Hb (g/dL)	10,2 ± 1,0	11,1 ± 1,3	0,0634
	RBC (10^6/μl)	5,3 ± 0,5	5,8 ± 0,7	0,0383
	HCT (%)	31,9 ± 3,5	34,2 ± 4,1	0,1150
	MCV	59,5 ± 3,8	58,7 ± 5,0	0,6273
	MCH	19,1 ± 1,6	19,0 ± 1,8	0,8487
	HbA2	4,5 ± 0,7	4,7 ± 0,4	0,3243
	HbF	2,5 ± 2,4	2,2 ± 3,0	0,8286



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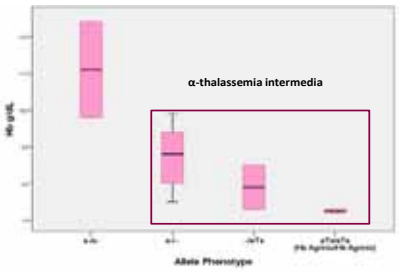
Severity of anemia in HbH patients due to deletional and non-deletional defects



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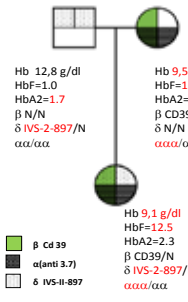
Thalassemia Intermedia
β + δ globin gene mutations + ααα



	R5488	R5488/M	R5488/T
HBB	Cd39(C>T)/ N	Cd39(C>T)/N	Normal
HBA	**ααα**/αα	**ααα**/αα	αα/αα
HBD	IVS-II-897(A>G)/N	Normal	IVS-II-897(A>G)/N
HBB Haplotype	II/V	II/II	V/IX
mRNA expresion β : γ	55.3:44.7	100:0	100:0
mRNA expresion Gγ : Aγ	58:42	60.1: 39.9	/
mRNA expresion AγT : Aγl	55:45	/	/
5' HBB -530 (rs140533502)	$(AT)_7(T)_9/(AT)_7(T)_7$	$(AT)_7(T)_9/(AT)_7(T)_7$	$(AT)_7(T)_9/(AT)_8(T)_5$
5' HBB -551 (rs35755129)	T/T	T/C	T/C
5' HBB -703 (rs11036364)	T/T	T/C	T/C
HBS-1L-MYB (rs9399137)	C/T	C/T	C/T
BCL11A (rs11886868)	T/C	T/T	T/C
XmnI (rs7482144)	C/C	C/C	C/T
Pre-Gγ Haplotype	TGA/TGG	TGG/TGG	TGA/TAG
5'δ Haplotype	R/T	T/T	R/T
KLF1	Normal	Normal	Normal
AHSP	Normal	c.*18T>C	Normal

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Treatment options for TI

- Transfusion therapy
- Splenectomy
- Iron chelation therapy
- Modulation of fetal hemoglobin production
- Hematopoietic stem cell transplantation

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Hb H disease due to homozygosity for Hb Agrinio in Romani population from the R. Macedonia

	R3911	R3911/M	R3911/F	R5669	R5669/M	R5669/F
Sex/ Age	F/11	F/32	M/33	F/3	F/18	M/23
Hb	7.8	12.6	13.0	7.7	13.9	13.6
RBC	3.7	5.4	5.5	3.3	5.1	5.5
HCT	23.0	38.0	38.0	22.6	39.6	38.7
MCV	62.0	70.0	70.0	69.1	78.3	70.1
MCH	21.1	23.3	23.6	23.5	27.5	24.7
SeFe	/	/	/	/	15.9	20.2
Hb A2	2.0	2.6	2.5	1.9	2.1	2.4
α Genotype	$\alpha^{Agr}\alpha/\alpha^{Agr}$	$\alpha^{Agr}\alpha/\alpha\alpha$	$\alpha^{Agr}\alpha/\alpha\alpha$	$\alpha^{Agr}\alpha/\alpha^{Agr}$	$\alpha^{Agr}\alpha/\alpha^{anti3.7}$	$\alpha^{Agr}\alpha/\alpha\alpha$

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Hb Agrinio

α^{Agr}	Hb Agrinio
Common name	Cd 29 CTG>CCG [Leu>Pro]
HGVS nomenclature	NM_000517.4:c.89T>C NP_000508.1:p.Leu30Pro
Allele phenotype	α^+
Stability	Hyperunstable
Occurrence	Greece, Cypriot and Spain

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Ackgnowledgements

RCGEB "Georgi D. Efremov"
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ТРАНСФУЗИОННО НЕЗАВИСИМА ТАЛАСЕМИЯ

Валерия Калева

Трансфузионно независима таласемия

- Трансфузионно независима таласемия (ТНТ) е понятие, което дефинира група от таласемии, които, за разлика от таласемия майор (ТМ), не изискват редовни хемотрансфузии, но на по-късен етап от своя живот може да имат нужда от епизодични или редовни трансфузии при задълбочаване на анемичния синдром или поява на клинични усложнения
- Форми на ТНТ
  - β-thalassaemia intermedia
  - haemoglobin E (HbE)/β-thalassaemia (mild/moderate)
  - α-thalassaemia intermedia (haemoglobin H (HbH) disease)
  - haemoglobin S (HbS)/β-thalassaemia
  - haemoglobin C (HbC)/β-thalassaemia

Weatherall DJ. *Blood Rev* 2012; 26S: S3-6.

Класификация на таласемиите

Хетерогенна група от вродени болести

- **Намалена или пълна липса на синтез на една или повече глобинови вериги →**
  - **-- нарушена еритропоеза**
  - **-- хемолиза**
  - **-- хронична анемия**
- **Класифицират се според типа на засегнатата глобинова верига**
- **7 вида глобинови вериги: ξ, α, ε, Gγ, Aγ, δ, β**
- **7 вида таласемии**

Muncie HL, Campbell JS. *Am Fam Physician* 2009; 80: 339-44.

Класификация на таласемиите

Хетерогенна група от вродени болести

Намалена или пълна липса на синтез на α-вериги на Hb → **α-таласемии**

Намалена или пълна липса на синтез на β-вериги на Hb → **β-таласемии**

Muncie HL, Campbell JS. *Am Fam Physician* 2009; 80: 339-44.

Субкласификация на α- и β-таласемии

- α-Thalassaemias[1]
  - α-thalassaemia 2 trait (single α-gene deletion)
  - α-thalassaemia 1 trait – minor (double α-gene deletion)
  - haemoglobin constant spring (reduced output of α-globin)
  - HbH (triple α-gene deletion)
  - hydrops fetalis with Hb Bart's (absence of α genes)
- β-Thalassaemias[2]
  - β-thalassaemia major
  - β-thalassaemia intermedia
  - β-thalassaemia minor
  - β-thalassaemia with Hb anomalies
    - HbC/β-thalassaemia
    - HbE/β-thalassaemia
    - HbS/β-thalassaemia
  - hereditary HbF and β-thalassaemia
  - β-thalassaemia associated with
    - trichothiodystrophy
    - x-linked thrombocytopenia

1. Muncie HL, Campbell JS. *Am Fam Physician* 2009; 80: 339-44.
2. Galanello R, Origa R. *Orphanet J Rare Dis* 2010; 5: 11.

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HbF = fetal haemoglobin.

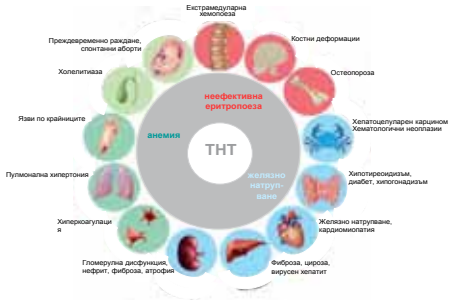
1. Muncie HL, Campbell JS. *Am Fam Physician* 2009; 80: 339-44.
2. Galanello R, Origa R. *Orphanet J Rare Dis* 2010; 5: 11.

Трансфузионно независима таласемия

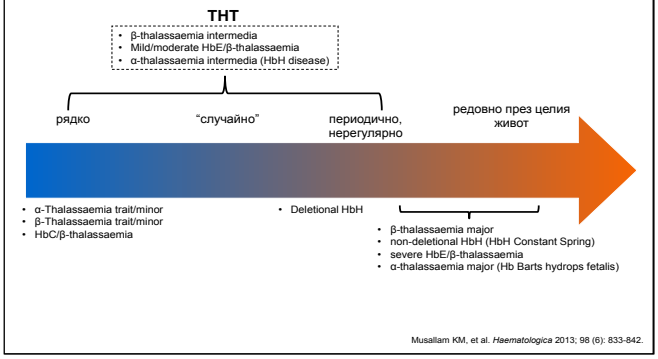
β-Thalassaemia intermedia (β-таласемия интермедия, β-ТИ)	• Резултат от унаследяване на 1 или 2 леки алела на β-таласемия или коунаследяване на генетични модификатори, които намаляват тежестта, свързана с унаследяването на 2 тежки алела на β-таласемия
HbE/β-thalassaemia (HbE/β-таласемия)	• Резултат от коунаследяване на структурния вариант, известен при HbE и много различни алели на β-таласемия • Степента на клинична вариабилност зависи от това дали наследственият β-таласемичен алел е от тежък, или лек вид и дали са включени други генетични или негенетични модификатори
HbH disease (HbH болест)	• Причинява се от инактивиране на 3 α-глобинови гени • Тежестта е свързана с подлежаща молекулярна патология: -- тези, които унаследяват делеционни форми на α+ и α^0 таласемия, обикновено се проявяват с лека клинична проява -- тези, които унаследяват един делеционен и един неделеционен алел, обикновено имат по-тежко заболяване

Weatherall DJ. *Blood Rev* 2012; 26S: S3-6.

Клинично представяне на ТНТ



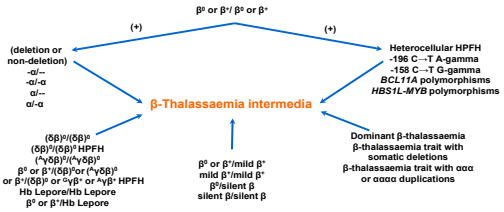
Необходимост от трансфузии при таласемия



Musallam KM, et al. *Haematologica* 2013; 98 (6): 833-842.

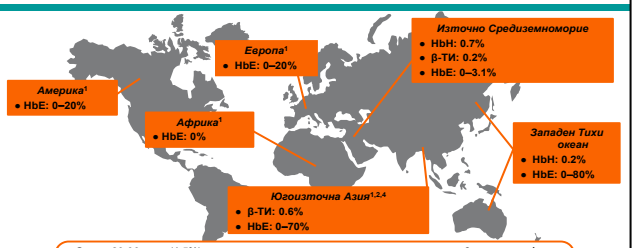
β-таласемия интермедия

- Клинично понятие за характеризиране на клинична и хематологична находка при пациенти с β-таласемия, чийто клиничен фенотип варира от трансфузионно зависима β-таласемия майор до β-таласемия минор[1, 2]
- Клиничният спектър е разнообразен и се дължи на множество различни генотипни комбинации [1, 2, 3]



[1] Taher A, et al. Blood Cells Mol Dis. 2006;37:12-20. [2] Taher AT, et al. Blood Rev. 2012;26S:S24-7.
[3] Musallam KM, et al. In: Weatherall D, et al., eds. Hemoglobin and its diseases. Cold Spring Harbor Laboratory Press, NY, USA; 2012.

Разпространение на ТНТ



- Около 80-90 млн. (1.5%) от населението на света са носители на гена на β-таласемия[1]
  -- около 50% произхождат от Югоизточна Африка[1]
- Около 50% от тежките форми на β-таласемия съдържат HbE генотип[7]
- Нарастващо разпространение на HbE/β-thalassaemia (над 1 милион)[3]
- Миграцията увеличава разпространението на таласемия в страните от западния свят

1. Weatherall DJ. Ann NY Acad Sci. 2005;1054:11-7. 2. Weatherall DJ, Clegg JB. Bull WHO. 2001;79:704-12.
3. Qadri MI, Islam SA, Saudi Med J. 2000;21:666-71. 4. Sachdev R, et al. Ind J Pathol Microbiol. 2010;53:57-62.
5. Lau Y-L, et al. N Engl J Med. 1997;336:1298-301. 6. Ashtiani MT, et al. J Clin Pathol. 2009;62:924-5.
7. Olivieri N, et al. Indian J Med Res. 2011;134:522-31. 8. Vichinsky E. Hematology Am Soc Hematol Educ Program. 2007;79-83.

Глобално разпространение на β-таласемия интермедия

Нарушения на Hb	Раждания годишно
β-таласемия майор	22,989
β-таласемия интермедия	недостатъчно данни

β-таласемия интермедия се наблюдава с ниска и променяща се честота при популациите с висока честота на β-таласемия и е особено разпространена в районите на Африка, където преобладават леки алели на β-таласемия

Weatherall DJ. *Blood* 2010; 115: 4331-6.
Weatherall DJ. *Blood Rev* 2012; 26S: S3-6.

Патофизиология и клинични последствия

Musallam KM, et al. *Hematologica* 2013; 98: 833-44.

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Нарушения на Hb	Раждания годишно
β-таласемия майор	22,989
β-таласемия интермедия	недостатъчно данни

Нарушения на Hb	България (пациенти)
β-таласемия майор	284
β-таласемия интермедия	29

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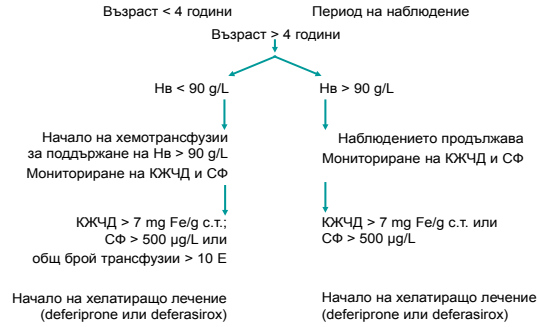
Weatherall DJ. *Blood* 2010; 115: 4331-6.
Weatherall DJ. *Blood Rev* 2012; 26S: S3-6.

β-таласемия интермедия
клинична характеристика

- Клиничният спектър включва:
  - напълно асимптоматични пациенти, представящи се само с лека анемия (Hb 70-100 g/L), изискваща рядко хемотрансфузии при специфични ситуации
  - пациенти с по-тежка форма, при които симптомите започват да се проявяват обикновено във възрастта между 2 и 6 години и изискват епизодични или редовни хемотрансфузии
- За разлика от β-таласемия майор и β-таласемия минор, липсата на специфична клинична картина прави диагнозата по-трудна и често остава дълго време неразпозната
- Дефиниция в практически аспект:
  пациент с таласемия със или без спленомегалия, при който след 2-годишна възраст се установява Hb 70-100 g/L.

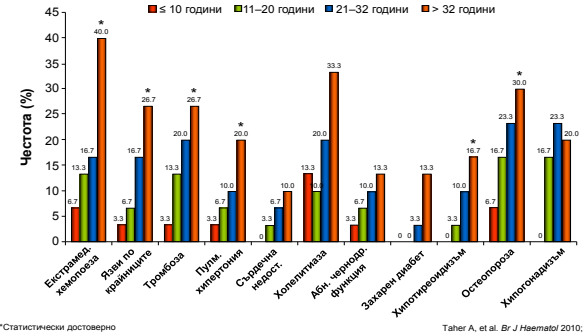
Taher A, et al. *Blood Cells Mol Dis* 2006; 37: 12-20.
Taher AT, et al. *Blood Rev* 2012; 26S: S24-7.

Алгоритъм за поведение при желязно натрупване при β-таласемия интермедия



Taher A, et al. *Br J Hematol* 2009; 147: 634-40.

β-таласемия интермедия
клинични усложнения



*Статистически достоверно

Taher A, et al. *Br J Haematol* 2010; 150: 480-97.

β-таласемия интермедия
терапевтични опции

- ***Hydroxyurea***
-- индуктор на HbF
-- повлиява диференциацията на стволови клетки в костния мозък чрез стимулиране на постнатална експресия на γ-глобиновите гени и повишава производството на HbF
-- повишаването на Hb варира между 6 и 25 g/L, но в 25% се наблюдава липса на успех
-- предпазва от екстрамедуларна хемопоеза, пулмонална хипертония, язви на краката, хипотиреоидизъм и остеопороза
-- ефективността се повишава при комбинация с хемотрансфузии и желязо-хелатираща терапия
-- краткотрайното прилагане се толерира добре от пациентите, но ефективността намалява при продължителна употреба
-- стартираща доза – 10 mg/kg с ескалиране на дозата от 3-5 mg/kg на всеки 8 седмици до максимална толерираща доза, не надвишаваща 20 mg/kg
-- допълнителен прием на фолиева киселина

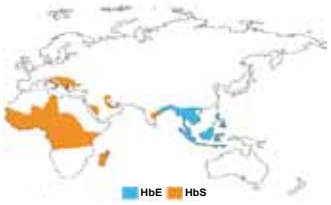
Taher AT, et al. Blood. 2010;115:1886-92. Taher AT, et al. Br J Haematol. 2011;152:512-23.
Viprakasit et al. Orphanet Journal of Rare Diseases 2014, 9:131

β-таласемия интермедия
терапевтични стратегии

- **Терапевтично поведение – предизвикателство за клиницистите:**
  - **голям брой, различни комбинации и различна степен на тежест на потенциалните усложнения**
  - **липса на ясни насоки за лечение на основното заболяване**
- **Терапевтични стратегии:**
  - **Спленектомия**
  - **Хемотрансфузии**
  - **Хелираща терапия с желязо**
  - ***Hydroxyurea***

[1] Taher A, et al. *Blood Cells Mol Dis* 2006; 37: 12-20.
[2] Aessopos A *et al. Transfusion* 2007;47:792–800. [3] Borgna-Pignatti C. *Br J Haematol* 2007;138:291–304.

Глобално разпространение на HbE/β-таласемия



Нарушения на Hb	Раждания годишно
HbE/ β-таласемия	19,128

Нарушения на Hb	България (пациенти)
HbE/ β-таласемия	-

HbE/β-таласемия е най-разпространената форма на тежка таласемия в популациите на Източна Индия, Бангладеш и Югоизточна Азия

Weatherall DJ. *Blood* 2010; 115: 4331-6.
Weatherall DJ. *Blood Rev* 2012; 26S: S3-6.

β-таласемия интермедия
терапевтични опции

- **Спленектомия**
-- показана при пациенти, които се нуждаят от чести хемотрансфузии, хиперспленизъм и симптоматична спленомегалия
-- асоциирана е с повишен риск от тромбоемболични усложнения, пулмонална хипертония и субклинични мозъчни инфаркти
- **Хемотрансфузии**
-- вземането на решение е трудно и е голямо предизвикателство за клиницита
-- показани при Hb < 50 g/L, изоставане в растежа, скелетни деформации, невъзможност за провеждане на физически упражнения, спадане на Hb следствие на нарастваща спленомегалия, инфекция, бременност и др. специфични за болестта усложнения (тромбоза, мозъчни инфаркти и др.)
-- предпазва от тромбоза, екстрамедуларна хемопоеза, пулмонална хипертония, сърдечна недостатъчност, холелитиаза и язви на краката
-- по-добро качество на живот
-- повишен риск от ендокринни усложнения
- **Хелираща терапия с желязо**
-- препоръчва се при пациенти с критерии за железен свръхтовар
-- серумен феритин (СФ) не е реален показател за натрупано желязо в организма
-- препоръчва се мониториране на концентрацията на желязо в черния дроб (ЖЧД) чрез биопсия, МРТ или ферискан на всеки 1-2 години

Taher AT, et al. Blood. 2010;115:1886-92. Taher AT, et al. Br J Haematol. 2011;152:512-23.
Viprakasit et al. Orphanet Journal of Rare Diseases 2014, 9:131

Фенотипът на HbE/β-таласемия е повлиян от множество генетични фактори

Вариант	Генотип	Фенотип
HbE-носителство	β^E/β	Асимптоматично състояние без клинично значение
HbE болест	β^E/β^E	Обикновено напълно асимптоматични, без анемия и хемолиза
HbE/ β-thalassaemia	β^E/β^+,β^E/β^0	Тежестта е много различна; клиничната картина варира от таласемия майор до таласемия минор
Компаунд хетерозиготи	β^E/β^S	Наподобява сърпоклетъчна анемия, но обикновено с редки вазооклузивни кризи

Gurkan E. *Am J Hematol* 2006; 81: 149-56.
Vichinsky E. *Hematology Am Soc Hematol Educ Program* 2007; 79-83.

HbE/β-таласемия
клинична характеристика

- HbE/β-таласемия е асоциирана с много различен клиничен фенотип
- 50% от случаите съответстват на β-таласемия майор и 50% – на β-таласемия интермедия

HbE/β-таласемия	Клиничен фенотип
Лека	• Нива на Hb между 90 and 120 g/L • Обикновено не се развиват клинично значими проблеми
Умерено тежка	• Нива на Hb между 60 and 70 g/L • Клиничните симптоми наподобяват тези на β-таласемия интермедия
Тежка	• Нива на Hb 40–50 g/L • Клиничните симптоми наподобяват тези на β-таласемия майор

Galanello R, Origa R. *Orphanet J Rare Dis* 2010; 5: 11.

Фенотипът на α-таласемия е повлиян от множество генетични фактори

Вариант	Генотип	Фенотип
Нормален	αα/αα	Нормален
"Тих" носител	-α/αα	Незначителни хематологични отклонения
Минор	-α/-α , --/αα	Гранична анемия с микроцитни и хипохромни червени кръвни клетки
HbH-болест	--/,-α , --/α^{cs}α	Умерена анемия с изразена микроцитоза и хипохромия
Хидропс феталис с Hb Bart's	--/--	Повечето развиват *хидропс феталис синдром* и умират в утробата по време на бременност или скоро след раждане

Muncie HL, Campbell JS. *Am Fam Physician* 2009; 80: 339-44.
Harteveld C, Higgs D. *Orphanet J Rare Dis* 2010; 5: 13.

Лечение на HbE/β-таласемия

- Няма препоръки за лечение на пациенти с HbE/β-таласемия
- Хемотрансфузии
  - Стабилно задържащи се нива на Hb в гранични стойности определят нуждата от редовни трансфузии
  - По принцип пациентите не трябва да получават редовни трансфузии, без да са провели за по-продължителен период (≥ 3-6 месеца без интеркурентни заболявания) мониториране на растежа, пубертетното развитие (ако е приложимо), качеството на живот, симптомите и признаците на анемия, вкл. промяната в размера на слезката
  - Костномозъчната експанзия и нарастването на размера на слезката са по-слабо изразени при възрастни пациенти; преходните трансфузии през периода на по-голяма експанзия могат да бъдат полезни
- Спленектомия
  - Изглежда има положителен ефект за растежа и развитието, но е асоциирана с повишена морбидност и смъртност от инфекции

Olivieri N, et al. Indian *J Med Res* 2011; 134: 522-31.

HbH-болест
клинична характеристика

- HbH-болест е най-тежката нефатална форма на α-таласемия[2]
  - Пациентите с HbH имат различен фенотип[1]
    - анемия (26–133 g/L) с различни количества на HbH (0.8–40%)
    - обичайна спленомегалия
    - непостоянен иктер
    - изоставане в растежа при деца
    - други усложнения: инфекции, язви по краката, жлъчни камъни
  - Пациентите с неделеционен тип HbH, които имат по-тежък фенотип, изискват чести трансфузии (10-12 годишно)[2]
    - повече клинични симптоми и по-висок серумен феритин[3]
    - по-възрастните пациенти често имат висок железен товар

1. Harteveld C, Higgs D. Orphanet J Rare Dis. 2010;5:13.
2. Fucharoen S, Viprakasit V. Hematology Am Soc Hematol Educ Program. 2009:26-34.
3. Laosombat V, et al. Ann Hematol. 2009;88:1185-92.

Глобално разпространение на HbH-болест

Нарушения на Hb	Раждания годишно
HbH болест	9,568

Нарушения на Hb	България (пациенти)
HbH болест	1

HbH-болест е най-разпространена в Югоизточна Азия и се среща спорадично в повечето от останалите тропически региони

Weatherall DJ. *Blood* 2010; 115: 4331-6.
Weatherall DJ. *Blood Rev* 2012; 26S: S3-6.

HbH-болест
терапевтичен алгоритъм

HbH пациенти
Препоръчва се добавяне на фолиева киселина, особено при деца[1]

Делеционен тип
Може да има нужда от интермитентни хемотрансфузии[2] (29–50% от пациентите)[3, 5, 6]

Неделеционен тип
Изискват по-чести хемотрансфузии (31–43% от пациентите)[3, 4, 6] и задължителна спленектомия

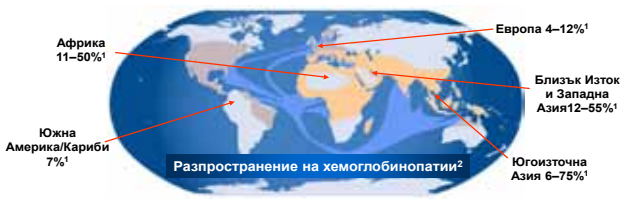
Ако има хемолитични кризи[1], нивата на Hb падат много бързо и е необходима незабавна интервенция

↓

Хемотрансфузия
Адекватна хидратация
Корекция на електролити
Контрол върху телесната температура
Идентифициране на причина за инфекция и лечение

1. Fucharoen S, Viprakasit V. Hematology Am Soc Hematol Educ Program. 2009:26-34. 2. Harteveld C, Higgs D. Orphanet J Rare Dis. 2010;5:13.
3. Origa R, et al. Br J Haematol. 2007;136:326-32. 4. Kanavakis E, et al. Br J Haematol. 2000;111:915-23.
5. Lal A, et al. N Engl J Med. 2011;364:710-8. 6. Cohen AR, et al. Hematology Am Soc Hematol Educ Program. 2004;14-34.

Миграцията увеличава разпространението на таласемия в западния свят: α-таласемия



HbH, HbH-Constant Spring и хомозиготната α-таласемия засягат поне 1 млн. от населението на света[3]

1. http://emedicine.medscape.com/article/206397-overview#a0199.
2. Harteveld C, Higgs D. Orphanet J Rare Dis. 2010;5:13.
3. Cohen A, et al. Hematology Am Soc Hematol Educ Program. 2004:14-34.

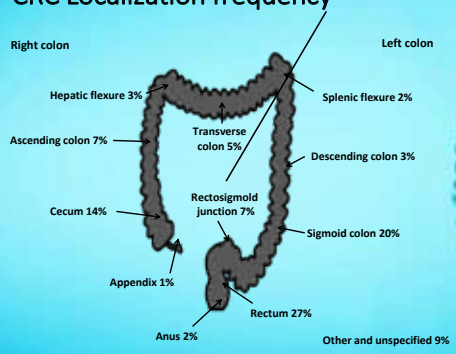
ПОСЛАНИЯ ЗА ВКЪЩИ

- Трансфузионно независима таласемия (ТНТ) – скоро въведено понятие, което се използва за таласемични фенотипове, които не се нуждаят от редовни хемотрансфузии
- Поставянето на навременна диагноза е от съществено значение за предпазване на засегнатите деца от провеждане на редовна доживотна трансфузионна терапия
- Голяма част от пациентите се развиват нормално с относително по-ниски нива на Hb, особено с HbE/β-таласемия
- Клиничните усложнения са разнообразни и се дължат на хроничната анемия, екстрамедуларната хемопоеза и желязното натрупване в организма
- Риск от железен свръхтовар съществува и при пациенти, които не се трансфузират, поради повишена гастроинтестинална абсорбция на желязо в резултат на неефективна еритропоеза
- Стойностите на серумния феритин при β-таласемия интермедия в повечето случаи дават неточна информация за степента на желязно натрупване в организма, поради което се препоръчва директна оценка на концентрацията на желязо в черния дроб
- Редовната оценка на желязното натрупване е задължителна, от която се определя началото, видът и дозовият режим на хелатиращата терапия

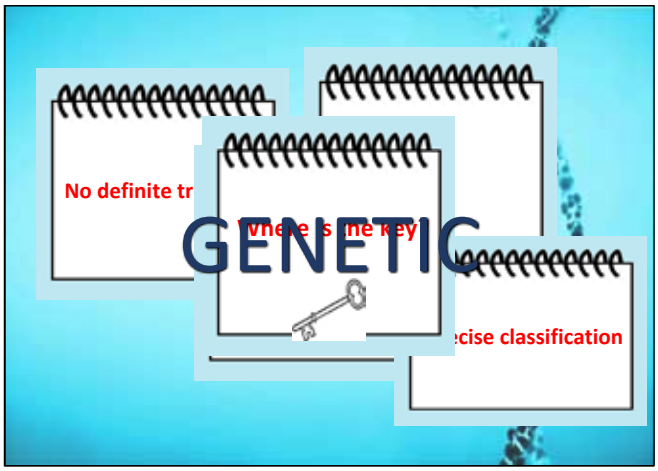
COLORECTAL CARCINOMA – FROM GENETIC MARKERS, RESPONSE PREDICTORS FOR THE TREATMENT TO PERSONALIZED THERAPY AND NEW GENETIC CLASSIFICATION

Lyubov Simeonova, Galina Kurteva

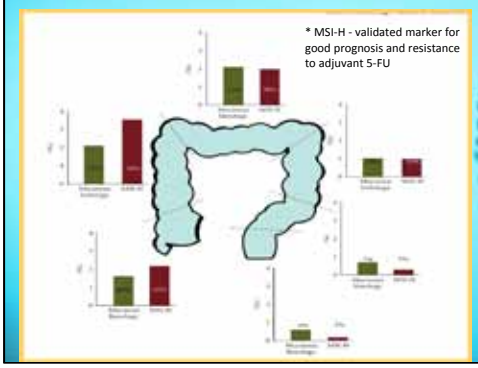
CRC Localization frequency



http://www.gponline.com/Clinical/article/1137481/Clinical-Review---Colorectal-cancer/



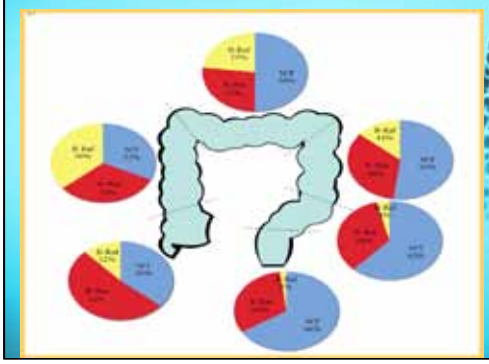
Distribution of mucosal histology and high microsatellite instability (MSI-H *)



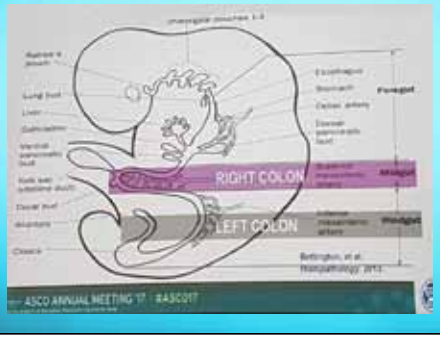
In cases of colorectal carcinoma (CRC), the established chemotherapy treatment regiments with or without a combination of target molecules, do not always cause the expected therapeutic response. Optimal therapeutic treatment can be identified based on the attained individual molecular subtype, correlating prognosis and drug sensitivity of the patients.

Studying said genetic profile can improve the prognosis of the progression of the condition as well as the personalized treatment approach.

Distribution of KRAS- and BRAF-mutations according to primary tumor localization



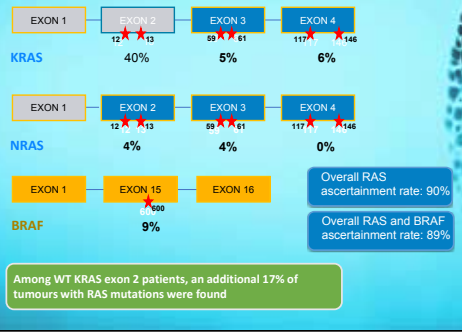
Differences begin from the very start



RAS proteins

- RAS proteins regulate cellular responses to many extracellular stimuli, including soluble growth factors
- RAS proteins are activated when GTP is bound
- RAS–GTP binds to various effector proteins to stimulate signalling pathways that regulate
  - Cytoskeletal organisation
  - Survival
  - Proliferation
  - Vesicle trafficking
  - Calcium signalling

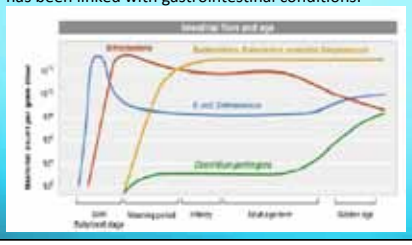
KRAS, NRAS and BRAF mutation hotspots



Among WT KRAS exon 2 patients, an additional 17% of tumours with RAS mutations were found

The role of the microbioma in carcinogenesis and therapeutic approaches

The human gut microbiota has become the subject of extensive research in recent years and our knowledge of the resident species and their potential functional capacity is rapidly growing. Our gut harbours a complex community of over 100 trillion microbial cells which influence human physiology, metabolism, nutrition and immune function while disruption to the gut microbiota has been linked with gastrointestinal conditions.



Approximately 48% of colorectal tumours harbour KRAS or NRAS mutations

Organ	Tumour type	Mutations (%) HRAS	KRAS	NRAS
Colon/rectum[1]	Adenocarcinoma	0	42*	5*
Biliary tract	Adenocarcinoma	0	35	2
Bladder	Transitional cell carcinoma	12	4	2
Liver	Hepatocellular carcinoma	0	4	4
Lung	Large cell carcinoma	4	21	4
	Non-small cell carcinoma	0	16	1
Pancreas	Ductal adenocarcinoma	0	69	1
	Endocrine tumour	0	1	75
Skin	Malignant melanoma	1	2	20

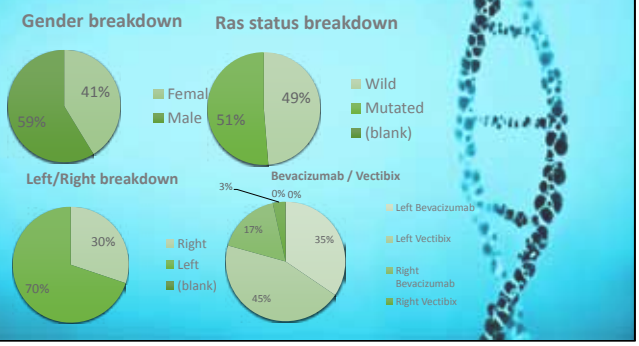
Role of the microbioma in therapeutic aproaches

- It is clear that not all probiotics are equivalent and that species effective in one disorder may be ineffective in another. In cytotoxic therapy associated mucositis, both clinical and preclinical studies support efficacy for Lactobacillus probiotics.
- Probiotic bacteria exert many beneficial functions which may be able to counter the underlying pathophysiology of mucositis. For example, they activate cytoprotective pathways in epithelial cells, counteract reactive oxygen species, displace pathogenic bacteria and interact with tight junctions to enhance mucosal integrity.
- There is another project in our clinic related to the role of microbioma in the therapeutic approach in patients with colorectal carcinoma.

Examples of predictive biomarkers in oncology

Tumour type	Biomarker	Drug
Breast cancer	HER-2 overexpression	Trastuzumab[1], lapatinib[1]
Gastric cancer	HER-2 overexpression	Trastuzumab[1]
CML	BCR/ABL fusion gene	Imatinib[1]
GIST	c-KIT mutation	Imatinib[1]
NSCLC	EGFR mutation	Gefitinib[1], erlotinib[1]
mCRC	RAS mutation status	Panitumumab[1], cetuximab[1]
Melanoma	BRAF V600	Vemurafenib[1]
NSCLC	ALK positive	Crizotinib[1]

2016 USHATO's statistics related to CRC



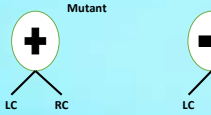
Based on up to date international research of genetic biomarkers and scientific attempts at subtyping, it is considered that CRC can be segmented into the following 5 subtypes:

- **CMS1**, (immune microsatellite instability, 14%), hyper-mutated microsatellite instability and strong immune response, BRAF (V600E) mutations;
- **CMS2,** (canonical, 37%), epithelial microsatellite stable, Tp53;
- **CMS3,** (metabolic, 13%), epithelial, metabolic dysregulation, multiple KRAS mutations;
- **CMS4**, (mesenchymal, 23%), microsatellite instability, insufficient mismatch repair protein (MRR), stromal invasion and angiogenesis;
- **CMS5**, 13% of the CRC patients cannot be assigned to the previously listed subtypes, due to the fact that they exhibit a combination of the listed genetic marker subtypes.

Conclusions can be made for the potential benefits of the application of specific treatment regiments and target molecules based on the genetic expression, common for the different subtypes.

Our project

- First line treatment for metastatic CRC disease
- 80 patients / 2 groups separate by RAS status
- Detailed genetic panel analysis of 160 genes as – KRAS, NRAS, BRAF, PTEN, ,MSH2, MSH6 etc.



Treatment scheme: XELOX (Capecitabin + Oxaliplatin) + Bevacizumab (Avastin)

Treatment scheme: XELOX (Capecitabin + Oxaliplatin) + Panitumumab (Vectibix)

Regular activities:
Every three weeks:
- Blood testing;
- Tumor marker testing;
- Infusion of the Oxaliplatin.

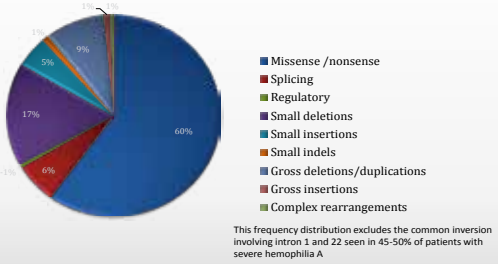
Every twelve weeks:
- Computer imaging;
- ctDNA testing.

In the case of progression or complete response, the listed analysis will be conducted again.
Estimated project duration – 2 years.
Results are expected in the period of 2018 - 2019

MOLECULAR DEFECTS DETERMINED AMONG HEMOPHILIA PATIENTS IN REPUBLIC OF MACEDONIA

Sukarova Stefanovska E, Bozhinovski Gj., Trajkova Z., Dejanova V., Plaseska-Karanfilska D.

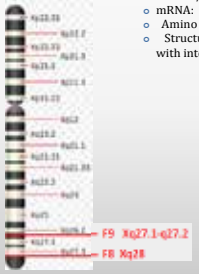
Extreme heterogeneity in disease-causing mutations reported in factor VIII and factor IX genes

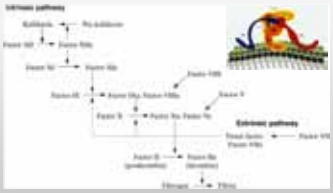


This frequency distribution excludes the common inversion involving intron 1 and 22 seen in 45-50% of patients with severe hemophilia A

EAHAD-DB Coagulation Factors Databases (F7, F8, F9)
Factor VIII gene (F8) variant database;
http://www.factorviii-db.org/
Factor IX gene (F9) variant database;
http://www.factorix.org/

- ***Hemophilia A***
  - X-linked bleeding disorder
  - Deficiency or dysfunctional coagulation factor VIII
  - Incidence: 1:5.000 male
  - Factor VIII gene
    - Xq28
    - 186 kb / 26 exons
    - mRNA: 9kb
    - Amino acids: 2370
    - Structure: three domains with internal homology
- ***Hemophilia B***
  - X-linked bleeding disorder
  - Deficiency or dysfunctional coagulation factor IX
  - Incidence: 1:25.000 male
  - Factor IX gene
    - Xq27.1
    - 34 kb / 8 exons
    - mRNA: 2.8kb
    - Amino acids: 460





IMPORTANCE OF GENETIC TESTING

- Precise genetic diagnosis
- Prediction of clinical course of disease
- Determining the risk of inhibitor development
- Determining the carriers of hemophilia genes in affected families
- Genetic counseling
- Prenatal diagnosis

- Characterization of mutations will help to identify amino acids or regions with essential functional or structural properties, and therewith clarify the mechanism of pathogenesis.

CLINICAL PRESENTATION OF HEMOPHILIA

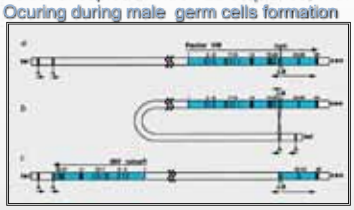
Classification	Coagulation factor level (VIII:C or IX:C)	Clinical phenotype
Severe	**<0·01 IU/mL** (<1% of normal activity)	Spontaneous soft tissue and musculoskeletal bleeding
Moderate	**0·01–0·05 IU/mL** (1–5% of normal activity)	Bleeding into joints and muscles after minor injuries or after surgical intervention
Mild	**>0·05–0·40 IU/mL** (5–40% of normal activity)	Post-operative and post-traumatic bleeding only

Material

- 70 Hemophilia A patients
  - severe 46
  - moderate 11
  - mild 13
- 25 Hemophilia B patients
  - severe 6
  - moderate 8
  - mild 11
- members of affected families
  - carrier determination
  - prenatal diagnosis

INVERSION IN INTRON 22

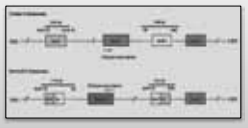
- Determined among 45% of patients with severe form of hemophilia A
- Aditional copies of intron 22 sequence, with 98% identity
- Ocuring during male germ cells formation



Model for intron 22 inversion formation

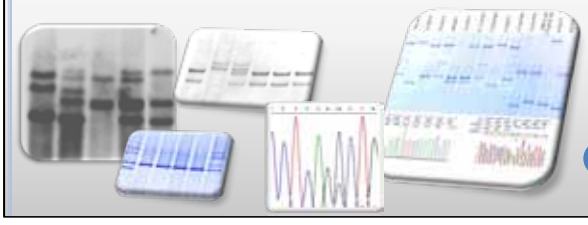
INVERSION IN INTRON 1

Determined among 0.6-5.0% of patients with severe form of hemophilia A



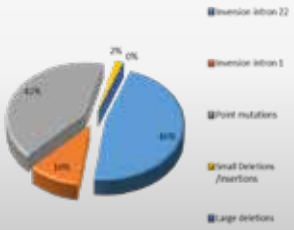
Methods

- ❑ Southern blot analysis
- ❑ Long range PCR
- ❑ PCR amplification of exons
- ❑ Restriction enzyme analysis of the amplified fragments
- ❑ Single Strand Conformation Polymorphism (SSCP)– Bio-Rad DeCode System
- ❑ Denaturation Gradient Gel Electrophoresis (DGGE)
- ❑ DNA sequencing (Big Dye 1.1, Life Technologies)



Molecular characterization of Hemophilia A

Factor VIII gene defects:	Number	Percentage
Inversion intron 22	28	46,4
Inversion intron 1	6	10,0
Point mutations	25	41,7
Small Deletions /Insertions	1	1.8
Large deletions	0	0

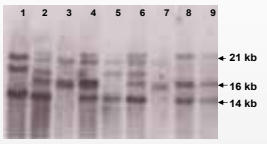


Detection rate: 60/70 (85,7%)

Molecular characterization of Hemophilia B in Republic of Macedonia

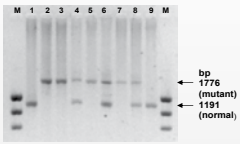
Nucleotide change (HGVS)	Amino acid Change (HGVS)	Location	Mutation Type:	Mutation Effect:	Protein Domain:	Severity of disease	No. of detected mutations
c.230_231delTT insA	p.Val77Aspfs*27	Exon b	in/del	Frameshift	Gla	Severe + inh	1*
c.316G>A	p.Gly106Ser (60)	Exon d	point	missense	EGF-1	Mild	4*
c.339T>G	p.Asn113Lys (67)	Exon d	point	missense	EGF-1	Mild	4*
c.536G>C	p.Gly179Ala (133)	Exon f	point	missense	Linker	Severe	1*
c.580A>G	p.Thr194Ala	Exon g	point	missense	Serine protease	Mild	1
c.835G>A	p.Ala279Thr (233)	exon g	point	missense	Serine Protease	Moderate	7
c.875A>C	p.Gln292Pro (246)	Exon h	point	missense	Serine Protease	Moderate	1*
c.849_850 delTA or c.850_851 delAT	Ile284* fs (238)	Exon h	deletion	frameshift	Serine Protease	Severe	1*
c.1095delA	Ser365Ser fs*3 (319)	Exon h	deletion	frameshift	Serine Protease	Severe	1*
c.1135C>T	p.Arg379* (333)	Exon h	point	nonsense	Serine Protease	Severe	1
c.1150C>T	p.Arg384* (338)	Exon h	point	nonsense	Serine Protease	Severe	1
c.1183T>C	p.Phe395Leu (349)	Exon h	point	missense	Serine Protease	Severe	1
c.1215T>G	p.Asp405Glu	Exon h	point	missense	Serine Protease	Severe	1*

INVERSIONS IN INTRON 22 AND INTRON 1 OF THE FACTOR VIII GENE AMONG MACEDONIAN HEMOPHILIA A PATIENTS



Southern blot analysis for detection of intron 22 inversion

Line 1&5. patients with inversion type 1;
line 2&6. carriers for inversion type 1;
line 3&7. patients with inversion type 2;
line 4. carrier for inversion type 2,
line 8&9. normal control.

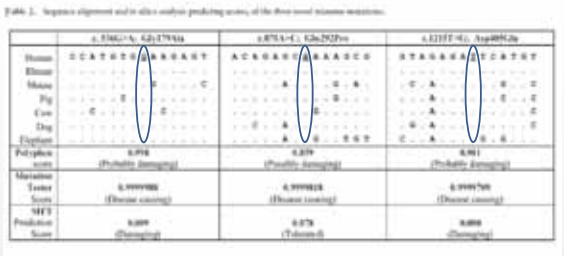


Multiplex PCR reaction for detection of intron 1 inversion

line 1&9. normal control;
line 2, 3, 5& 7. patients with inversion in intron 1;
line 4, 6 & 8. carrier for inversion in intron 1.

Inversion in intron 22: 28 (40,0% of all patients, or 60.9% of severely affected patients)
Inversion in intron 1: 6 (8.6% of all patients, or 13.0% of severely affected patients)

Sukarova Stefanovska E., et al. Acta Haematologica 120:192-194, 2008



- Each new missense variant is within a highly conserved sequence of the factor IX gene.
- Prediction of the pathogenicity was performed with "in silico" analysis

Sukarova-Stefanovska E. et al., Haemophilia, 21 (2), e144-e146, 2015

Nucleotide substitutions

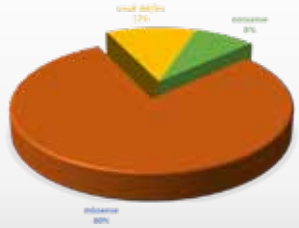
Nucleotide change (HGVS)	Amino acid Change (HGVS)	Location (exon)	Type of mutation	Domain	No of chromo-somes	Severity
c. 2T>G,	p.Met+1Arg (-19), *	Int Cd.	missense	Signal peptide	1	severe
c.43C>T	p.Arg15* (-5)	1	nonsense	Signal peptide	1	severe
c. 291G>C;	p. Ala97Pro(78)*	3	missense	A1	1	severe
c.553_5 insTC	p. Lys185Ile fs4 *	4	frameshift	A1	1	severe
c.761A>G,	p.Asn235Ser(216)	6	missense	A1	1	severe
c.871G>T;	*p. Glu291* (272)*	7	nonsense	A1	1	severe
c.1172G>A;	p.Arg391His (372)	8	missense	A2	1	moderate
c.1241A>G;	p.Tyr414Cys (395)	8	missense	A2	1	mild
c.1405G>A,	p.Gly469Arg (450)	9	missense	A2	1	severe
c. 1475A>G	p.Tyr492Cys(473)	10	missense	A2	1	mild
c.1729 T>C	p.Ser577Pro (558)	11	missense	A2	1	moderate
c.1735 G>A;	p.Asp579Asn (560)	11	missense	A2	1	mild
c.2167G>A;	p.Ala723Thr (704)	14	missense	A2	1	mild
c.2214C>A,	p.Tyr738*(719)	14	nonsense	A2	1	severe
c.5122C>T;	Arg1708Cys (1689)	14	missense	a2	1	moderate
c.5143C>T;	p.Arg1715* (1696)	14	nonsense	A3	1	severe
c.6532C>T	Arg2178Cys (2159)	23	missense	C1	5	mild
c.6545G>A;	p.Arg2182His (2163)	23	missense	C1	1	mild
c. 6577T>G,	p.Cys2193Gly (2174) *	24	missense	C2	3	moderate
c.6823T>G	p.Tyr2275Asp(2256) *	25	missense	C2	1	mild

Sukarova-Stefanovska E., BJMG, 5 (3&4), 27-35, 2002

FAMILY STUDIES

- Analysis for the carrier status in the affected families, revealed eighteen sisters of patients with HA to be carriers for the mutation and ten sisters carriers for HB.
- Fifteen prenatal diagnosis for HA and one for HB were successfully performed.
- ***Establishing the national database of molecular defects in both hemophilia A and B is important for precise genetic diagnosis and possibility for carrier determination and prenatal diagnosis in the affected families.***

Molecular characterization of Hemophilia B in Republic of Macedonia



Factor IX gene defects:	Number	Percentage
nonsense	2	8.0
Missense	20	80,0
Small Deletions /Insertions	3	12.0
Large deletions	0	0

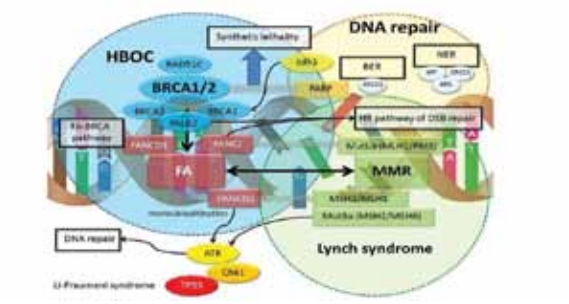
Detection rate: 25/25 (100%)

Thank you

MOLECULAR PROFILING OF HEREDITARY BREAST AND OVARIAN CANCER IN BULGARIA

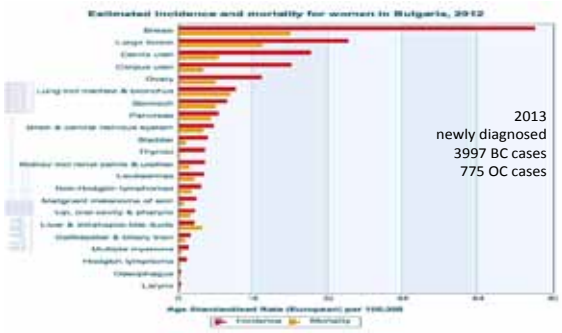
R. Kaneva, R. Dodova, A. Mitkova, D. Pencheva, S. Valev, M. Taushanova, A. Vlahova, T. Dikov, M. Vassileva, T. Sedloev, S. Christova, K. Timcheva, V. Mitev

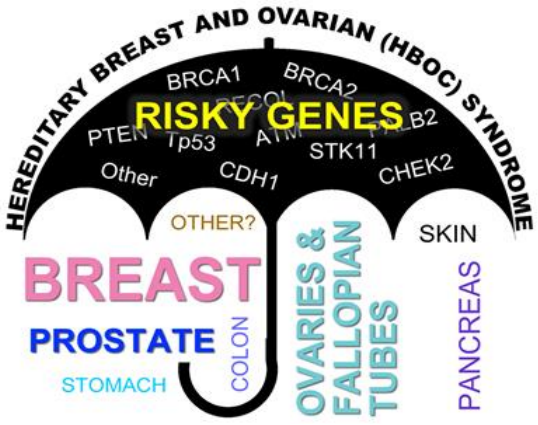
Role of DNA repair network of genes in oncogenesis



Kobayashi et al. ONCOLOGY REPORTS 30: 1019-1029, 2013

Breast and Ovarian Cancer in Bulgaria





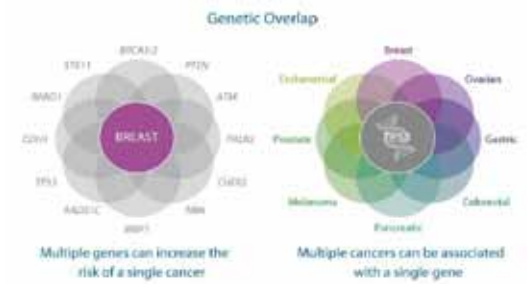
Frequency of hereditary cancer syndromes

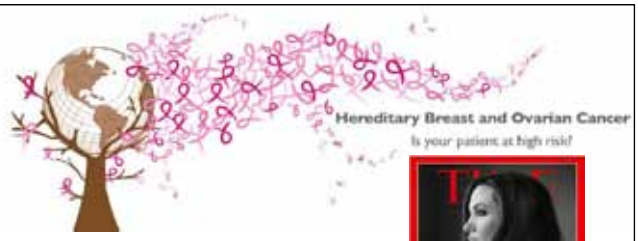
The absolute cancer risks (up to age 70) for individuals with HBOC

- Breast cancer: 46% to 71% in females, up to 2.8% in males
- Ovarian cancer: 17% to 46%.
- Prostate cancer: Up to 7.5%.
- Pancreatic cancer: 1% to 7%.
- Melanoma: 0.1% to 2.4%.

National Comprehensive Cancer Network. NCCN Guidelines: Genetic/Familial High-Risk Assessment: Breast and Ovarian

Hereditary cancer syndromes – genetic overlap



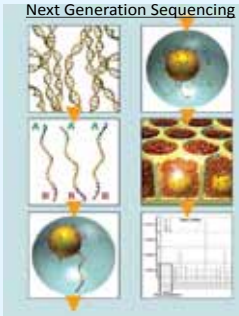


Establishing a molecular diagnosis can help guide preventive measures, direct surgical options and estimate personal and familial cancer risk.

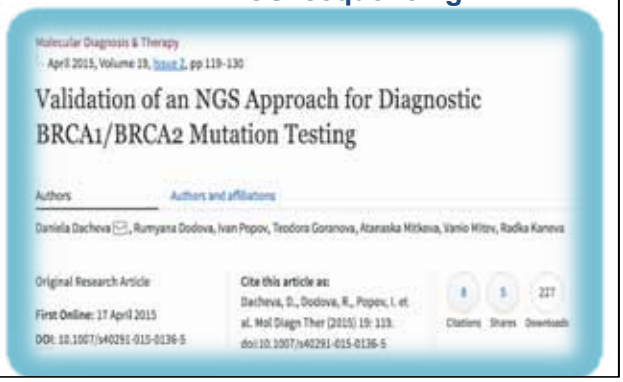
Technologies for sequencing BRCA genes

First Generation Sequencing

Next Generation Sequencing



GDL works with validated method for BRCA NGS sequencing



NGS sequencing

Illumina MiSeq

Genome Diagnostics Laboratory, Molecular Medicine Center MF, MU- Sofia

Life Technology Ion Torrent

Participation in International Quality Control Schemes

- **Laboratory of Genome Diagnostics participates in the EMQN Scheme for**
- **BRCA analysis of germline mutations, 2016**
- **NGS sequencing, 2016, 2017**
- **Guaranties high quality and reliability of the results**

Molecular testing of HBOC in Bulgaria

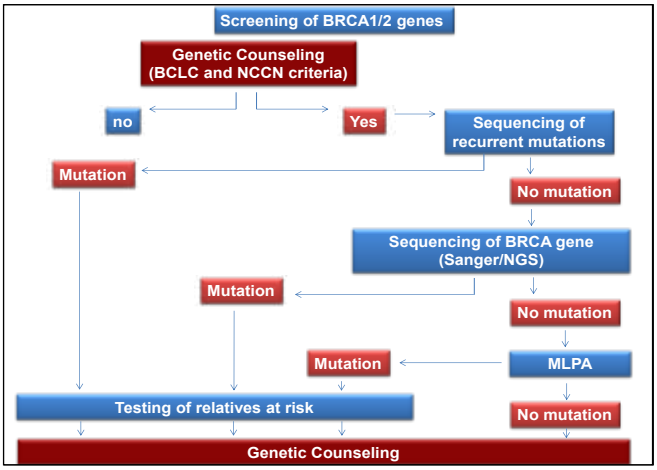
- 2009-2015 — **200** HBOC cases
- 2015-2017 — **200** HBOC cases BRCA1/2 Sanger/NGS
  125 OC cases recurrent BRCA mutations
  70 HBOC cases with Multiple Gene panel NGS sequencing

Positive

- Expertise and capacity for rapid, efficient, cost-effective diagnostic texting
- Increased awareness among patients and society about the genetic testing
- Possibility for personalised treatment for OC BRCA positive patients (PARP inhibitors)

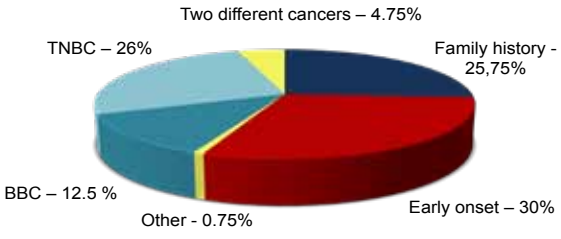
Negative

- No reimbursement from the National Healthcare System



BRCA genetic testing in Bulgaria

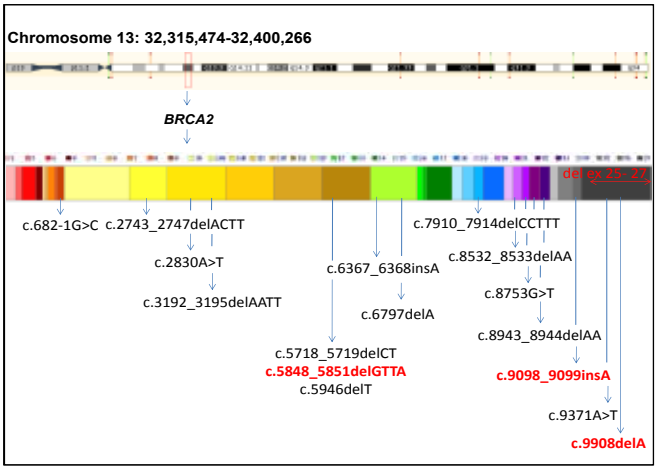
Breast cancer - 400 patients

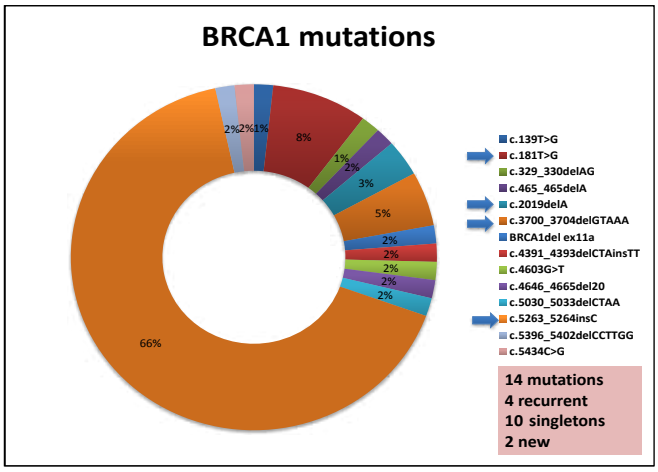


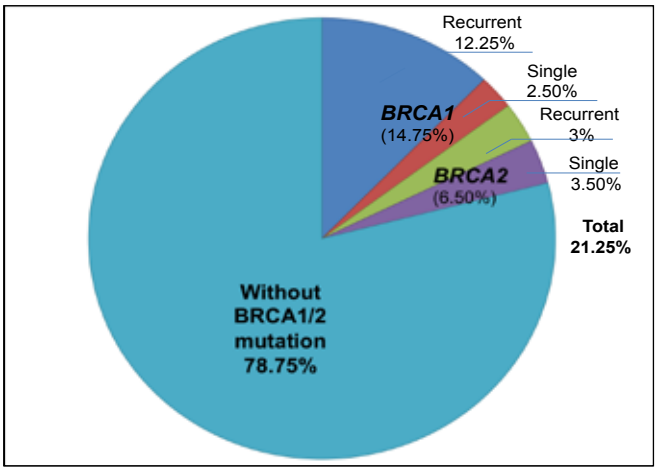
ALL VARIANTS = 92

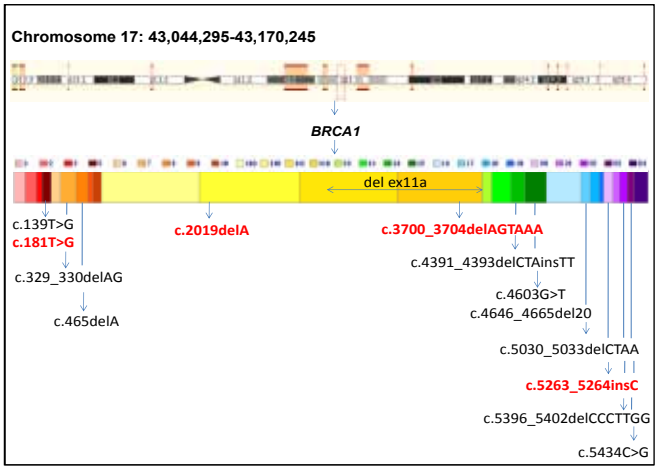
PATHOGENIC AND LIKELY PATHOGENIC = 28

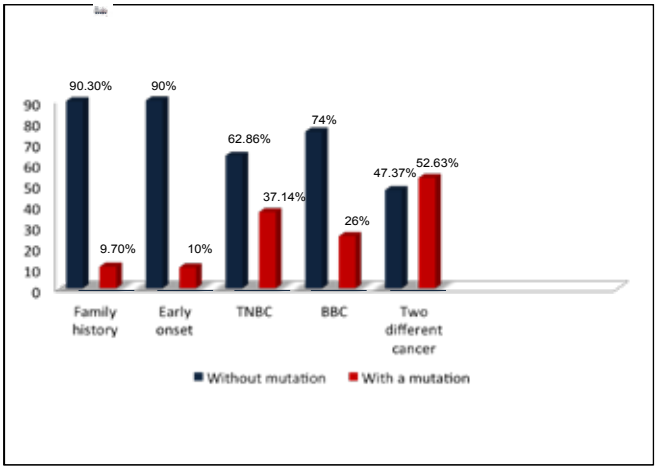
GENE	TYPE	NUMBER	
BRCA1	FS	9	42
	M	20	
	SG	1	
	SYN	4	
	IVS	8	
BRCA2	FS	12	50
	IFD	1	
	M	13	
	SG	3	
	Splice-3	1	
	UTR-3	1	
	URT-5	1	
	SYN	16	
	IVS	12	

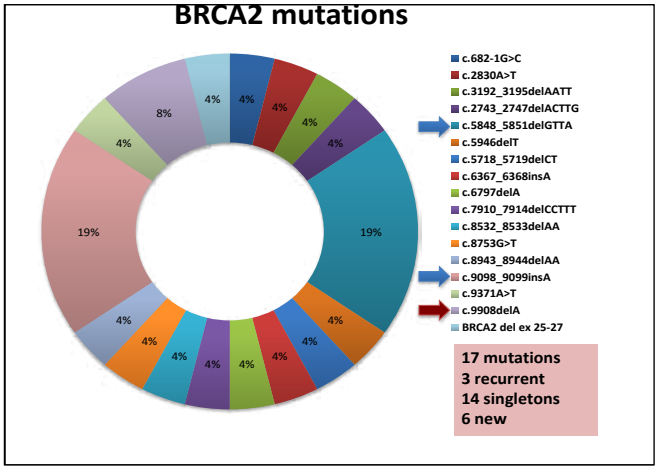


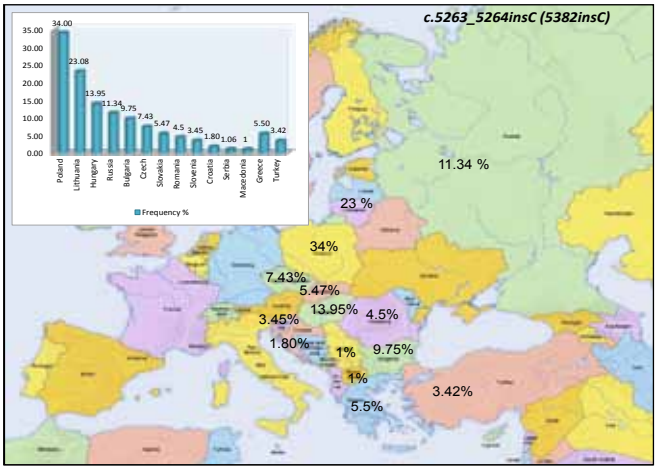












NGS sequencing in Bulgarian HBOC patients

70 HBOC patients (BCLC/ NCCN criteria)

- **31 *BRCA* (-)**
- **39 negative for the recurrent *BRCA1/2* mutations**
- **Early onset; Family history of BC/OC with other types of tumours**

- **TruSight Cancer Panel**

illumina

Pathogenic mutations found in HBOC patients with TrueSight Cancer Panel

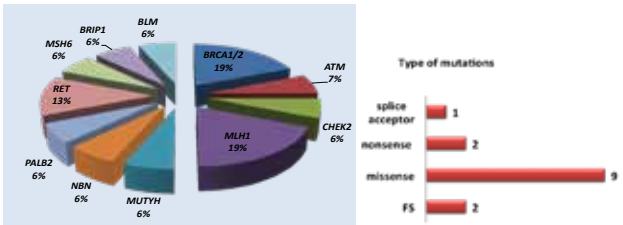
Gene	Position cDNA (HGVS)	Change	Type	Frequency	Effect
MLH1	c.1852A>G	p.Lys618Glu	missense	2.9% (2/70)	Pathogenic
MLH1	c.2093C>A	p.Ser698Ter	stop gained	1.4% (1/70)	Likely Pathogenic NEW
MSH6	c.3191C>T	p.Ala1064Asp	missense	1.4% (1/70)	Likely pathogenic
RET	c.2372A>T	p.Tyr791Phe	missense	2.9% (2/70)	Pathogenic
BLM	c.2205_2206insT	p.Tyr736Leufs	FS	1.4% (1/70)	Likely pathogenic

TruSight Cancer Panel Illumina

Breast/ Ovarian Cancer Related Gene Panel

TruSight Cancer Target Genes				
AIP	CYLD	FANCG	**NF1**	SDHAF2
ALK	DDB2	FANCI	NF2	SDHB
APC	DICER1	FANCL	NSD1	SDHC
ATM	DIS3L2	FANCM	**PALB2**	SDHD
BAP1	EGFR	FH	PHOX2B	SLX4
BLM	EPCAM	FLCN	PMS1	SMAD4
BMPR1A	ERCC2	GATA2	**PMS2**	SMARCB1
BRCA1	ERCC3	GPC3	PRF1	**STK11**
BRCA2	**ERCC4**	HNF1A	PRKAR1A	SUFU
BRIP1	ERCC5	HRAS	PTCH1	TMEM127
BUB1B	EXT1	KIT	**PTEN**	**TP53**
CDC73	EXT2	MAX	**RAD51C**	TSC1
CDH1	EZH2	MEN1	**RAD51D**	TSC2
CDK4	FANCA	MET	RB1	VHL
CDKN1C	FANCB	**MLH1**	RECQL4	WRN
CDKN2A	FANCC	**MSH2**	RET	WT1
CEBPA	FANCD2	**MSH6**	RHBDF2	XPA
CEP57	FANCE	**MUTYH**	RUNX1	XPC
CHEK2	FANCF	**NBN**	SBDS	

Distribution of pathogenic mutations



Variants found in HBOC cases with TruSight Cancer Panel

Clinical significance	Known	New	Total
Pathogenic Likely pathogenic	13	1	14
Benign and Likely Benign	30	4	34
VUS	53	6	59
Total	96	11	107

Mutations found in 22.86 % (16/70) HBOC patients
Pathogenic mutations in 11,94% in *PALB2, CHEK2, NBN, MUTYH, MLH1,* and *RET*
Probably pathogenic mutations in 7,46% in *ATM, MLH1, MSH6, BRIP1, BLM.*

Conclusions

- BRCA mutations explain 21.25 % of Bulgarian HBOC cases
- Recurrent mutations in BRCA1/2 genes account for 72.26 % of all mutations in the two genes.
- Screening of recurrent mutations is the first step in the diagnostic algorithm
- The 20 gene panel for NGS is efficient choice for genetic testing in BRCA negative families
- Pathogenic and likely pathogenic mutations have been found in 18.57 % of the BRCA negative cases
- Optimal cost-effective strategy for the routine diagnostic testing is offered, allowing genetic counseling, risk assessment and risk management in HBOC families.

Pathogenic mutations found in HBOC patients with TrueSight Cancer Panel

Gene	Position cDNA (HGVS)	Change	Type	Frequency	Effect
BRCA1	c.2019delA	p.Glu673Aspfs	FS	5.1% (2/39)	Pathogenic
BRCA2	c.682-1G>C		splice acceptor	2.6% (1/39)	Pathogenic
ATM	c.7463G>A	p.Cys2488Tyr	missense	1.4% (1/70)	Likely pathogenic
CHEK2	c.599T>C	p.Ile200Thr	missense	1.4% (1/70)	Pathogenic
BRIP1	c.1255C>T	p.Arg419Trp	missense	1.4% (1/70)	Likely pathogenic
NBN	c.511A>G	p.Ile171Val	missense	1.4% (1/70)	Pathogenic
PALB2	c.48G>T	p.Lys16Asn	missense	1.4% (1/70)	Pathogenic
MUTYH	c.1187G>A	p.Gly396Asp	missense	1.4% (1/70)	Pathogenic

Thank you for the attention!

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Collaborators
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University Hospital "Alexandrovska"
Prof. S. Hristova, Alexandrina Vlahova, Tihomir Dikov
Mariela Vassileva
University Hospital "Tzaritza Joanna – ISUL"
Assoc. Prof. T. Sedloev

DIFFERENTIAL EXPRESSION OF 12 MICRORNAS IN BREAST CANCER AND THEIR POTENTIAL USE AS MARKERS FOR DIFFERENT CLINICOPATHOLOGIC FEATURES

Popovska-Jankovic Katerina, Noveski Predrag, Kubelka-Sabit Katerina, Stojanovska Liljana, Karagjozov Mitko, Arsovski Andrej, Plaseska-Karanfilska Dijana

Methods

- RNA extraction – Qiagen RNeasy kit
- Microarray analysis (Agilent)
- qPCR analysis (Life Technologies)
  - TaqMan microRNA assays:
  - Statistical analysis – DataAssist software v.3.01 –ΔΔCt method, two-tailed Student–t test, p-value<0.05, $\log_2$FC>1.5, BH correction
  - SPSS v.19 for construction of Receiver operating characteristic (ROC) curves to calculate specificity and sensitivity of 7 miRNAs (miR-21, miR-205, miR-210, miR-142-3p, miR-139-5p, miR-146a, and miR-320c)

Breast cancer and microRNA

- Breast cancer is heterogenous disease with differences in clinical, molecular and biological characteristics and is one of the most prevalent cancers which incidence continue to grow
- Histological type, grade, tumor size, lymph node involvement, and estrogen (Er), progesterone (Pr) and HER2-receptor statuses, all influence prognosis and the probability of response to systemic therapies
- MicroRNAs (miRNAs) are small RNA molecules (~22 nt) that regulate gene expression post-transcriptionally, playing a key role in diverse biological processes, including development, cell proliferation, differentiation, and apoptosis;
- Altered miRNA expression contribute to human disease, including cancer

Selection of the miRNAs

Table 1. Significantly differentially expressed microRNAs in the microarray analysis

MicroRNA	$\log_2$FC	p value	MicroRNA	$\log_2$FC	p value
hsa-miR-21	**3.735**	**8.00E-04**	hsa-miR-320a	-1.082	0.02081
hsa-miR-146a	**2.505**	**0.00065**	hsa-miR-1268	-1.146	0.02951
hsa-miR-1260	**2.491**	**0.03203**	hsa-miR-320b	-1.198	0.01465
hsa-miR-155	**2.19**	**0.00014**	hsa-miR-557	-1.204	0.00718
hsa-miR-197	1.918	0.02074	hsa-miR-671-5p	-1.273	0.03173
hsa-miR-16	1.88	0.01765	hsa-miR-320c	-1.291	0.01006
hsa-miR-342-3p	1.78	0.00394	hsa-miR-145	-1.365	0.01811
hsa-miR-21*	1.666	0.0055	hsa-miR-1275	-1.513	0.01134
hsa-miR-652	1.383	0.04908	hsa-miR-205	-1.621	0.01023
hsa-miR-150	1.362	0.03643	hsa-miR-654-5p	-1.78	0.01933
hsa-miR-193a-5p	-0.915	0.04049	**hsa-miR-486-5p**	**-2.144**	**0.00846**
hsa-miR-1225-5p	-0.941	0.04377	**hsa-miR-139-5p**	**-3.244**	**4.00E-04**
hsa-miR-320d	-1.079	0.02476			

Based on the microarray analysis:
- miR-21,
- miR-155,
- miR-146a
- miR-139-5p.

Based on literature data:
- miR-125b,
- miR-142-3p,
- miR-145,
- miR-181c
- miR-200a
- miR-205,
- miR-210
- miR-320c

Iorio et al. 2005, Mattie et al. 2006, Yan et al. 2008, Hui et al. 2009, Blenkiron et al. 2007, Lowery et al. 2009

Aims of the study

- Differences in the differential expression of the miRNAs, not just between normal and breast cancer tissue, but also between different breast cancer subtypes, could contribute to the tumor diagnosis and systemic therapy
- The differential expression of 12 miRNAs (miR-21, miR-125b, miR-139-5p, miR-142-3p, miR-145, miR-146a, miR-155, miR-181c, miR-200a, miR-205, miR-210 and miR-320c) in breast cancer patients were analyzed with an aim to determined their potential use as biomarkers for different clinicopathologic features.

Results – comparison of malignant and normal samples

Table 2. Differential expression of the microRNAs obtained from the comparison of malignant and normal tissue

MicroRNA	Linear fold change	p-value	Logarithmic fold change ($\log_2$FC)
miR-125b	0.7498	0.1693	-0.41542
miR-139-5p	**0.2871**	**1.00E-04**	**-1.80037**
miR-142-3p	**5.258**	**0.0021**	**2.394514**
miR-145	0.3029	0.0577	-1.72309
miR-146a	3.2324	0.0577	1.692606
miR-155	**6.2934**	**0.0112**	**2.65384**
miR-181c	2.5981	0	1.377451
miR-200a	**9.0739**	**0.0021**	**3.181723**
miR-205	**0.3171**	**1.00E-04**	**-1.65699**
miR-21	**19.6841**	**0**	**4.298959**
miR-210	1.7942	0.0577	0.843341
miR-320c	0.5106	1.00E-04	-0.96973

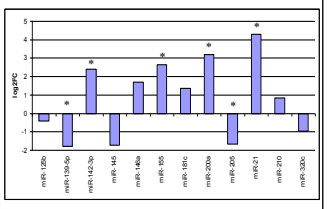
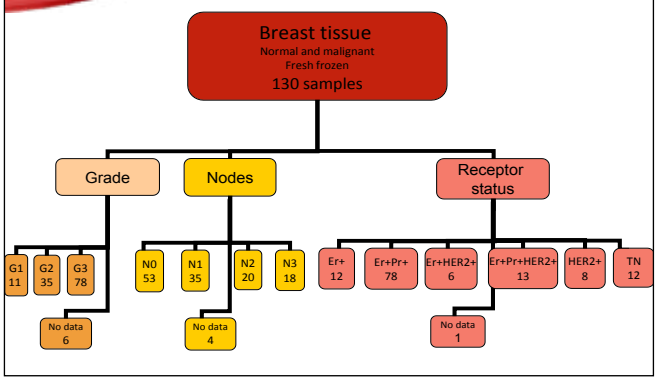


Figure 1. Differential expression of the microRNAs presented as logarithmic fold change with base 2 ($\log_2$FC) of the analysed microRNAs in malignant compared to normal breast tissue

Materials



Results - differential expression in different grade

Figure 2. Differential expression of the microRNAs presented as $\log_2$FC in samples with different tumor grades compared to normal breast tissue

Results - differential expression in different grade

Table 4. Calculated AUC for 7 selected microRNAs as markers for discrimination of the grade 3 compared to other grades

MicroRNA	Area	Standard error[a]	Asymptotic significance [b]	Asimptotic 95% Confidence interval (CI 95%)	
				Lower Bound	Upper Bound
miR-21	,578	,055	,153	,471	,685
miR-205	,455	,056	,405	,345	,564
miR-210	**,648**	,053	**,007**	,545	,751
miR-142-3p	,583	,053	,130	,478	,687
miR-139-5p	,437	,055	,248	,330	,544
miR-146a	,580	,052	,143	,478	,683
miR-320c	,494	,054	,912	,389	,599

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

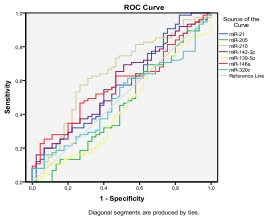


Figure 3. Cumulative ROC curve for 7 selected microRNAs as markers for discrimination of the grade 3 compared to other grades

Conclusions

- MiR-21, miR-155, miR-200a, miR-205, miR-142-3p and miR-139-5p could be used as a panel for distinguishing tumor from normal samples
- Progressive upregulation of the expression with the progress of the tumor grade was obtained for miR-142-3p, miR-200a and miR-21;
- Progress in differential expression with the progress of the nodal statuses was observed for miR-142-3p↑, miR-146a↑, miR-200a↑, miR-21↑ and miR-320c↓. Only miR-320c showed significance in N3;

Results - differential expression in different nodal status

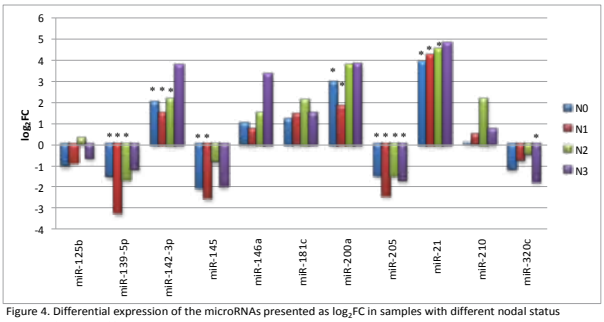


Figure 4. Differential expression of the microRNAs presented as log_2FC in samples with different nodal status compared to normal breast tissue

Conclusions

- Differential expression of the miRNAs in groups with different receptor status reveal that
  - miR-125b was significantly downregulated in HER2+ and Er+HER2+ groups
  - miR-21 was the most significant upregulated miRNA in TN group
  - miR-210 had highest expression in TN group
  - miR-320c was down regulated in all receptor groups except in TN
- Construction of ROC curves showed that
  - miR-210 could be considered as a marker to separate G3 form G1 and G2
  - three miRNAs i.e. miR-210, miR-320c and miR-21 could be considered as biomarkers that separate TN from other receptor statuses
- The results from this study could contribute to the global knowledge of microRNA expression in breast cancer and could potentially improve the management of patients with breast cancer.

Results - differential expression in different receptor status

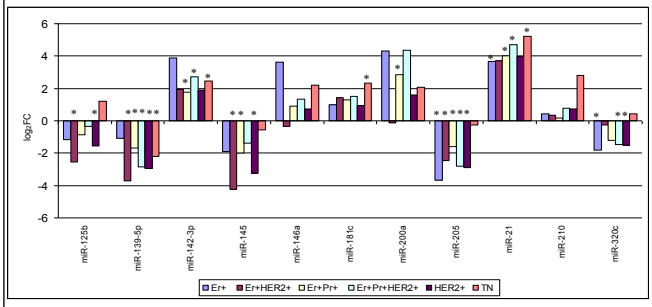


Figure 5. Differential expression of the microRNAs presented as log_2FC in samples with different receptor status compared to normal breast tissue

THANK YOU FOR YOUR ATTENTION

Results - differential expression in different receptor status

Table 7. Calculated AUC for 7 selected microRNAs as markers for discrimination of the TN compared to other receptor statuses

MicroRNA	Area	Standard error[a]	Asymptotic significance [b]	Asimptotic 95% Confidence interval (CI 95%)	
				Lower Bound	Upper Bound
miR-21	**,703**	,061	**,026**	,584	,823
miR-205	,566	,100	,468	,371	,762
miR-210	**,785**	,066	**,002**	,655	,915
miR-142-3p	,652	,084	,096	,488	,817
miR-139-5p	,473	,102	,772	,273	,674
miR-146a	,679	,085	,051	,513	,845
miR-320c	**,737**	,090	**,010**	,562	,913

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

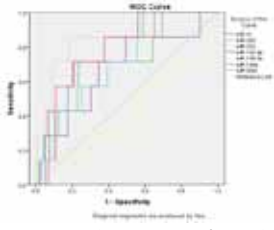


Figure 6. Cumulative ROC curve for 7 selected microRNAs as markers for discrimination of the TN compared to other receptor statuses

SESSION 5-II

Moderators: Emilia Severin, Giovanni Neri

- **TTR FAP in Balkan countries**
  I. Tournev
- **TTR FAP in Bulgaria**
  S. Sarafov
- **Cardiac involvement in patients with hereditary transthyretin amyloidosis associated with Glu89Gln mutation and its impact on prognosis**
  M. Gospodinova
- **Genetic profile of TTR-FAP in Bulgaria: parent-of-origin difference in penetrance**
  A. Todorova
- **Genetic screening for transthyretin amyloidosis in Bulgaria. Genetic profile of TTR-FAP: Glu89Gln founder effect**
  A. Kirov

TTR FAP IN BALKAN COUNTRIES – GENETIC AND CLINICAL VARIABILITY

Ivailo Tournev

TURKEY

28 TTR FAP patients were diagnosed in Turkey – 19 in Istanbul and 9 – in Ankara.

- Val30Met – 11 patients
- Glu89Gln – 5 patients
- Gly47Glu – 4 patients
- Gly53Glu – 3 patients
- Thr49Ser – 2 patients
- Glu54Lys – 2 patients
- Glu54Gly – 1 patient

Introduction

- Transthyretin familial amyloid polyneuropathy (TTR-FAP) is autosomal dominant, life-threatening, and progressive hereditary disease caused by TTR mutations that lead to amyloid accumulation in nervous tissue and various organs.
- Amyloid accumulation causes peripheral sensorimotor neuropathy, autonomic dysfunction, restrictive cardiomyopathy, and death within an average of 10 years from symptom appearance.
- TTR amyloidosis was first identified in northern Portugal, where it was found to be associated with a Val30Met mutation of the TTR gene. This variant has now been found worldwide, is the most widely studied TTR variant, and has served as a prototype for variant-sequence ATTR

Turkey - Val30Met mutation

- Eleven patients from six Turkish families have Val30Met mutation, 6 of them are diagnosed in Istanbul and 2 – in Ankara. Eight of the patients are heterozygous for the mutation and one patient is homozygous.
- Val30Met constitutes 52.9 % of whole TTR FAP cohort in Turkey. The patients diagnosed by Prof. Parman are all from inner Anatolia and the patients diagnosed by Prof. Erdem-Ozdamar are from Ankara and Maras (a city in Southeastern Turkey)

Introduction

- Portugal, Brazil, Japan, and Sweden are considered endemic regions. The usual age of disease onset among TTR V30M gene carriers in Portugal, Brazil, and Japan is in the third to fourth decade of life. However, there are late-onset cases (as seen in Sweden) in which disease onset is in the fifth to sixth decade of life.
- The phenotype of TTR amyloidosis is typically either neurologic (characterized by peripheral sensorimotor neuropathy), cardiac (characterized by cardiomyopathy), or a mixed neurologic and cardiac phenotype.
- There exist significant genetic and clinical heterogeneity. More than 120 different mutations were identified. Most variants that cause familial ATTR are rare, but a few are common in certain populations.

Turkey - Val30Met mutation

- The clinical phenotype is of a late-onset disease in non-endemic region. The mean age of the disease is 57.9 yrs. (44 yrs. – 82 yrs.). All the patients have axonal sensory-motor and autonomic polyneuropathy associated with left ventricular hypertrophy and left atrial enlargement. Also, the most of them have alternating constipation-diarrhea, impotence, orthostatic hypotension and urinary incontinence as a late clinical feature.
- The disease severities and clinical presentations differed within the individual family.

TTR FAP in Balkan countries: 145 patients with 10 different mutations

- **Turkey** – Val30 Met, Glu89Gln, Glu54Gly, Gly53Glu, Gly47Glu (Greek family), Glu54Lys, Thr49Ser
- **Romania** – Glu54Gln
- **Bulgaria** - Glu89Gln, Val30Met, Ser77Phe, Gly47Glu, Ser52Pro
- **Macedonia** - Glu89Gln
- **Kosovo** - Glu89Gln
- **Croatia –** 13 patients are selected for genetic testing
- **Serbia** – 3 patients from the territory of Voivodina, Northern Serbia were selected for genetic testing

Turkey -Thr49Ser

- The family from Ankara with two affected patients is described (Can Ebru Bekircan-Kurt et all., 2015)
- The index case of 36 year-old man has suffered from abdominal pain and constipation.
- An endoscopic duodenal biopsy was compatible with amyloidosis.
- The neurological examination was normal but the electrophysiological examination revealed bilateral carpal tunnel syndrome.
- The echocardiography showed diastolic heart failure with left ventricular hypertrophy.
- The patient has followed up as a heart transplantation candidate, but he died at age of 38 yrs.

Turkey -Thr49Ser

- The daughter of the index case has complaints of constipation and orthostatic hypotension at age of 18 yrs.
- Her neurological examination showed vibration sensation lost below her wrists and ankles and she demonstrated orthostatic hypotension.
- The patient's ENMG results were normal but her HRV indicated sympathetic system dysfunction.
- The patient's cardiological and ophthalmological examination were normal but her ejection fraction was 70 % with mild tricuspid insufficiency. Holter monitoring revealed paroxysmal ventricular extrasystoles, her heart rate varied 56-138 beats per min. Her BNP level was normal.
- The findings suggest that the Thr49Ser mutation causes a predominantly cardiac phenotype.

Turkey -Glu54Lys

- The family from Mersin (a city in Mediterranean shore) with two patients was described (Can Ebru Bekircan-Kurt et all., 2015)
- Early onset – 28-30 yrs.
- First manifestation – numbness in distal legs, leg weakness, incontinence, orthostatic hypotension
- Neurological manifestation – sensorimotor and autonomic polyneuropathies
- Systemic involvement – heart failure
- ECG – inferolateral T-wave negativity
- Echocardiography – left ventricular hypertrophy, atrial and ventricular wall thickness with septal granulation, left ventricular diastolic dysfunction
- Ophthalmological – vitreous opacity

Turkey - Glu89Gln

- Two families with five affected patients were diagnosed (Durmus-Tekce H. et all, 2016).
- The families with this mutation originated from Balkan countries.
- The average onset of the disease is 51.8 yrs. (37 yrs. – 62 yrs.)
- Two of the patients have died: one – at 60 yrs. by cardiac failure and one – at 57 yrs. by cardiac and renal failure.
- The presenting symptoms are paresthesia in the feet and constipation.
- The clinical phenotype include hypoesthesia, deep tendon reflexes abolished in the lower limbs, decreased vibration sensation in lower limbs, distal and prominent weakness of all limbs, early impotence, severe orthostatic hypotension, alternating constipation-diarrhea, weight loss, restrictive cardiomyopathy, 2 years later urinary incontinence. One of the patients has severe neuropathic pain. Hypophonia due to vocal cord involvement and severe obstructive sleep apnea were described in one patient.

Turkey - Gly47Glu

- TTR FAP family with four affected patients was described (Durmus-Tekce H. et all, 2016).
- The family originated from Xanthi district of Northern Greece.
- The average onset of the disease is 28.75 yrs. (25 yrs. – 39 yrs.).
- Genetic anticipation in the age at onset of the disease was observed.
- Two of the patients have died at 33yrs. and 46 yrs. by cardiac failure.

Turkey - Gly47Glu

- The presenting symptoms were fatigue, paresthesia, nausea and vomiting.
- The clinical phenotype include early and severe gastrointestinal manifestations (severe vomiting, alternating constipation-diarrhea), weight loss, distal and prominent weakness of all limbs, deep tendon reflexes abolished in the lower limbs, decreased vibration sensation in lower limbs, early impotence, severe orthostatic hypotension, infiltrative cardiomyopathy, 2 years later urinary incontinence. One of the patients reported severe neuropathic pain.

Turkey - Gly53Glu

- TTR FAP family with three affected patients was described (Durmus-Tekce H. et all, 2016).
- The family originated from Western Turkey.
- The average onset of the disease is 28.3 yrs. (21yrs – 33yrs.)
- The presenting symptoms are paresthesia in the feet, dizziness, diarrhea, weight loss.

Turkey - Gly53Glu

- The clinical phenotype include distal and prominent weakness of all limbs, deep tendon reflexes abolished in the lower limbs, decreased vibration sensation in lower limbs, early impotence, severe orthostatic hypotension, cardiac failure, alternating constipation-diarrhea, 2 years later urinary incontinence.
- Central nervous system involvement was observed in two of the patients. Both patients presented with transient ischemic attack-like episodes of dysarthria and hemihypoesthesia.
- One of the patients has died at 36 yrs.

Turkey - Glu54Gly

- The patient is from Southeastern part of Turkey.
- The onset of the disease is early, at 21 yrs.
- The main complaints – paresthesia in the hands, cramps in the lower extremities, constipation
- The clinical phenotype includes early and severe gastrointestinal manifestations – nausea and vomiting, constipation-diarrhea syndrome, orthostatic hypotension, predominant autonomic polyneuropathy. There was not observed cardiac involvement.

ROMANIA

- Four TTR FAP cases were diagnosed in four different towns: Satu Mare, Galatie, Radauti and Barlad. Three patients are from the Northern part of the country and one patient is from center-eastern part of the country
  - All of them have the same mutation **Glu54Gln**
  - The mean age of onset of the disease is 43 yrs.
  - The clinical phenotype includes paresthesias of the lower limbs, a progressive peripheral sensory and motor polyneuropathy, autonomic dysfunction, and a restrictive cardiomyopathy, orthostatic hypotension, dysphagia, and chronic diarrhea. There was rapidly progressive cardiac dysfunction, heart failure and pulmonary hypertension. One of the patients has bilateral carpal tunnel syndrome.

BULGARIA

110 TTR FAP patients and 94 asymptomatic carriers from 80 affected families were identified.

- **Glu89Gln** - 63 families
- **Val30Met** – 8 families
- **Ser77Phe** – 6 families
- **Gly47Glu** – 2 families
- **Ser52Pro** – 1 family

Bulgaria - Glu89Gln

- **Glu89Gln** is the most frequent TTR mutation in Bulgaria- 86%
- There is endemic region in Bulgaria – Two districts, Blagoevgrad and Kjustendil, in South-Western part of the country
- The average onset of the disease is 51,7 yrs. with small male/female differences.
- The clinical phenotype includes: Carpal tunnel syndrome; Sensorimotor and autonomous neuropathy; Painful dysesthesias more pronounced in the feet; Loss of vibration and temperature perception; Sensory ataxia, Autonomous dysfunction; Distal weakness; Restrictive cardiomyopathy; Constipation/Diarrhea, Weight loss, Cachexia.

Bulgaria - Glu89Gln

- Polyneuropathy is the predominant initial symptom in a large proportion of the affected. In more rare cases peripheral nerve, cardiac and gastrointestinal symptoms can be observed concurrently in the beginning of the disease broadening the differential diagnosis. In only 5 patients cardiomyopathy was reported to precede the other symptoms for years. Gastrointestinal involvement was found as the initial sign in only 6 of the affected patients.
- The patients with Glu89Gln developped earlier and more severe restrictive cardiomyopathy than the patients with Val30Met.
- Based on the variable presentation, genetic screening may be helpful for early detection of at-risk family members.

Bulgaria - Val30Met

- 8 families with 9 affected patients and 8 carriers.
- The most of the patients originated from Smolyan region.
- The clinical phenotype is of a late-onset disease in non-endemic region. The mean age of the disease is 63.8 yrs. (55.4 yrs. – 76 yrs.). All the patients have axonal sensory-motor and autonomic polyneuropathy associated with left ventricular hypertrophy and left atrial enlargement. Also, the most of them have alternating constipation-diarrhea, impotence, orthostatic hypotension and urinary incontinence as a late clinical feature.

Bulgaria - Ser77Phe

- 6 families with 8 affected patients and 6 carriers
- All the families with this mutation originated from a village of Vakarel, located 25 km from the capital.
- The clinical phenotype is of a late-onset TTR FAP. The mean age of the disease is 57.2 yrs. (52.3 yrs. – 61.8 yrs.). All the patients have axonal sensory-motor and autonomic polyneuropathy associated with restrictive cardiomyopathy. Also, the most of them have alternating constipation-diarrhea, impotence, orthostatic hypotension and urinary incontinence as a late clinical feature.

Bulgaria - Gly47Glu

- Two large Gypsy families in 3 generations was identified in Russe district in North-Eastern part of the country. We examined 3 affected patients and collected the data for other 10 patients, who have died.
- The average onset of the disease is 33.6 yrs. (30 yrs. – 38 yrs.) in the last generation. In the male patients the age of onset is earlier (30-32 yrs.) than the only female patient (37-38 yrs.). One of the patients from the last generation has died at 33 yrs. In the first and the second generations the disease has started later (average age of onset – 40 yrs.) and three patients have died at 46 yrs. Genetic anticipation in the age at onset of the disease was observed.

Bulgaria - Gly47Glu

- The presenting symptoms were pain in the feet, nausea, vomiting, loss of weight till 50 kg, diarrhea. The clinical course of the disease is rapidly progressive.
- The clinical phenotype include early and severe gastrointestinal manifestations (severe vomiting, alternating constipation-diarrhea), weight loss, distal and prominent weakness and hypotrophy of all limbs, deep tendon reflexes abolished in the lower limbs, decreased vibration sensation in lower limbs, early impotence, severe orthostatic hypotension, restrictive cardiomyopathy, heart failure, microalbuminuria.

Bulgaria - Ser52Pro

- One affected patient from Veliko Tarnovo region was examined. The patient has a mother with the same disease, died at 63 yrs.
- The age of onset is 44.2 yrs. She died at 53 yrs.
- The clinical phenotype include axonal sensory-motor and autonomic polyneuropathy, diarrhea, weight loss, severe orthostatic hypotension, restrictive cardiomyopathy, heart failure, albuminuria, renal insufficiency, secondary anemia.

CONCLUSIONS

- **TTR FAP with early onset (18 yrs -39 yrs.)** was caused by five different mutations: Gly47Glu (25 yrs. – 39 yrs.), Gly53Glu (21yrs. – 33 yrs.), Glu54Gly (21 yrs.), Glu54Lys (28 yrs. - 30 yrs.), Thr49Ser (18 yrs. – 36 yrs.)
- **TTR FAP with intermediate onset (40 yrs - 54 yrs.)** was caused by several mutations: Glu54Gln (average age – 43 yrs.), Ser52Pro (age onset– 44 yrs.), Glu89Gln (average age – 51 yrs.
- **TTR FAP with late onset (above 55 yrs.)** was caused by the following mutations: Ser77Phe (average age – 57.2 yrs) and Val30Met (average age – 57.9 yrs. in Turkey and 63.8 yrs. in Bulgaria)

CONCLUSIONS

- **Glu89Gln-** specific regional Balkan-Mediterranean mutation, identified in Turkey, Bulgaria, Macedonia, Kosovo, Italy (Sicily). South-Western part of Bulgaria is an endemic region for this mutation.
- **Gly47Glu** – second regional Balkan-Mediterranean mutation, identified in Italy, Greece, Bulgaria. In Bulgaria the mutation was identified in Roma (Gypsies). Are other patients with the same mutation are Roma (Gypsy)?
- **Thr49Ser** mutation causes a predominantly cardiac phenotype of the disease.
- **Glu54Lys and Gly47Glu** mutations usually started with early and severe gastrointestinal manifestations (nausea, severe vomiting and diarrhea)

Acknowledgments

I acknowledge all colleagues for sending the clinical, genetic and epidemiological data:

- Prof. Yesim Parman, Turkey
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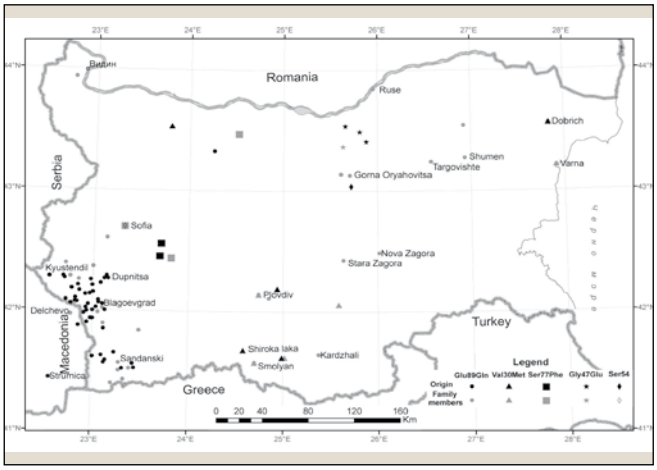
- Prof. Albena Todorova, Bulgaria
- Dr. Stayko Sarafov, Bulgaria
- Dr. Daniel Coriu, Romania
- Prof. Zorica Stevic, Serbia
- Prof. Sanja Milenkovic, Serbia
- Dr. Ervina Bilic, Croatia

Thank you for your attention!

TRANSTHYRETIN FAMILIAL AMYLOID POLYNEUROPATHY IN BULGARIA (TTR-FAP)

Stayko Sarafov



FAMILIAL AMYLOID POLYNEUROPATHY

Rare AD disease (for USA fr,1:1500 and >, for EU fr.1:2000 and > (www. Rare disorders page) found all over the world due to a mutation in the TTR (**Transthyretin = transports thyroxine and retinol**) gene. Firstly described by the Portuguese neurologist Mário Corino da Costa Andrade in 1952 and known as a Portuguese disease till 1985, when the gene was sequenced. More than 100 point mutations till now with variable penetrance. Most of them provoke TTR-FAP, some of them stabilize the molecule. Val30Met is the most often all over the world mainly in Portugal, Sweden, Brasil, Japan, USA and accepted as an endemic mutation.

Clinical onset – for all mutation found

1. Polyneuropathy syndrome in 2/3 of cases. In 50% the PNP starts as a single typical carpal-tunnel syndrome, progresses to a typical polyneuropathy syndrome .
2. Different Heart disturbances in 1/3 of cases expressed with rhythm and conductive disturbances. US restrictive type cardimyopathy.
3. Different gastro-intestinal symptoms from upper GIT and/or lower GIT in 7-10% **.** GI symptoms are similar to the symptoms in many other common GI diseases affecing the upper and lower GI tract.
4. Other (arterial hypotension, body weight loss, eyes & kidneys involvement, erectile dysfunction) and mixed up to 10%**.** They could be found in many other conditions.

CLINICAL FEATURES of BG TTR – FAP PATIENTS – all mutations

TTR - FAP is multi systemic disease affecting all organs and systems as a whole with clinically / population - genetic correlations.
Predilection organs affected by the type of mutation - peripheral nerves, heart, kidneys, eyes, liver, bones, gastrointestinal tract, genito-urethral tract, skin, etc.
The most affected systems are essential for the diagnosis.
Specific clinical phenotype in individual mutations.
The involvement of individual organs and systems in specific mutations is different and it is important for the clinical diagnosis.
Differences in age of onset, sex, survival.
Intra familial heterogeneity, also in homozygous twins !

Phenotypic–genetic correlations according to

Mutation type, mean age of onset (years), (+) familial history / penetrance & mean survival (years)

Mutation / patients (n)	Mean age of onset (y)	Onset range	Increasing the mean age of onset ↓ / Increasing the (+) familial history ↑	(+) familial history	Mean survival (y) →	Survival range (y)
Gly47Glu (3)	**35.3**	31.3–38.1		**100**	**~ 5**	5 ?
Ser52Pro (1)	**44.2**	-		**100**	**9,4**	9.4
Glu89Gln **(106)**	**52.7**	35.3–70.3		**~ 85**	**8,0**	2.8–20.8
Ser77Phe (7)	**57.6**	50.6–62.3		**~ 75**	**7,0**	4.5–8.8
Val30Met (10)	**66.0**	54.7–74.2		**~ 30**	**7,9**	5.7–9.2

CURRENT DATA

Since 2008, there are five mutations found till now:
- Glu89Gln, (64 families)
- Ser77Phe, (6 families)
- Val30Met, (9 families)
- Ser52Pro, (1 family)
- Gly47Glu (2 families - gypsies)

Selective genetic screening program is performing now for the affected families with more than 100 asymptomatic carries.
One patient carried Glu89Gln/Val30Met
Homozygous twins – one sick, the second asymptomatic carrier till now.

REMARKS

Glu89Gln is the most often mutation in BG population. The clinical phenotype is strict with defined clinical-genetic correlations:
- mean onset 52,73 with small male/female differences.
- cases with onset before 45 and later than 60 are comparatively rare; more than 3/4 of the pts have an onset between the ages 45-60, the main part after 50, so Glu89 is mainly late onset ≥ 50, than early onset ≤ 50. In the last 1-2 years there is a tendency for raising the number of patients with onset after 50.
- Clinically the disease is more severe for males, the result of the Tafamidis therapy is not so expressed than in females (preliminary data)

REMARKS

- The presented mutations have different mean of onset, but similar survival, except Gly47Gln.
- Ser77Phe and Glu89 has similar clinical phenotype, except the onset for Ser77 which is a few years latter.
- Gly47Gln is specific for the gypsy population, not found in other ethnic groups till now. The BG gypsies are not explored for TTR-FAP in the past, so we have to expect more patients / families inside this population during the next years.
- Val30Met presents as a "sporadic" due to highest age of onset, "lack" of (+) familial history & "soft" clinical phenotype - highest mean age of onset, dominated PNP with slightly affected heart & other. Usually in those cases the symptoms (especially the heart's) are accepted by GPs and other specialists as an age related.
- Interfamilial heterogeneity is presented for all mutations.
- The DNA screening reveals new pts. earlier, which statistically decrease the age of onset.

Clinical onset – for all mutation found

1. Polyneuropathy syndrome in 2/3 of cases.
In 50% the PNP starts as a single typical carpal-tunnel syndrome, for a long time (0,5- > 15 years / average 6 years), after than progresses to a typical polyneuropathy syndrome (own observations). The patients with CTS onset have longer survival (preliminary data).
2. Different Heart disturbances in 1/3 of cases expressed with rhythm and contuctive disturbanses. US restrictive type cardimyopathy (increased thickness walls & septum, decreased cavities). The most difficult for early diagnosis if they are the only clinical manifestation. The accumulation of amyloid in the heart preceeds with several years typical cardiac echo ultrasound changes !!! (own single cases)

REMARKS

1. We couldn't say that all carriers with Glu89Gln have been found, so the possibility to have patient/s in double homozygous state for Glu89Gln could not excluded.
2. Having a patient with Glu89Gln / Val30Met in heterozygous state, other patients in heterozygous state need to be expected.
3. Circulating the SOD1 mutation for genetic ALS form and TTR Glu89Gln in the region of Blagoevgrad give the risk of combination of those both mutations in a same patients.
4. Often but not always, crossing the sex between a sick parent and a carrier child when the parent has two children with different sex: sick father – daughter carrier, sick mother – son carrier (Glu89).
5. In a case of two kids with the same sex, no rule who will accept the mutation – the older or the younger.

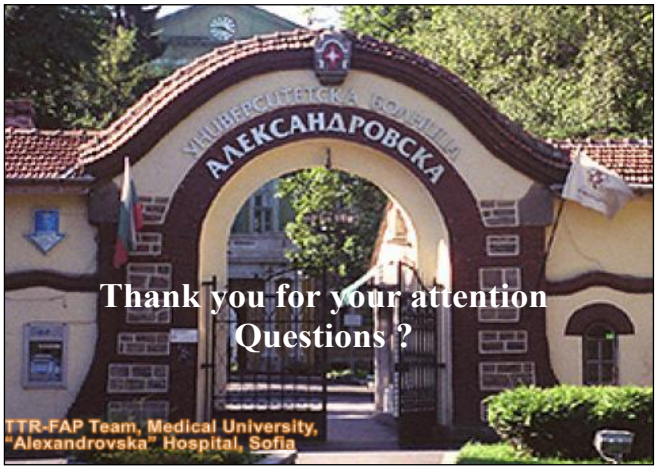
Clinical onset – for all mutation found

3. Different gastro-intestinal symptoms from upper GIT (early satiate, epigastritic pain, belching, vomiting, heartburns) or lower GIT (constipation / diarrhea (diarrhea after feeding) or both alternating) in 7-10% : the early diagnosis is difficult in case of single GI clinical manifestation. GI symptoms are similar to the symptoms in many other common GI diseases affecing the upper and lower GI tract.
4. Other (arterial hypotension, body weight loss, eyes & kidneys involvement, erectile dysfunction) and mixed up to 10%. They could be found in many other conditions, which decreas their diagnostic value.

CONCLUSION

Our results present that TTR-FAP is not so rare and we expect new cases in the next years. Founder effect is possible for all mutations except for the Val30Met. Our work could be a good base for developing better TTR-FAP epidemiology and diagnosis in neighbor countries.

One patient (Blgrd) with Glu89Gln / Val30Met in heterozygous
state. One family of homozygous twins Glu89Gln – one of them is
sick, the other is asymptomatic carrier up to date. The father
conducted the mutation. The disease started with CTS and
amiloydotic cardiomyopathy, but isn't RNA expression and other
clinical events up to date !



CARDIAC INVOLVMENT IN HEREDITARY TRANSTHYRETIN-RELATED AMYLOIDOSIS, ASSOCIATED WITH GLU89GLN MUTATION AND ITS IMPACT ON PROGNOSIS

Mariana Gospodinova

Cardiac amyloidosis

- Restrictive (infiltrative) cardiomyopathy
  - Predominant diastolic dysfunction
  - Preserved EF with early impairment of LV longitudinal systolic function
  - Heart failure symptoms
  - Rhythm and conduction disturbances

Overview

- Hereditary ATTR is a progressive multi-system disorder with predominant neurological and cardiac involvement.
- It causes a significant impairment in quality of life and death in several years from the onset of symptoms.
- Gastro-intestinal tract manifestations are common.

Diagnostic tools

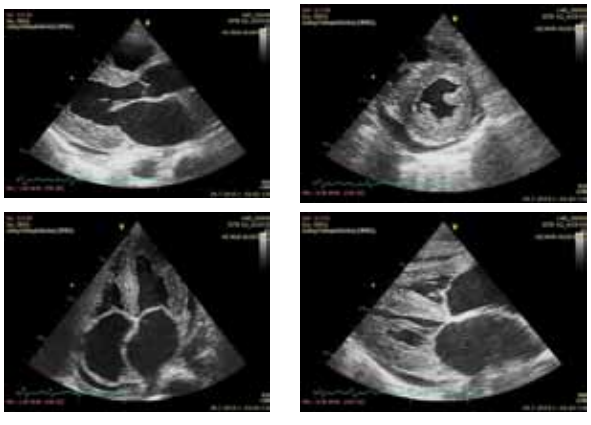
- ECG
- Echocardiography
- CMR - LGE, T1 mapping
- 99mTC-DPD scintigraphy
- Biomarkers – Troponin, T- pro BNP
- Biopsy – Congo red, Imunohistochemistry
- Molecular-genetic analysis – definite diagnosis

TTR cardiac amyloidosis

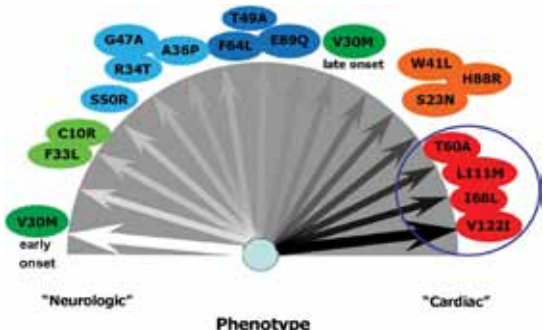
- Extracellular deposition of TTR amyloid fibrils in the:
  - myocardium
  - conduction system
  - heart valves
  - atria
  - vessels.

Cardiac involvement depends on the TTR mutation and geographic area

- THAOS (June 2017) – cardiac phenotype (34%) from non-Val30Met patients.
- Mutations with predominant or exclusively heart involvement – Thr60Ala, Leu111Met, Ile68Leu, Val122Ile
- Val122Ile affects the greatest world population- 3–4% of the US African American population.



Spectrum of genotype–phenotype correlations in transthyretin-related amyloidosis.



Claudio Rapezzi et al. Eur Heart J 2013;34:520-528

Background

- Glu89Gln is the most common mutation in Bulgarian patients with ATTR.
- Mixed phenotype – cardiac and neurological.

Purpose

- To evaluate cardiac involvement, morbidity and mortality in patients with ATTR associated with Glu89Gln mutation.

Patients and methods

- 63 patients with Glu89Gln
- 29 males/34 females
- Mean age at diagnosis - 58±7 years
- A clinical examination, 12-lead ECG, and Echocardiography were performed.
- Follow up for 31 months (from 1 to 72 months)

Multisystem involvement

Gene mutations	Glu89Gln
Cardiac symptoms at onset (n,%)	16 (25)
Cardiac involvement at diagnosis (n,%)	63(100)
Neuro symptoms at onset (n,%)	39 (62)
Neuro symptoms at diagnosis (n,%)	63 (100)
Autonomic neuropathy at diagnosis (n,%)	43 (68)
Carpal tunnel (n,%)	25 (40)
Gastrointestinal symptoms at onset (n,%)	6 (10)
Gastrointestinal symptoms at diagnosis (n,%)	43 (68)
Unintentional weight loss	41 (65)

Electrocardiogram

Gene mutations	Glu89Gln
Pathological ECG, n (%)	51 (81)
Infarct pattern, n (%)	34 (53)
LAFB, n (%)	24 (38)
Low QRS voltage, n (%)	22 (35)
A-V block I degree, n (%)	15 (24)
Pace-maker, n (%)	4 (6)
Atrial fibrillation, n (%)	6 (10)
LBBB, n (%)	6 (10)
RBBB, n (%)	6 (10)

Echocardiography

Gene mutations	Glu89Gln
IVS thickness, mm	18±3
PW thickness, mm	17±2
LVEF %	58±11
LA diameter, mm	41±6
RV free wall, mm	8±2
Restrictive filling pattern, n (%)	25 (40)
s septal, cm/s	5±2
s lat., cm/s	6±2
Pericardial effusion, n (%)	17 (27)

Follow-up
31 months (from 1 to 72 months)

Gene mutations	Glu89Gln
Death, n (%)	15 (24)
Advanced heart failure, n	11
Ischemic stroke, n	2
Sudden death, n	2
HF NYHA class III-IV, n (%)	19 (30)
Ischemic stroke (total), n (%)	3 (5)
Progressive walking disability	32 (51)
Diarrhea/constipation	35 (56)

Conclusion

- A significant cardiac involvement was found in all the evaluated patients with ATTR, associated with Glu89Gln mutation.
- Progressive LV dysfunction with heart failure symptoms and rhythm and conduction disturbances were the main cause of death.
- The quality of life was profoundly impaired by both cardiac and extra-cardiac (neurologic and gastrointestinal) symptoms.

GENETIC PROFILE OF TTR-FAP IN BULGARIA: PARENT-OF-ORIGIN DIFFERENCE IN PENETRANCE

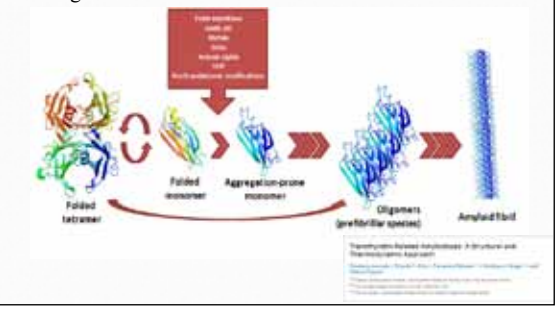
Albena Todorova, Andrey Kirov, Tihomir Todorov, Stayko Sarafov, Teodora Chamova, Mariana Gospodinova, Ivailo Tournev

Disclosures

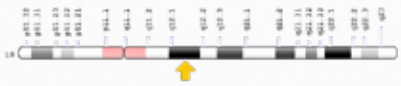
The study is supported by Pfizer in the context of Investigator-Initiated Research Agreement from 15.11.2016

The protein transthyretin

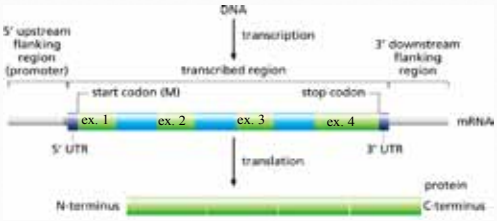
127 aminoacids
Transport protein
Primarily synthesized in liver
The native TTR exists as a tetrameric complex composed of four single chain TTR monomers.



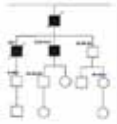
The destabilization is usually caused by mutations in the TTR gene, localized on chromosome 18q12.1.



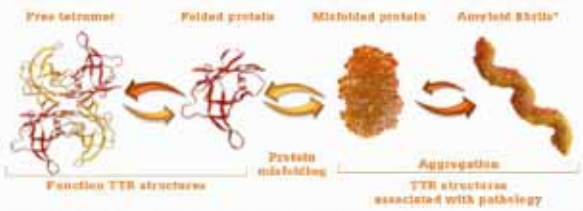
The TTR gene is relatively simple, composed of only 4 exons.



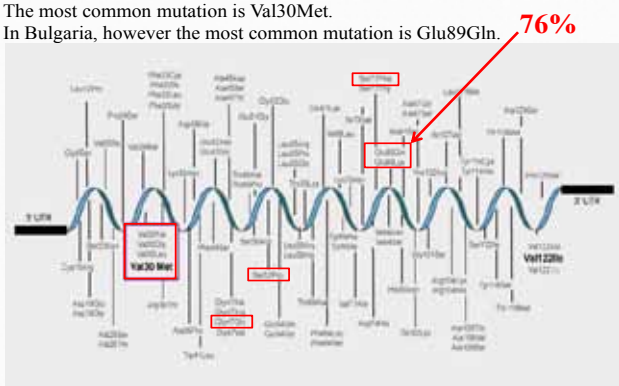
Transthyretin (TTR) amyloidosis is an autosomal dominant systemic disorder.



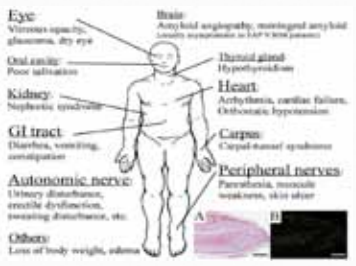
Mutant transthyretin misfolds in amyloid fibrils deposited in the extracellular space.



More than 100 mutations in the TTR gene have been found to cause transthyretin amyloidosis.
Nearly all of them are missense mutations.
The most common mutation is Val30Met.
In Bulgaria, however the most common mutation is Glu89Gln.



TTR amyloidosis is the most common form of hereditary amyloidosis. It can present as familial amyloid polyneuropathy (TTR-FAP), or as familial amyloid cardiomyopathy (TTR-FAC).



Ueda and Ando. Translational Neurodegeneration 2014, 3:19

Characterized by:
Systemic amyloid deposition in different organs, such as heart, peripheral nerves, kidneys, etc.

Peripheral somatic and autonomic neuropathy associated to multiorgan dysfunction

Firstly identified in Portugal, now reported worldwide

Genotype-Phenotype correlations
We are in the genotype-phenotype puzzle

"Wooden neurons" (from https://www.flickr.com/photos/gagilas/6787143138 under a Creative Commons License)

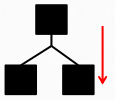
Parent-of-origin difference in the disease penetrance
Anticipation ??????

- So far, we have studied our patients in the context of every single individual, but TTR-FAP is a familial pathology which requires genetic counselling.
- Markedly different penetrance according to the gender of the transmitting parent has been reported [Hellman et al., 2008; Bonaiti et al., 2009].

Parent-of-origin difference in the disease penetrance
Bulgarian experience

- In Bulgarian Glu89Gln mother-son pair we found more than 5 years earlier age at onset in the son.

- Surprisingly, we also found anticipation in father-to-sons transmission: a twin with neurological onset at the age of 36, the second brother not affected at this age



age at onset 46 carpal tunnel syndrome operation and cardiac manifestation at 65;
Diagnosed after genetic testing
10 years difference in the disease onset

AIM
to better understand the difference in the disease penetrance by evaluating the ratio of mutant versus wild type transcripts

- Study design: to sequence the *TTR* RT-PCR product and to compare the mutant versus wild type transcript in families with markedly different age at onset.

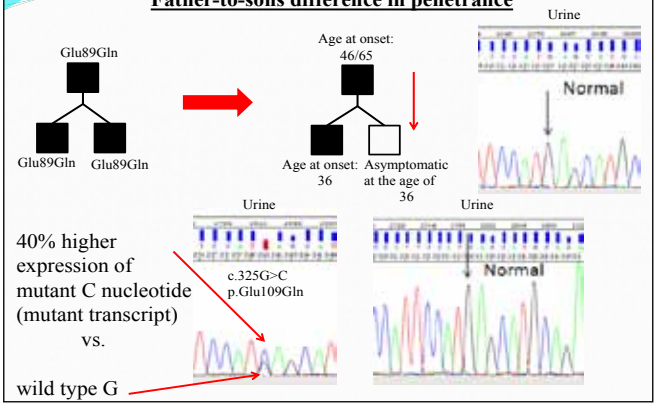
The RNA was extracted from plasma and urine (non-invasive specimens)

!!!

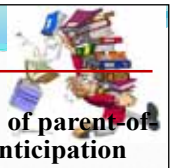
USING AQUEOUS NONTOXIC LIQUID STORAGE REAGENT WHICH:

- **Preserves genetic integrity of Nucleic acids (DNA and RNA) during sample storage/transport at RT**
- **Eliminates the need for refrigeration or specialized equipment**
- **Inactivates DNA and RNA nucleases and infectious agents**

RESULTS
Father-to-sons difference in penetrance



Take home messages



1. **The obtained results from our studies of parent-of-origin difference in penetrance and anticipation showed different expression levels of mutant versus wild type transcripts, which coincides with the disease onset and phenotypic manifestation in a single family.**
2. **Anticipation needs to be considered in genetic counselling and in follow-up of mutation carriers.**
3. **Such an experiment to assess mutant versus wild type *TTR* transcripts has not been performed so far.**

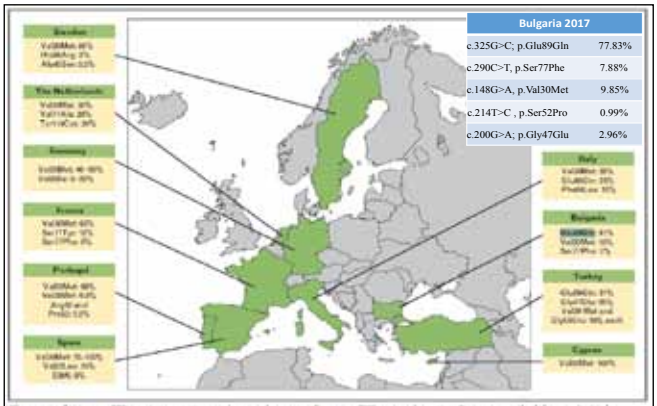
Thank you for your attention

GENICA

Genome Center Bulgaria

GENETIC SCREENING FOR TRANSTHYRETIN (TTR) AMYLOIDOSIS IN BULGARIA. GENETIC PROFILE OF TTR-FAP: GLU89GLN FOUNDER EFFECT

Andrey Kirov, Albena Todorova, Tihomir Todorov, Stayko Sarafov, Teodora Chamova, Mariana Gospodinova, Ivailo Tournev



The most frequent TTR point mutations observed across Europe. TTR, transthyretin. Data compiled from clinical experience of the European Network for TTR-FAP (ATTReuNET) in March 2014.

Transthyretin familial amyloid polyneuropathy in Europe [Parman et al. 2016]

Disclosures

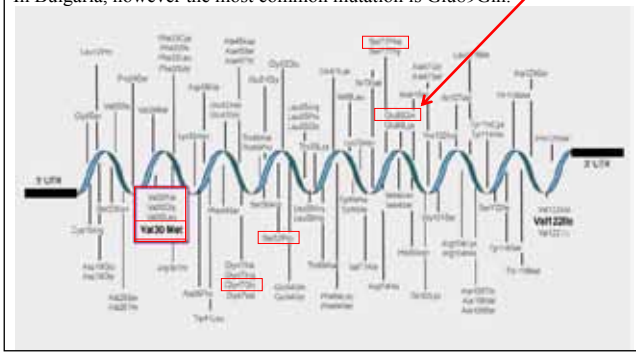
The study is supported by Pfizer in the context of Investigator-Initiated Research Agreement from 15.11.2016

More than 100 mutations in the *TTR* gene have been found to cause transthyretin amyloidosis.

Nearly all of them are missense mutations.

The most common mutation is Val30Met.

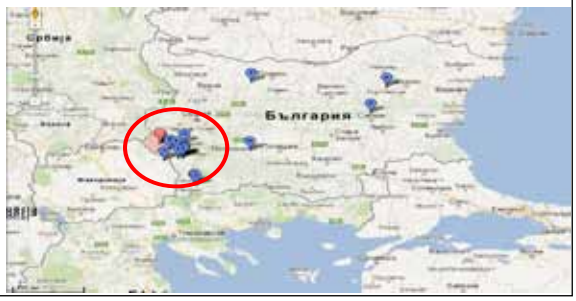
In Bulgaria, however the most common mutation is Glu89Gln. **76%**



Summary

- ✓ **Distribution of the *TTR* mutations across the world and in Bulgaria**
- ✓ **Endemic region in Bulgaria**
- ✓ **Genetic screening for transthyretin (*TTR*) amyloidosis in Bulgaria**
- ✓ **Founder effect of Glu89Gln mutation in Bulgarian population**
- ✓ **Pitfalls of haplotype analysis Preliminary results from our research**

We have clinically well described and genetically screened endemic region in the South-West part of the country.



Transthyretin (TTR) amyloidosis is an autosomal dominant systemic disorder caused by the formation of amyloid fibrils in the extracellular space. This is usually caused by mutations in the *TTR* gene (18q12.1).

TTR amyloidosis is the most common form of hereditary (familial) amyloidosis. It can present as familial amyloidotic polyneuropathy - TTR-FAP, or as familial amyloid cardiomyopathy – TTR-FAC.

What is the distribution of the *TTR* mutations across the world?

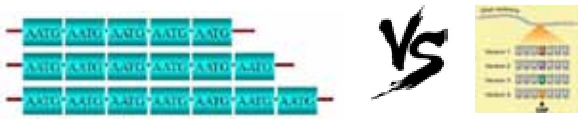
Genetic screening for transthyretin (*TTR*) amyloidosis in Bulgaria

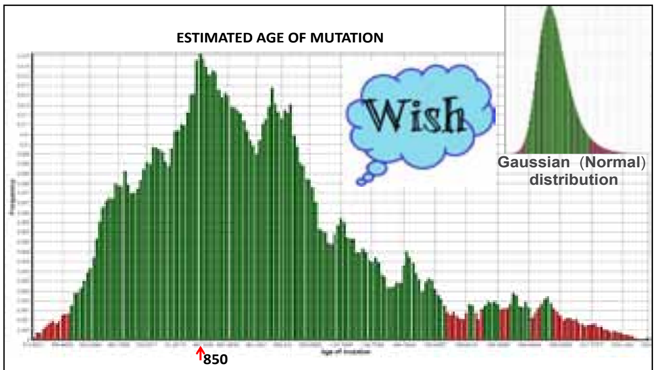
We performed genetic screening including 75 affected families involving 203 patients mainly (78%) with Glu89Gln mutation. We also screened more than 100 random anonymous newborn DBS samples which originated from the endemic region, but no mutation carriers were detected outsides the affected families.

TTR variants	Number of tested people	[%] of all tested people	[%] of patients positive for TTR mutation
c.325G>C; p.Glu89Gln (p.Glu109Gln)	158	33.69%	77.83%
c.290C>T, p.Ser77Phe (p.Ser97Phe)	16	3.41%	7.88%
c.148G>A, p.Val30Met, (p.Val30Met)	20	4.26%	9.85%
Compound heterozygous carrier: c.148G>A, p.Val30Met, (p.Val30Met) + c.325G>C; p.Glu89Gln (p.Glu109Gln)	1	0.21%	0.49%
c.214T>C , p.Ser52Pro (p.Ser72Pro)	2	0.43%	0.99%
c.200G>A; p.Gly47Glu (Gly67Glu)	6	1.28%	2.96%
Normal results	266	54.05%	-
Total	469	100%	-

In population genetics, the founder effect is the loss of genetic variation that occurs when a new population is established by a very small number of individuals from a larger population.

STRs SNPs





Age of p.Glu89Gln (*TTR* gene) mutation estimated by DMLE+ v.2.3 software based on the founder haplotype. Green bars delimit the 95% confidence interval. X-axis: age of mutation in generations (one generation = 25 years). Y-axis: frequency for each estimation out of a total of 1,000,000 iterations. The highest frequency was obtained for about 850 generations. The 95% confidence interval stretches from 580 to 1331 generations.

Glu89Gln *TTR* mutation is linked to the normal alleles of the selected SNPs

PRELIMINARY DATA

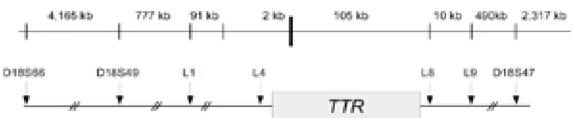
Patient ID	Glu89Gln status	rs3764479 c.-2041A>G	rs3764478 c.-1383G>T	rs723744 c.70-383G>T	rs1800458 c.76G>A	rs1080094 c.200+806A>G	rs3764476 c.336+1242C>A	rs3794884 c.337-1560T>G
114-3	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
114-4	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
114-5	negative	A/G - heterozygous	G/G	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
114-6	negative	A/A	G/G	G/G	G/G	A/A	C/C	T/T
114-7	positive	A/G - heterozygous	G/G	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
92-3	positive	A/G - heterozygous	G/G	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
92-4	positive	A/G - heterozygous	G/G	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
92-5	negative	G/G - homozygous	G/G	T/T - homozygous	G/G	G/G - homozygous	A/A - homozygous	G/G - homozygous
92-6	negative	G/G - homozygous	G/G	T/T - homozygous	G/G	G/G - homozygous	A/A - homozygous	G/G - homozygous
92-7	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
92-8	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
92-9	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
92-10	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
89-3	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
89-4	negative	A/G - heterozygous	G/T - heterozygous	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
89-5	negative	A/A	G/G	G/G	G/G	A/A	C/C	T/T
89-6	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
89-7	negative	A/G - heterozygous	G/T - heterozygous	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
89-8	positive	A/G - heterozygous	G/T - heterozygous	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
41-3	positive	A/G - heterozygous	G/G	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
41-4	positive	A/G - heterozygous	G/G	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
41-5	negative	G/G - homozygous	G/G	T/T - homozygous	G/G	G/G - homozygous	A/A - homozygous	G/G - homozygous
41-6	negative	A/G - heterozygous	G/G	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
41-7	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
41-8	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
41-9	negative	A/G - heterozygous	G/G	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
41-10	negative	A/G - heterozygous	G/T - heterozygous	G/T - heterozygous	G/A - heterozygous	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
41-11	negative	A/G - heterozygous	G/G	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
41-12	negative	A/G - heterozygous	G/T - heterozygous	G/T - heterozygous	G/A - heterozygous	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous
41-13	negative	G/G - homozygous	T/T - homozygous	T/T - homozygous	G/G	G/G - homozygous	A/A - homozygous	G/G - homozygous
41-14	negative	A/A	G/G	G/G	G/G	A/A	C/C	T/T
41-15	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
41-16	positive	A/A	G/G	G/G	G/A - heterozygous	A/A	C/C	T/T
41-17	positive	A/A	G/G	G/G	G/G	A/A	C/C	T/T
41-18	negative	A/G - heterozygous	G/T - heterozygous	G/T - heterozygous	G/G	A/G - heterozygous	C/A - heterozygous	T/G - heterozygous

Estimated mutation age

Ancient mutation with very limited geographic distribution?

Spread across the world???

STRs



Schematic representation of the distribution of the microsatellites in the human *TTR* gene

Haplotypes of the 36 Glu89Gln probands were reconstructed using PHASE.

Take home messages

1. **Glu89Gln is the most frequent *TTR* mutation in Bulgaria- 76%**
2. **In Bulgaria the disease is clustered in endemic regions**
3. **The preliminary results from our studies of the founder effect and estimated mutation age showed: clearly proved founder effect and estimated age - 850 generations, which is approximately 21250 years.**

PRELIMINARY DATA

Patient ID	Glu89Gln status	D18S66	D18S49	L1	L4	TTR gene mutation Glu89Gln	L8	L9	D18S47
41-3	positive	254	115	245	267		292	324	198
41-4	positive	254	115	245	267		292	324	198
41-7	positive	254	115	245	267		292	324	198
41-8	positive	254	115	245	267		292	324	198
41-15	positive	250	115	245	267		292	324	198
41-16	positive	254	115	245	267		292	324	198
41-17	positive	256	115	245	267		292	324	198
89-3	positive	256	115	249	267		292	324	202
89-6	positive	256	115	249	267		292	324	202
89-8	positive	254	113	249	267		292	324	202
78-3	positive	254	115	249	267		292	324	202
78-4	positive	254	115	249	267		292	324	202
78-5	positive	254	115	249	267		292	324	202
78-6	positive	254	115	245	267		292	324	202
11-6	positive	256	115	245	267		292	324	202
11-7	positive	254	115	245	267		292	324	202
11-9	positive	254	115	245	267		292	324	202
11-10	positive	254	115	245	267		292	324	202
11-11	positive	254	115	245	267		292	324	202
11-12	positive	256	115	245	267		292	324	202
11-13	positive	256	115	245	267		292	324	202
11-14	positive	256	115	245	267		292	324	202
88-3	positive	254	115	245	267		292	324	202
88-4	positive	254	115	245	267		292	324	202
88-7	positive	254	115	245	267		292	324	202
68-2	positive	256	115	245	267		292	324	202
68-3	positive	256	115	245	267		292	324	202
68-4	positive	256	115	245	267		292	324	202
59-3	positive	252	115	245	267		300	322	202
59-5	positive	252	115	245	267		300	322	202
59-6	positive	250	115	245	267		292	324	198
59-7	positive	256	115	245	267		292	324	198
59-8	positive	250	115	245	267		292	324	202
133-3	positive	256	115	245	267		292	324	202
133-5	positive	256	115	245	267		292	324	202
133-7	positive	254	113	249	265		292	320	202
Freq									
Mb		7.314	3.149	2.372	2.272	0	0.105	0.115	0.605
θ		0.07314	0.03149	0.02372	0.02272	0	0.00105	0.00115	0.00605

Haplotypes of the 36 Glu89Gln probands were reconstructed using PHASE.

Thank you for your attention

Genome Center Bulgaria

SESSION 6-I

Moderators: Albena Todorova, Dimitrina Konstantinova

- **Neuromuscular disorders in Roma (Gypsies)**
  I. Tournev
- **Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases**
  R. Penchovsky

Oral presentations:

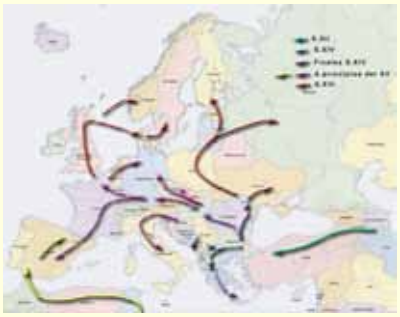
- **Pleven registry of congenital anomalies in the EUROCAT network**
  K. Kovacheva
- **Genetic study of achondroplasia in Serbian population**
  S. Radunovic
- **Dysregulated pathways in autism spectrum disorder**
  H. Ivanov
- **Genetically proven cases of Wolman disease in Bulgaria and mutation screening of two presumable endemic regions**
  A. Mandadzhieva
- **NPC1 and NPC2 gene analysis in Serbian patients with Niemann-Pick disease type C**
  M. Brankovic

NEUROMUSCULAR DISORDERS IN GYPSY (ROMA) POPULATION

Ivailo Tournev

Origins and Early History

Meanwhile, the remaining Gypsies continued their journey north and west and, by the end of the 15th century, had reached all parts of Europe (Fraser 1992). These three major migrations continue to define broad relationships between the multitude of endogamous groups that comprise the current Gypsy population.



BRIEF ROMA HISTORICAL BACKGROUND

The history of repression and ethnic discrimination was worst in Romania where Roma peoples spent more than 600 years in slavery the fourteenth to the mid-nineteenth century. Depending on the owner, Gypsy slaves were split into categories with different degrees of freedom and "privileges". Within each category, further subdivisions were based on trade, with some Romani groups retaining their professional ethnonyms (e.g. basket-weaves, spoon-makers etc.) to the present day.

Origins and Early History

The Roma (Gypsies) are a transnational minority with an overall population size estimated between 10 and 15 million (Liegeois, 1994). There are 8 million Roma in Europe, with close to 70 % resident in Central and Eastern Europe, mainly in the Balkans.

Data provided by the social sciences and genetic research suggest that Roma represent a conglomerate of genetically isolated founder populations.

Present status of Roma in an expanding Europe

- Roma are the most prominent poverty risk group in many of the countries of Central and Eastern Europe.
- Housing segregation – a form of geographic exclusion. Lack of basic sanitary conditions. Roma neighborhoods are frequently extremely overcrowded and destitute. Some Roma slums have evocative nicknames; for example, "Abyssinia" and "Cambodja", are extremely impoverished areas within Roma ghettos. Lack of water, gas, electricity, and public services such as waste collection bedevils many Roma neighborhoods.
- Economic exclusion, employment discrimination and very limited employment opportunities. Because of their low education levels, Roma were most frequently employed in low-skilled manufacturing industries.

Origins and Early History

- Ethnolinguisticly the Roma share a common Hindi/Sanskrit core language (Brearly, 1996). However, the more than 100 dialects of Romani illustrate the divergent paths that the various Roma groups have taken in their travels (Brearly, 1996).Traces of Middle Eastern languages, Armenian, Persian, Turkish and Kurdish are found in the various dialects. Feder's review (1989) of Traveller Roma characterizes this group as heterogeneous in nature, changing with and adapting in new cultures and places as they travel.
- The arrival of the Gypsies in Europe is estimated to have occurred, in the 11th-12th century (Fraser 1992; Marushiakova and Popov 1997), at which point a large fraction of the population settled permanently in the Balkans. Others , referred to as the Vlax Gypsies, crossed the Danube into present-day Romania where they were enslaved until the mid-1800s.

Present status of Roma in an expanding Europe

- Roma children often are excluded from education in mainstream public schools in Central and Eastern Europe and instead relegated to schools for the mentally retarded.
- The different surveys demonstrate lower educational attainment among Roma. Most Roma have primary education or below. In Bulgaria 89 % of Roma had primary education and only 10 % had some secondary education. Only 1 % of Roma continued past secondary school.
- The cultural differences and traditions are observed in some Roma communities. Our research showed that 60 % of marriages among the Kalajdjii group, 45 % among the Kalderas Roma amd 27.8 % among the Rudari are endogamous. Endogamy and inbreeding, which are most common among the Vlax Roma groups lead to the accumulation of the hereditary disorders.

Present status of Roma in Bulgaria

- **Poverty** rates: **Roma: 84.3%**; Turks: 40%; Bulgarians: 31.7%.
- **Unemployment** rates - **between 50% and 80%,** depending on the definition of unemployment.
- **Life expectancy**: **Roma - 66.6 years**, Bulgarians - 75.3 years.
- The results of the poor living conditions, malnutrition, and lack of sanitation: **prevalence of infectious diseases**, including tuberculosis and hepatitis (rates are significantly higher among the Roma population).
- **50%** of the Roma families have a **chronically ill** family member, and 20 % have two.
- Percentage of Roma with some kind of **disability**: **6 times higher** than the Bulgarian population.
- The tradition of large families and **early marriages** places women and young girls at increased health risk.

Other Mendelian disorders of the Roma caused by private mutations

Disorder	OMIM	Inheritance	Map Location	Gene	Mutation
Private congenital glaucoma	613086	AR	14q24.3	LTBP2	c.895C→T; R299X
Galactokinase deficiency	230200	AR	17q24	GK1	P387K
CMT4C	601596	AR	5q32	SH3TC2	R1109X
Glanzmann thrombasthenia	273800	AR	17q21	ITGA2B	IVS15DS
Polycystic kidney disease	173900	AR	4q21-q23	PKD2	R306X
Niemann-Pick disease B	607616	AR	11p.15.4	SMPD1	W391G
Hereditary spastic paraplegia7	607259	AR	16q24.3	SPG7	p.L78X
Cerebellar ataxia	613726	AR	3p22.1	ANO10	c.1150_1151del
Congenital ataxia	604473	AR	6q24	GRM1	c.2652_2645del and c.2660+2T>G

Population Genetics

Similar to other genetically isolated founder populations, such as the Finns and the Ashkenazi Jews, the Roma harbour a number of unique or rare autosomal recessive (AR) disorders, caused by “private” founder mutations.

Oppressive policies of persecution, exclusion, containment and forced assimilation practiced towards the Roma in most, if not all, European countries, together with Roma adherence to an ancient social tradition, have acted together to result in endogamy and isolation, making the Roma one of the Europe’s largest genetic isolates (Kalaydjieva L., 2001).

Hereditary Motor and Sensory Neuropathy Type Lom – Clinical Characteristics

- onset of gate disturbances at age 4 to 10 yrs
- upper limb weakness in the 2nd decade
- distally accentuated sensory loss involving all modalities
- hearing loss in the 3rd decade
- skeletal deformities
- NCV into the demyelinating range

Population Genetics

Collaborative studies have identified novel single-gene disorders and private mutations among the Roma drawing attention to this previously ignored founder mutation: CMS, due to mutation ε1267delG in CHRNE; HMSN Lom due to R148X in NDRG1 gene; CCFDN due to IVS6+ 389C>T mutation in CTDP, LGMD2C due to mutation C283Y in SGCG, HMSN Russe due to a mutation in an alternative untranslated exon of hexokinase 1 gene; GNE myopathy due to p.Ile587Thr mutation in the GNE gene. The identification of a growing number of novel Mendelian disorders and private mutations in the Roma (Gypsies) points to their unique genetic heritage.

As a result, these unique disorders, often occurring at high frequencies and presenting a public health problem, have escaped the attention of European medicine.

HMSNL Patients

Mendelian Neuromuscular Disorders of the Gypsy Caused by Private Founder Mutations

Disorder	OMIM	Inheritance	Map Location	Gene	Mutation
Hereditary motor and sensory neuropathy - Lom	601455	AR	8q24	NDRG1	R148X
Congenital cataracts facial dysmorphism neuropathy syndrome	604168	AR	18qter	CTDP1	IVS6 +389C>T
Hereditary motor and sensory neuropathy - Russe	605285	AR	10q23	HK1	G>C in alternative untranslated exon (Alt†2) G>A in adjacent intron
Limb girdle muscular dystrophy type 2C	253700	AR	13q12	SGCG	C283Y
Congenital myasthenia type Ia	254210	AR	17p13	CHRNE	1267delG
GNE myopathy	603824	AR	9p12-p11	GNE	I587T

HMSNL Patients

Nerve conduction studies CV in m/s

- Motor nerve conduction velocity is measurable only in young patients and subsequently unobtainable
- peroneal nerve at ages 2, 4.5 and 7 yrs: 34.4, 19.6 and 20.4 m/s
- tibial nerve
  - at age 2 yrs: 29.5 m/s
  - at ages 4.5, 7 and 8 yrs: 19 - 10.8 m/s
- median nerve
  - at age 2 yrs: 30.6 m/s
  - at ages 4.5 - 14 yrs: 20 - 10.8 m/s
- ulnar nerve at ages 7-15 yrs: 20.5 - 4.3 m/s

Animal Model of HMSNL

Okuda et al. (2004) found that myelination of Schwann cells in the sciatic nerve of Ndrg1-deficient mice was normal for 2 weeks after birth, but the sciatic nerve degenerated with demyelination at about 5 weeks of age. Ndrg1-deficient mice showed muscle weakness, especially in the hind limbs, but retained complicated motor skills. In wildtype mice, Ndrg1 was abundantly expressed in the cytoplasm of Schwann cells rather than the myelin sheath. Okuda et al. (2004) concluded that NDRG1 is essential for maintenance of the myelin sheaths in peripheral nerves.

Auditory function in HMSNL

- hearing loss of a pure neural type
- BAEP - central and peripheral abnormalities
- abnormal BAEP – at ages 4.5-11 years
- earlier involvement of the central auditory pathways, followed later by mixed peripheral and central abnormalities

Congenital cataracts facial dysmorphism, neuropathy syndrome – clinical features

1. Eye symptoms
   - congenital bilateral zonular cataracts – 100%
   - microcorneae – 100%
   - microphthalmos – 86%
   - nystagmus – 80%
   - strabismus – 76%
2. Facial dysmorphism – prominent midface, thickened perioral tissues, hypognathism
3. Short stature
4. Delayed motor milestones

Ultrastructural Changes in Peripheral Nerve in HMSNL

- considerable reduction in myelinating fiber density and extensive endoneurial collagen deposition
- severe progressive axonal loss
- hypertrophic small onion bulb changes are present in the younger patients which later regress
- accumulation of pleomorphic material in the adaxonal Schwann cell cytoplasm
- presence of intra-axonal accumulations of irregularly arranged curvilinear profiles similar to those reported in experimental vitamin E deficiency

Congenital cataracts facial dysmorphism syndrome – main clinical features

7. Predominant motor peripheral neuropathy
8. Mild mental retardation (IQ 55-65)
9. Hypogonadotropic hypogonadism
10. Kyphoscoliosis, foot deformities (pes cavus) and claw hand
11. CNS involvement – pyramidal signs, mild chorea and ataxia
12. Parainfectious rhabdomyolysis

HMSNL Genetics

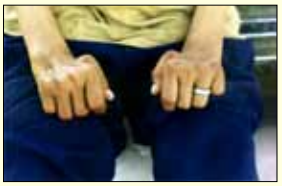
The R148X mutation in NDRG1 gene has been detected in the homozygous state in all HMSNL patients.

NDRG1 appears to play a role in growth arrest and cell differentiation, possibly as a signaling protein shuttling between the cytoplasm and the nucleus. Kalaydjieva et al. (2000) demonstrated that expression in peripheral nerve is particularly high in Schwann cells. Taken together, the findings pointed to NDRG1 having a role in the peripheral nervous system, possibly in Schwann cell signaling necessary for axonal survival.

CCFDN Patients

CCFDN Patients

CCFDN Patients



CCFDN Patients

Nerve Conduction Studies

Motor nerve conduction velocity (MNCV) was reduced into demyelinating range and distal motor latency prolonged, both for proximal and distal nerves. MNCV in the ulnar nerve on recording from abductor digiti minimi was reduced at a mean value of 24.7 ± 3.9 m/s with a prolonged mean distal latency at 4.3 ± 1.8 ms. Mean distal latency for the facial nerve was prolonged at 8.3 ± 2.0 ms. Mean sensory action potential values were of normal amplitude in the upper and lower limbs, but again, conduction velocity was reduced into the demyelinating range in both.

CCFDN Patients

HORMONAL FINDINGS

The results for endocrine investigation indicated hypogonadotropic hypogonadism, as demonstrated by low testosterone (6.29 ± 1.94 nmol/ml; normal: 9 -30 nmol/ml) and subnormal FSH levels (1.54 ± 0.86 mIU/ml; normal: 2 -10 mIU/ml) in males and by low estradiol (19.21 ± 4.6 pq/ml; normal 30 -90 pq/ml) and subnormal LH levels (3.75 ± o.25 ng/ml; normal: 4-12 ng/ml) in females. GH levels were in the low normal range with a pronounced increase after insulin-induced hypoglycemia. This suggests a regulatory deficiency of GH. The other hormonal results were normal. Bone mineral density was decreased into the osteopenic/osteoporotic range.

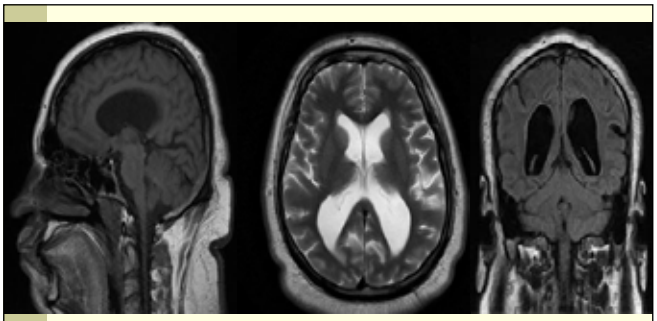
CCFDN Patients

Cognitive impairment in CCFDN

- Twenty two CCFDN patients underwent a formal neuropsychological assessment, consisting of the following tests:
  - Mini-Mental State Examination (MMSE); general intelligence assessment by Raven progressive matrices
  - Memory tests (Rey Auditory Verbal Learning Test, including subtests of immediate and delayed recall and forced-choice recognition and Digit Span forward)
  - Language tests (Phonological Word Fluency and Semantic Word Fluency)
  - Frontal and executive tasks (Tower of London and Digit Span backward)
- Their results were compared to the results of a control group of 24 healthy subjects with the same social and educational background

Cognitive impairment in CCFDN

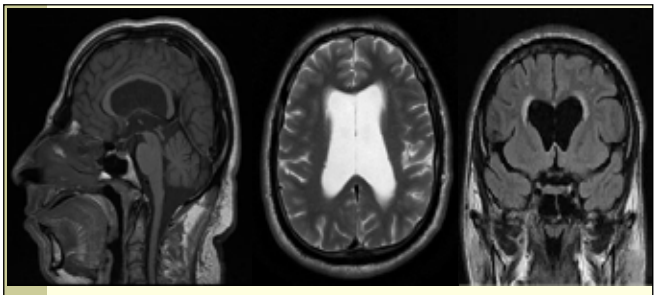
- In all psychometric tests, evaluating different cognitive domains, CCFDN patients had statistically significant lower scores when compared to the healthy control group
- Eighteen were classified as mild intellectual deficit, whereas 4 had borderline intelligence
- Language abilities were developed at a concrete and pragmatic level. Different cognitive domains (verbal memory, executive functions, attention and language skills) seemed similarly affected
- Patients were socially integrated, but for their everyday functioning they needed guidance and assistance



Patient with CCFDN, aged 33 y with MRI features of brain atrophy and ventricular enlargement

Cognitive impairment in CCFDN

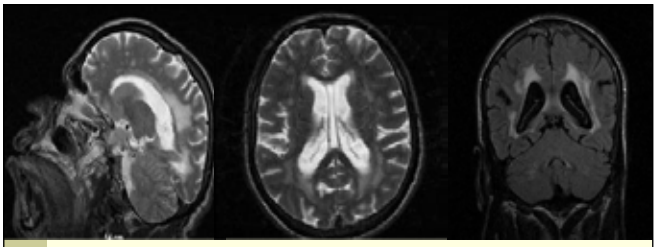
- As a conclusion cognitive impairment is a prominent feature of CCFDN as it is present in all patients, with a similar level of involvement of verbal and nonverbal skills, although the contribution of the visual impairment and social deprivation of the affected subjects should not be underestimated.



Patient with CCFDN aged 37 y with MRI features of brain atrophy, ventricular enlargement, hyperintense on T2 and FLAIR lesions, located in the frontal and parietal subcortical white matter

Brain imaging characteristics of patients with CCFDN

- Brain magnetic resonance imaging (MRI) was performed in 20 patients, using a 1.5T MR imager (MR Signa HDxt, GE Healthcare Milwaukee USA)
- Brain MRI demonstrated abnormalities in 19 out of 20 investigated patients (95%)
- Cerebral atrophy with enlargement of the lateral ventricles and dilatation of the subarachnoid spaces were observed in 18 of the affected (90%). Two of the affected without signs of cerebral atrophy belonged to the age group 4-8 years. Ventricular enlargement and cortical atrophy were evaluated as mild in 7 patients, as moderate in 6, and as severe in 5.
- Cerebellar atrophy was found in only one patient, who also had global cerebral atrophy with inner and outer CSF space widening.



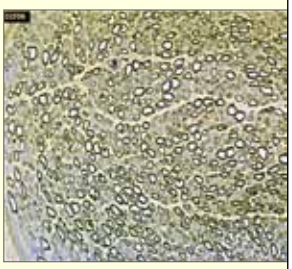
Patient with CCFDN, aged 46 y with MRI data of bilateral confluent hyperintense on T2 and FLAIR lesions in the frontal, parietal and occipital white matter as well as in the pons

Brain imaging characteristics of patients with CCFDN

- Thin corpus callosum was found in 3 CCFDN patients.
- Hyperintense lesions, varying from small single to multiple diffuse, encompassing periventricular white matter and brain stem, were observed in 18 patients. Hyperintensities were located predominantly in frontal periventricular white matter in 17 patients and in parietooccipital periventricular white matter in 9. Dense hyperintensisites were observed in frontal region (11/17) as well as in parietooccipital region (7/9). U – fibers in frontal (7/20) and in parietooccipital area (6/20) were the second common locations of hyperintense lesions. Only one patient had periventricular hyperintensities in temporal lobe.

NEUROPATHOLOGICAL DATA

Observations have been made on the peripheral nerve changes in four patients, ranging in age from 4 to 32 years, with CCFDN syndrome. The salient abnormality was diffuse hypomyelination, which in older patients, was associated with demyelination and then axonal degeneration. The morphological observations suggest the operation of a developmental process affecting myelination with a later superimposed degenerative disorder.



CCFDN Genetics

Firstly the gene has been mapped to the telomeric region of chromosome 18q. Later, it was found that the CCFDN is caused by a single-nucleotide substitution in antisense Alu element intron 6 of CTDP1 (C-terminal-domain-phosphatase of RNA polymerase II), considered to be universal component of eukaryotic basal transcription machinery with an essential role in different stages of the transcription cycle.

CCFDN thus belongs to a small group of disorders known as "transcription syndromes" and is the first poorly transcriptional defect identified that affects polymerase II-mediated gene expression.

Nerve biopsy

Sural nerve biopsies were obtained from four HMSNR patients aged from 6 to 43 years. Sural nerve biopsy specimens demonstrated a depletion of large myelinated nerve fibers, profuse regenerative activity and uniformly reduced thickness of the myelin sheath relative to axonal diameter, indicating hypomyelination. There was no indication of axonal atrophy.

Hereditary Motor and Sensor Neuropathy Type Russe – Clinical Characteristics

- Age of onset of distal lower limbs weakness was 8-16 yrs with an average of 11 yrs
- Age of onset of distal upper limbs weakness was variable with range of 10-43 yrs with an average of 22 yrs
- Steadily progressive clinical course leading to severe disability in the fifth decade of life
- Sensory loss affecting all modalities was a prominent feature.
- All patients showed foot deformities and most patients showed hand deformities
- Auditory functions and BAEP are normal in all the patients

HMSNR Genetics

We identified two sequence variants in Hexokinase 1 (HK1) gene in complete linkage disequilibrium, a G>C in a novel alternative untranslated exon (AltT2) and a G>A in the adjacent intron, segregating with the disease in affected families and present in the heterozygote state in only 5/790 population controls. The mutational mechanism and functional effects remain unknown and could involve disrupted translational regulation leading to increased anti-apoptotic activity (suggested by the profuse regenerative activity in affected nerves), or impairment of an unknown HK1 function in the peripheral nervous system (PNS).

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HMSNR Patients

Limb Girdle Muscle Dystrophy 2C (LGMD 2C with the γ–sarcoglycan deficiency)

- the most frequent LGMD in Gypsy.
- mean age at onset was 5.3 years.
- 50% had the same severe progression as the Duchenne patients, 25% of the patients had the intermediate phenotype and the remaining 25% had the Becker phenotype.
- Girdle, trunk and proximal limb flexor muscles had earlier and most severe involvement
- Calf hypertrophy, scapular winging, macroglossia, and lumbar hyperlordosis were common.

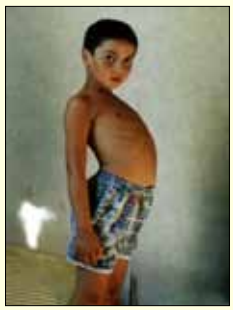
Nerve conduction studies

- Motor nerve conduction was unobtainable in the legs
- In the upper limbs, a peculiar combination of moderately reduced motor nerve conduction velocity (31.9±7.05 m/s for the ulnar nerve and 32.0±6.8 for the median nerve) and a greatly increased threshold for electrical nerve stimulation was observed. Sensory action potentials were absent.

Limb Girdle Muscle Dystrophy 2C (LGMD 2C with the γ–sarcoglycan deficiency)

- mean CK was 42±23 times normal correlating inversely with age and degree of disability
- intellectual development and cognitive functions were normal in all affected individuals
- cardiac involvement was present in 9/57 patients
- EMG demonstrated typical myopathic changes.

LGMD 2C Patients

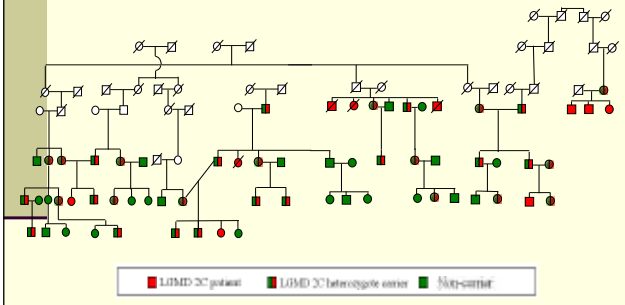


LGMD 2C Genetics

The LGMD 2C in Gypsy is caused by a "private" homozygous C283Y mutation on the same haplotype, suggesting a founder effect. LGMD 2C due to the C283Y mutation has a more severe presentation than that caused by the North African out of frame del521T mutation.

LGMD 2C Patients

LGMD 2C pedigree in Xoroxane Roma in Bulgaria



LGMD 2C Patients

GNE Myopathy

The mean age at onset was 24.40 ± 6.31 years, ranging between 10 and 42 years. In the majority of cases (36/50, 72%) the first symptoms appeared in the third decade. Onset was earlier (at age 10 - 19 years) in seven patients (14%), and later (at age 31 or older) in another seven (14%).

The initial manifestations in most patients (48/51) included difficulties in walking or running. Steppage gate was noted from the beginning. Initially, and more severely affected were mm. tibialis anterior, extensor digitorum brevis and abductor hallucis. In the advanced stages, there was no substantial difference in weakness of the anterior and posterior distal muscle groups of the lower limbs. In the course of the disease the thigh adductors became involved as well. At the initial stages, m. gluteus medius and minimus and neck flexors were slightly affected.

Muscle biopsy

Muscle specimens were undertaken from 9 LGMD 2C patients. The immunohistochemical results demonstrated the following pattern: α-SG reduced, but the intramembranous part well preserved; δ-SG partially preserved; β-SG vastly reduced; γ-SG lost completely. In addition α-dystroglycan is almost completely lost although β-dystroglycan is preserved.

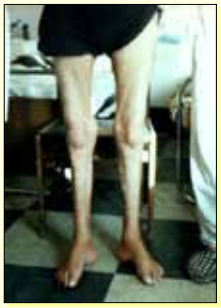
GNE myopathy Clinical Phenotype

- In 16/51 patients the muscle strength of the quadriceps femoris was fully preserved for 2 to 10 years after the clinical onset (5/5 on the MRC scale). In the remaining individuals, quadriceps femoris was relatively preserved compared to the other lower limb muscles: 3/5 in 22 and 4/5 in 13 patients.
- Weakness in the hands (in holding small objects) developed 5-11 years after onset, with m. extensor pollicis longus et brevis, interossei dorsales, pronator, flexor carpi radialis and biceps brachii affected first and most severely. Three patients reported weakness of the distal muscles of the upper limbs as the initial complaint, whereas the distal muscles of the lower limbs became involved later in the course of the disease.
- Scapular winging and lumbar hyperlordosis developed late in the course of the disease. Scoliosis was not present. The neck flexors were severely affected in 10 bed-ridden patients. The facial, extraocular and bulbar muscles were preserved in all affected subjects. Ankle contractures were noted in 32/51 patients.

GNE myopathy Clinical Phenotype

- In the course of the study (a 15-year follow-up period), 32 patients became non-ambulant. Loss of ambulation occurred on average 10.34 ± 4.31 years after the onset of muscle weakness, with a broad variation between 3 and 20 years. Sixteen patients became wheelchair-bound within 10 years of onset, while in the remaining 16 subjects (50%) ambulation was preserved for longer periods. There was no correlation between age at onset and years of preserved ambulation after the clinical onset (r=0.117, p=0.524), and between disease duration and current functional status (scores on the Modified Gardner-Medwin and Walton scale) in the 18 ambulant patients (r=0.441, p=0.067). We used Levene's test to examine the effect of modifying genetic factors (non-GNE) on the course and severity of the disease: intra-familial phenotype variation was compared to the variation between unrelated non-familial cases. Variation between affected relatives was not significantly different from that between unrelated patients as regards age at onset (p=0.719) and years of preserved ambulation (p=0.695).

GNE myopathy Patients




GNE myopathy Clinical Phenotype

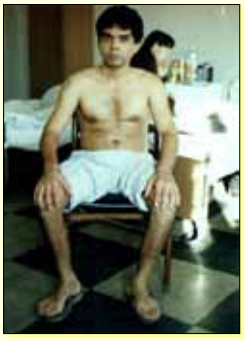
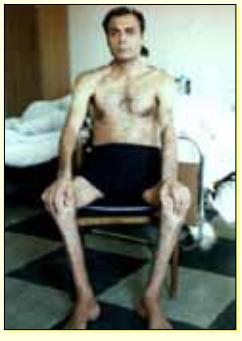
- EMG consistently showed myopathic changes with small motor unit potentials. CK values were within the normal range (20-200 UI/l) in 6 patients, while in the remaining 36 they were elevated 2 to 10 times above the upper limit of normal. There was no correlation between CK levels and disease progression, measured by the Modified Gardner-Medwin and Walton scale (r=-0.122,p=0.443).

GNE myopathy Patients


GNE myopathy Patients

GNE myopathy Patients


GNE myopathy Patients

GNE myopathy Cardiac Abnormalities

Six affected individuals had subjective cardiac complaints – three reported easy fatigue upon physical efforts, while another three had periodic palpitations. On physical examination there were no significant abnormalities, however pathological changes in the left ventricular (LV) function were evident by echocardiography in 11 out of 33 evaluated patients. In five (including two symptomatic), the cardiac abnormalities could be related to the myopathy, as there was no history of other cardiac or non-cardiac disorder. Three of these subjects had mild LV systolic dysfunction with ejection fraction (EF) below 55% and mild LV diastolic dysfunction. In the other two patients, only impaired relaxation was registered by Doppler echocardiography.

GNE myopathy Cardiac Abnormalities

- Another six patients, who had LV hypertrophy and mild LV diastolic dysfunction, suffered from arterial hypertension and one patient was diagnosed with diabetes. In four patients, mitral valve prolapse with non-significant mitral regurgitation was observed (Table 1). ECG abnormalities were observed in seven patients : three presented with repolarization abnormalites in the inferolateral leads, of whom two had arterial hypertension and LV hypertrophy, and one had no concomitant pathology. None had a history or symptoms of coronary heart disease. One patient with arterial hypertension and LV hypertrophy was diagnosed with ventricular extrasystolic arrhythmia and three displayed nonspecific intraventricular delay. The latter three had no concomitant disease.

CMS Ia Gypsy Patients

GNE myopathy Molecular Analysis Data

The mutation causing the disease was a single base substitution 1811 T→C changing Ile 587 Thr in GNE gene.

Despite the mutation homogeneity, the clinical course and severity of the disease show inter- and intra-familial variation, with no evidence of genetic modifying factors found in this study.

The screening of 660 ethnically matched controls detected 11 carriers, an overall carrier rate of 1.67%. Notably, all belonged to Roma/Gypsy groups from the Balkan migrational category. The carrier rate among the higher-risk Balkan groups was estimated at 2.84%, with a predicted incidence of affected births around 1 in 5000.

CMS Ia Gypsy Patient

Congenital Myasthenic Syndrome Type Ia - Clinical Phenotype

CMS occurs in diverse Gypsy groups (Abicht et al.) The founder mutation, 1267delG in CHRNE on 17p13, was identified originally in patients from India/Pakistan (Croxen et al. 1999).

93 CMS patients with the homozygous mutation 1267delG were clinically characterized recently. 45 of them demonstrated mild clinical course of the disease, 28 – moderate clinical course and 20 – severe clinical course; 47 of them had not fatigable weakness of the bulbar muscles, 36 of them had mild bulbar muscle weakness and 10 of them more severe bulbar weakness; 30 of them has not fatigable weakness of the proximal limb muscles; 33 has mild proximal weakness and 29 has moderate/severe weakness of the proximal limb muscles. 15 CMS patients were with normal FVC %, 55 CMS patients with FVC % varied between 70 % and 90 %; 18 – 50-60 % and 5 – under 50 %.

CMS Ia Gypsy Patient – severe form

CMS Ia Gypsy Patient

Mutation History of Roma (Gypsies)

- The Gypsies comprise a complex mosaic of culturally and socially distinct groups . We investigated the distribution of five autosomal recessive founder mutations previously identified in one or more Gypsy groups. These included a mutation in NDRG1 associated with hereditary motor and sensory neuropathy type Lom (HMSNL), CTDP1 associated with congenital cataracts facial dysmorphism neuropathy (CCFDN), CHRNE associated with congenital myasthenia (CMS), SGCG associated with limb girdle muscular dystrophy type 2C (LGMD2C) and GALK1 causing galactokinase deficiency.
- In our sample of 1363 Gypsy individuals from 14 distinct communities, we found four of the five mutations in historically separated populations.

CMS

- Haplotype analysis showed that a mutation associated with CMS in Gypsy and Indian individuals is identical providing evidence for an Indian origin of the Gypsies. The ages and distribution of the disease mutations supports the claim that the Gypsy population, founded 1000 years ago, arrived in Europe as a single group which subsequently split into numerous founder populations.
- A wide spread of the CMS mutation in the Indian subcontinent is suggested by the different places of origin, language groups, castes and religious affiliations represented in our small group of Indian/Pakistani patients. CMS 1267delG mutation was already present at the time of the founding of the proto-Gypsy population, 32-40 generation before present. The mean age of the mutation is 800-1000 years. CHRNE 1267delG mutation was found in all Gypsy groups at approximately equal frequencies.

Mutation frequencies

- Mutation frequencies differed significantly between the Vlax and Balkan populations in Bulgaria, where Gypsy group identity had been reliably defined. Although mutations were widely distributed among populations, intragroup frequencies also differed markedly. In many cases disease gene frequency was extremely high in one group but was absent or at a very low frequencies in other groups in the populations. This is exemplified by CCFDN (carrier frequency of ~7% the Rudari) and LGMD2C (5-6% among the Darakchii and Turgovzi) for which the mutation was virtually absent in other groups within the Vlax and Balkan populations respectively.

HMSNL

- HMSNL mutation R148X in NDRG1 gene was present in all migrational Gypsy categories.
- The R148X mutation was estimated to have been introduced 425 to 600 years ago in individual Vlax groups with the 95% confidence intervals suggesting a younger age of the mutation in the individual Gypsy groups. The carrier rate for HMSNL in General Roma is 2 %, but in some high risk groups – 16 %

Mutation frequencies

- Thirteen percent of the population was found to carry one of the mutations. i.e. 1 in 8 subjects was heterozygous for one of the mutations tested. This value is higher than estimates obtained for the Finnish and Ashkenazi Jewish populations, where testing for five high frequency mutations give carrier rates of 7.5% (AGU, APECED, CNFMajor, DTD and INCL; Pastinen et al. 2001) and 2.7% [GD, APC, FII (type II), FII (type II) and CX26; Risch et al. 2003] respectively. Thus, recessive mendelian disorders represent a considerable health concern in Gypsy populations. Given the high frequency of disease alleles and allelic homogeneity the Gypsies are an excellent candidate population for community based genetic screening. Group specific frequencies for individual founder mutations were as high as 16%.

LGMD2C

- LGMD2C and galactokinase deficiency are more restricted and represent the founding and expansion of the Gypsy migrational categories 20+ generations ago. LGMD2C was fully confined to the Balkan and Western European Gypsies and was not present in Vlax Gypsies. The mean age of C283Y mutation is 600 years with somewhat younger ages in the Balkan and West European populations where it has been detected (425 and 525 years respectively).

Epidemiological data

- **CMS** – Hungary, Bulgaria, Romania, Greece, Turkey, Macedonia, Serbia, Kosovo, Montenegro, Germany, India, Pakistan, Egypt
- **HMSNL** – Bulgaria, Romania, Hungary, Germany, Czech Republic, Serbia, Slovenia, Spain, France, Italy, Belgium
- **CCFDN**- Bulgaria, Romania, Hungary, USA, Italy, Germany, Austria, Greece, Czech Republic
- **LGMD2C** – Spain, Italy, France, Germany, Portugal, Bulgaria
- **HMSNR** - Bulgaria, Romania, Spain, France

CCFDN

- CCFDN falls into the most recent ”age group” , coinciding with the founding of individual subisolates. CCFDN mutation, which was found to occur mainly among the Rudari and was estimated to have originated around 500 years ago.

CLINICAL EPIDEMIOLOGY, COMMUNITY-BASED TESTING AND UPTAKE OF GENETIC SERVICES IN BULGARIA

Sources used for identification of Roma families with hereditary neuromuscular disorders

The main sources for collecting the epidemiological information were the field work studies.

A neurological screening of hereditary neuromuscular disorders was performed in 2500 towns and villages (having predominantly Roma population) in the country. It was performed according to the Modified Scale of Waldrop (Peter a Wynn Owen, 1996) and using the method "door to door". Those towns and villages where pedigrees with hereditary neuromuscular disorders resided were visited from 2 to 7 times with the aim of collecting pedigree information, blood samples for genetic studies and neurological examination of the patients. The field work studies covered a period of 20 years (1994-2014).

Sources used for identification of Roma families with hereditary neuromuscular disorders

2. Registers of the regional and a lot of municipal neurological departments and regional labour expert medical boards.
3. Registers of the regional pediatric departments.
4. Registers of the medical genetic consulting centers in Burgas, Pleven, Varna and St. Zagora.
5. Registers of the neurophysiological laboratories in the country.
6. Registers of the Clinic of Child Neurology, University Hospital "St. Naum"; the Neurological Department of the Pediatric Hospital, Medical University, Sofia; the Electromyography Laboratory, University Hospital "Queen Jovanna"; the Clinic of Child Ophthalmology, University Hospital "Alexandroff and the Laboratory of Molecular Pathology, University Hospital of Obstetrics and Gynaecology.

Sources used for identification of Roma families with hereditary neuromuscular disorders

7. Registers of orphanage and aged debilitated people homes in different regions of the country and the school for blind in "Nadejda" quarter, Sofia.

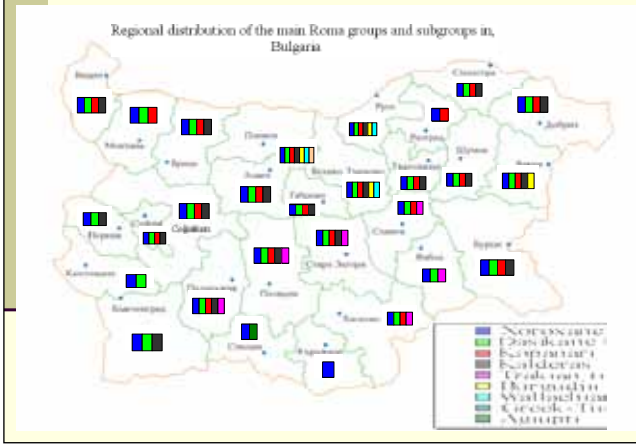
8. The data collected from the patients and their families were another important source of information concerning other affected individuals, residing elsewhere. Some of the more endogamous groups organize annual holiday meetings and have the opportunity to exchange information within the group and to preserve their native identity.

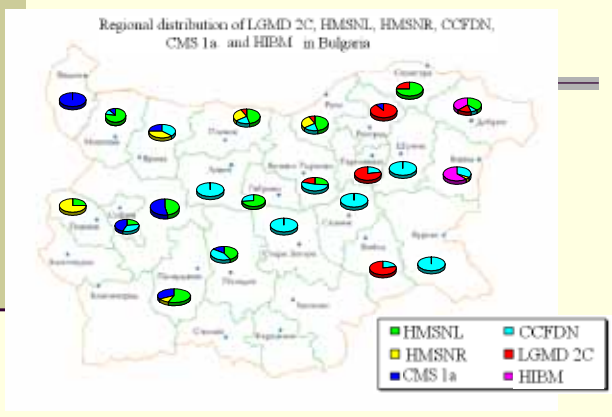
Sources used for identification of Roma families with hereditary neuromuscular disorders

1. Field studies. 95% of the Roma population, living in compact Gypsy quarters was encompassed. An ethnographical and linguistic examination was performed in every quarter using a semi-standard interview for identification of various Roma groups and subgroups. In those towns and villages where Roma people live in several quarters or more than one Roma group resided, the ethnographical and linguistic examinations were performed in every quarter and in every separate group. The field studies were performed with the support of the local "Roma" foundations and Roma coordinators from different parts of the country.

Analysis of the results

Hereditary neuromuscular disorders in Roma people were identified in 26 out of 28 regions in the country. There were 839 patients from 281 Gypsy families identified with various hereditary neuromuscular disorders, residing in 305 towns and villages. Five of the disorders turned out to be more frequent than the others, namely: Hereditary motor and sensory neuropathy type Lom (HMSNL); Congenital cataracts facial dysmorphism neuropathy syndrome (CCFDN); Congenital myasthenic syndrome Ia (CMS Ia); Limb-girdle muscular dystrophy, gamma sarcoglycanopathy (LGMD 2C); Spinal muscular atrophy (SMA).





We found the following distribution of neuromuscular disorders in Roma people in Bulgaria:

Congenital myasthenic syndrome Ia (CMS Ia) - 137 cases from 63 families in 49 towns and villages.

Hereditary motor and sensory neuropathy - Lom (HMSNL) - 120 cases from 45 families in 53 towns and villages.

Congenital cataracts facial dysmorphism neuropathy syndrome (CCFDN) - 85 cases from 34 families in 43 towns and villages.

Limb-girdle muscular dystrophy, gamma sarcoglycanopathy (LGMD 2C) - 57 cases from 32 families in 29 towns and villages.

The frequency of the hereditary motor and sensory neuropathies (all forms) is rather high even when taking into account the maximal total number of the Roma population – 51.3/100 000 people. That frequency considerably differs from that of the whole Bulgarian population - 5.2/100 000 people (V. Georgieva and M. Abadjiev, 1998).

We found the following distribution of neuromuscular disorders in Roma people in Bulgaria:

Spinal muscular atrophy - 115 cases from 47 families in 39 towns and villages:

type I – 35 affected
type II – 21 affected
type III – 59 affected

Hereditary spastic paraplegias - 42 cases from 17 families in 10 towns and villages. Mutations in spastin, atlastin and paraplegin were identified in 3 families. All the mutations in Gypsies are novel.

DMD/BMD - 51 cases from 37 families in 28 towns and villages.

Community-based carrier testing program in high-risk Roma groups

Public awareness campaign

A/ Informing the local medical staff.

Our experience so far has shown that the GPs and regional specialists /neurologists, therapists, pediatricians, gynecologists/ are not familiar with the Roma specific health problems and don't use the existing preventing potentials. That's why informing the local medical staff and promoting it's participation in the prophylactics activities by undertaking certain engagements will benefit the effect of the genetic prevention.

We made GPs and medical specialists familiar with the aims and stages of the genetic prevention program by both spreading newsletters and reading lectures at the regional healthcare centers.

We found the following distribution of neuromuscular disorders in Roma people in Bulgaria:

GNE myopathy - 51 cases from 13 families in 16 towns and villages.

FSHD - 58 cases from one large family.

Hereditary motor and sensory neuropathy type Russe (HMSNR) - 30 cases in 14 towns and villages.

Congenital myopathies - 19 cases from 8 families in 10 towns and villages.

Other LGMD - 8 cases from 3 families in 8 towns and villages.

Public awareness campaign

B/ Informing high-risk Roma groups.

The „door to door" health educational program was performed in the Roma communities, in order to reassure that all young people from all groups at risk would be covered. The main points in this educational program were: what the disease itself is, how it could appear, how it could be inherited and transmitted to the offspring, what the genetic analysis itself is, what could the detected carriers expect, what kinds of choices the couples of two carriers have and what the prenatal diagnosis means. Several common meetings were also performed in the suburb and amazingly a lot of young people were presented there. During the performance of the health educational program, a lot of medical exams were offered and performed for free by the doctors from Gypsy origin and the rest of the medical staff.

We found the following distribution of neuromuscular disorders in Roma people in Bulgaria:

Other CMT syndromes:

CMT 1A – 2 cases from one family
HNPP – 3 cases from two families
CMTX – 2 cases from one family
Others CMT forms – 11 cases

Public awareness campaign

C/ Providing information for the local Roma organizations implementing cooperative health prevention activities.




Selective screening for genetic prevention in a high risk Roma groups

A selective screening for hereditary neuromuscular disorders /LGMD 2C, HMSNL, CMS Ia, CCFDN, SMA/ was performed. The screening was a part of a National Program for genetic prevention and was performed in agreement with the Bulgarian Ministry of Health. The EMHP Foundation team in collaboration with Roma health mediators organized the collection of blood samples. The "door-to-door" health information activities were performed in Roma communities. A total of 5547 Roma volunteers at reproductive age were involved in the screening program during the extensive fieldwork. All individuals who took part in the screening program signed an informed consent for a voluntarily participation in the program.

Tasks of the Health mediator

- Health mediators have official Job description adopted by the Ministry of Health Care. Main tasks:
  - To collaborate with GPs for obtaining high immunization coverage (search for non-immunized children, inform parents about vaccine-preventable diseases).
  - To search for and identify people with disabilities and chronic illnesses, young mothers with many children, pregnant women that are not health insured.
  - To assist illiterate people to submit documents to health and social institutions.
  - To assist in the organization of prophylactic check-ups with mobile technique.

Individual genetic counseling

All participants in the screening were informed in writing about their carrier status and consultations were provided individually in person's houses. Genetic counseling was offered to the detected carriers. They were informed about the risk, in case of having a carrier partner, to deliver an affected child. It was explained to them what kind of choices they have and how they can reassure healthy offspring. Some of the individuals, detected by this program as being carriers, already addressed us, willing to know the carrier status of their partners. Genetic tests were proposed also to the relatives of the detected carriers.

Tasks of the Health mediator

- Health mediators have official Job description adopted by the state. Main tasks:
  - To organize meetings for increasing the health culture and awareness within the community, including in schools and kindergartens (in cooperation with local and regional health specialists and institutions).
  - To provide information and assistance to women who are willing to use IUDs (HMs receive free of charge IUDs from the Bulgarian Association for Family Planning).
  - To report cases of discrimination.

Prenatal diagnosis

In the last years 75 prenatal genetic tests were performed in Gypsy families with different hereditary neuromuscular disorders:

33 prenatal tests for SMA
21 prenatal tests for DMD
11 prenatal tests for LGMD 2C
7 prenatal tests for CMS Ia
3 prenatal tests for CCFDN

Background of Health mediators

- Women or men
- Graduated secondary education
- Belonging to the local vulnerable community
- Speaking the language of the community (Roma, Turkish)
- Communicative and well accepted by the community
- Computer literacy (Internet, Word)
- Free to travel for the initial training, annual meetings of HMs, willing to participate in additional trainings and meetings.

Development of the Roma Health Mediator Program in Bulgaria

- The Health mediator is an intermediary who facilitates the access of vulnerable/ isolated ethnic minorities to health and social services.
- The Health mediator is **woman or man** who belongs to the local community and speaks its language.
- The Health mediator is not an administrator but a **field worker** who works actively to identify the most vulnerable and marginalized community members.
- The Health mediator initiates communication with all local health and social institutions and specialists and offers them assistance.

Training of Health mediators

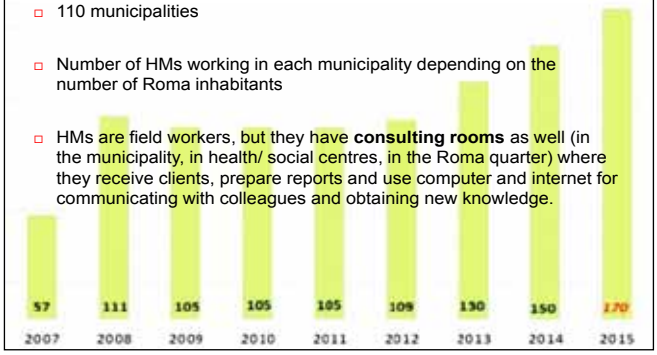
Training provided by **Medical University-Sofia** and **NNHM team:**

- Health and social legislation,
- System of health services,
- Patient's rights,
- Health and health problems – basic health information,
- Health and intercultural differences,
- Communication and advocacy,
- Case work,
- Professional role of the HM,
- Leadership and teamwork,
- Project preparation and management.

- Final exam.
- Receiving **Certificate** for successful graduation. *Health mediators without Certificate could not be appointed in the municipalities*
- Municipalities pay for the training.

Number of Health mediators & Settings

- 110 municipalities
- Number of HMs working in each municipality depending on the number of Roma inhabitants
- HMs are field workers, but they have **consulting rooms** as well (in the municipality, in health/ social centres, in the Roma quarter) where they receive clients, prepare reports and use computer and internet for communicating with colleagues and obtaining new knowledge.



NNHM Website
www.zdravenmediator.net

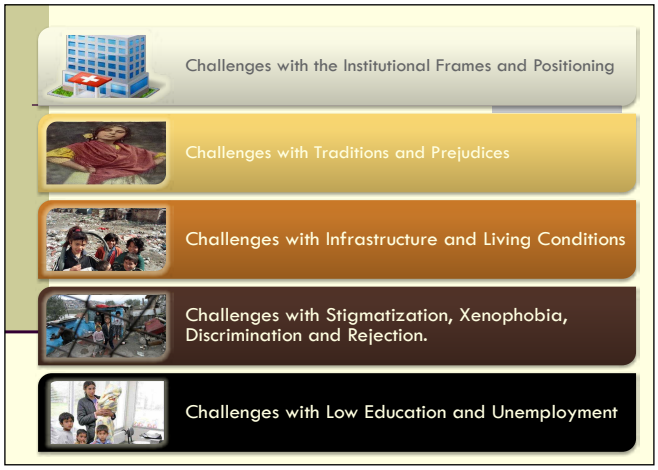
Health mediators on the map

Professional forum of Health mediators
www.zdravenmediator.net

Working relationships

- As a municipal employee the HM is subordinated to Deputy Mayor (smaller settlements) or Director of "Health and social activities" department.
- HMs communicate with:
  - GPs and other medical specialists
  - Hospitals/ Emergency Units
  - School/ kindergarten nurses
  - Regional Health Inspectorates (28 in Bulgaria)
  - Regional Health Insurance Fund
  - Social services
  - Child protection departments
  - Regional Commissions for Protection against Discrimination
  - Other social services providers and local NGOs

Challenges faced by the Health Mediator



2 Challenges with Traditions and Prejudices

There is a long list of unhealthy prejudices and traditions that the community continue to practice like:
Early marriages and early pregnancies, prejudices concerning abortions and vaccinations, non-healthy domestic practices during pre and post natal period, mistrust to the official medical care providers, mysticism concerning general health condition and pregnancy.



The health mediator searches and develops educational tools and activities in understandable for the community language and in accepted by the community form to provoke the establishment and sustainability of healthy behavior at individual and community level.


1 Main Challenges with the Institutional Frames

- Medical professionals complain that the majority of Roma are not health insured and therefore doctors are forced to deny provision of health care to them.
- GPs face difficulties to find their patients for vaccination, prophylactics or follow-up examinations.
- People from the community complain that institutions are not understanding and responding to their health needs.



3 Challenges with Infrastructure and Living Conditions

- Most of the Roma live in segregated communities, in illegal houses, built by themselves that do not meet the healthy standards for living, with limited access to clean water and electricity, lack or bad functioning canalization, on streets full of garbage with no green areas and with polluted air from the chimneys during the winter.



The health mediator is working in close collaboration with GPs, Regional Health Inspectorate, Local Hospitals, Social care institutions and Municipality to help both: The Roma community - to access adequate health care and Health and Social Care professionals - to get their job done.

The health mediators is working with the community - to build awareness about the common healthy standards of living conditions and with the concerned institutions to bring their attention to the problem and provoke decisions of policy makers to help improving the living conditions of Roma

4 Challenges with Stigmatization, Xenophobia, Discrimination and Rejection.

- Both Gadjo and Roma bare a heavy burden of centuries-long prejudices against each other.
- Unawareness, broken Communication, Fear from the un-known have built heavy layers of Stigmatization, and Hostility that result in Discrimination and Rejection in all aspects of live including access to health care.

Main functions of the health mediator

To support the integration of the Roma community trough healthcare.

To advocate with his life and work for general attitude and behavior change towards mutual respect and equal rights for Roma and Non-Roma.

To serve as a bridge between the Roma community and the social and health care institutions in order to facilitate the improvement of the health status of the Roma population.

The health mediator constantly tries to find ways to improve communication between both: social and health professionals- from one side and the Roma community- from the other side, in order to help them understand each other better and find their common healthy future together.

Training of Roma medical students

- During the last 3 years our team organized training courses for Roma young people to study medicine. At present time 106 Roma are students in medical universities, 22 of them study medicine, 84 – other medical specilties.

5 Challenges with Low Education and Unemployment

- Many Roma have limited access to health because of Unemployment.
- Many Roma are illiterate or with very low education that limits their communication with social and health care professionals.
- Many Roma children study in segregated schools and drop out at the age of 11-12 because of marriage arranged by their parents.

Training of Roma medical students

- The Health mediator motivates parents and children to invest in education and assist teachers in their endeavors to reflect adequately to the individual needs of the children and their families.
- The health mediator is fully aware of the fact that his personal live and his educational and professional achievements serve as a model for the community.
- His daily life is more than just a job but rather- a mission

Training of Roma medical students

Roma medical students

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Roma medical students

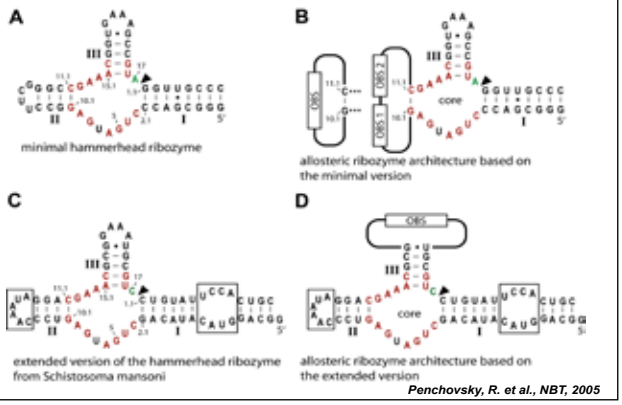
Thank you!

ENGINEERING INTEGRATED DIGITAL CIRCUITS WITH ALLOSTERIC RIBOZYMES FOR SCALING UP MOLECULAR COMPUTATION AND DIAGNOSTICS OF RARE DISEASES

Robert Penchovsky

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Secondary Structures of Allosteric Hammerhead Ribozymes



Penchovsky, R. et al., NBT, 2005

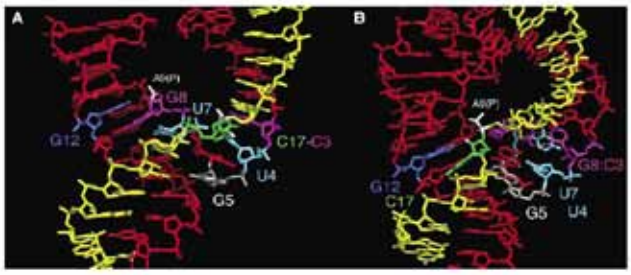
"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Trinucleotide repeat expansion disorders - Polyglutamine (PolyQ) diseases

Type	Gene	Normal PolyQ repeats	Pathogenic PolyQ repeats
DRPLA (Dentatorubropallidoluysian atrophy)	ATN1 or DRPLA	6 - 35	49 - 88
HD (Huntington's disease)	HTT	6 - 35	36 - 250
SBMA (Spinal and bulbar muscular atrophy)	AR	9 - 36	38 - 62
SCA1 (Spinocerebellar ataxia Type 1)	ATXN1	6 - 35	49 - 88
SCA2 (Spinocerebellar ataxia Type 2)	ATXN2	14 - 32	33 - 77
SCA3 (Spinocerebellar ataxia Type 3 or Machado-Joseph disease)	ATXN3	12 - 40	55 - 86
SCA6 (Spinocerebellar ataxia Type 6)	CACNA1A	4 - 18	21 - 30
SCA7 (Spinocerebellar ataxia Type 7)	ATXN7	7 - 17	38 - 120
SCA17 (Spinocerebellar ataxia Type 17)	TBP	25 - 42	47 - 63

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Side-by-Side Comparison of the Minimal and Full-Length Hammered Ribozyme Structures



In Vitro Selection vs Computational selection for engineering allosteric ribozymes

Martick, M. et al., Cell, 2006

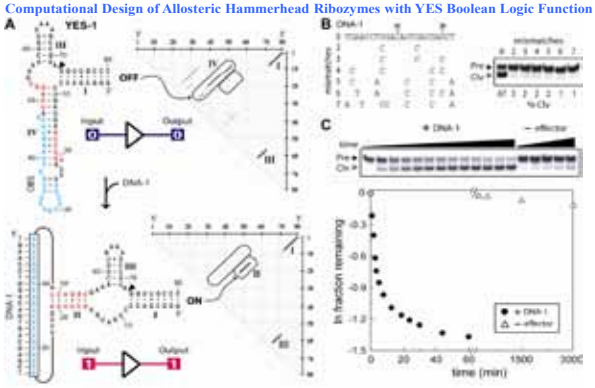
"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Trinucleotide repeat expansion disorders - Non-polyglutamine diseases

Type	Gene	Codon	Normal/wild type	Pathogenic
FRAXA (Fragile X syndrome)	FMR1, on the X-chromosome	CGG	6 - 53	230+
FXTAS (Fragile X-associated tremor/ataxia syndrome)	FMR1, on the X-chromosome	CGG	6 - 53	55-200
FRAXE (Fragile XE mental retardation)	AFF2 or FMR2, on the X-chromosome	CCG	6 - 35	200+
FRDA (Friedreich's ataxia)	FXN or X25, (frataxin—reduced expression)	GAA	7 - 34	100+
DM (Myotonic dystrophy)	DMPK	CTG	5 - 34	50+
SCA8 (Spinocerebellar ataxia Type 8)	OSCA or SCA8	CTG	16 - 37	110 - 250
SCA12 (Spinocerebellar ataxia Type 12)	PPP2R2B or SCA12	nnn On 5' end	7 - 28	66 - 78

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

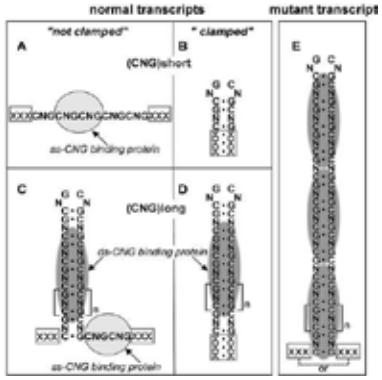
Computational Design of Allosteric Hammerhead Ribozymes with YES Boolean Logic Function



Penchovsky, R. et al., NBT, 2005

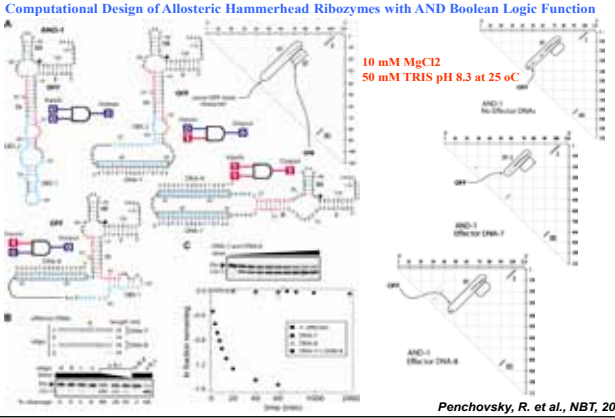
"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Trinucleotide repeat expansion disorders – secondary structures of mRNAs with different numbers of CNG codons



"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Computational Design of Allosteric Hammerhead Ribozymes with AND Boolean Logic Function



Penchovsky, R. et al., NBT, 2005

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

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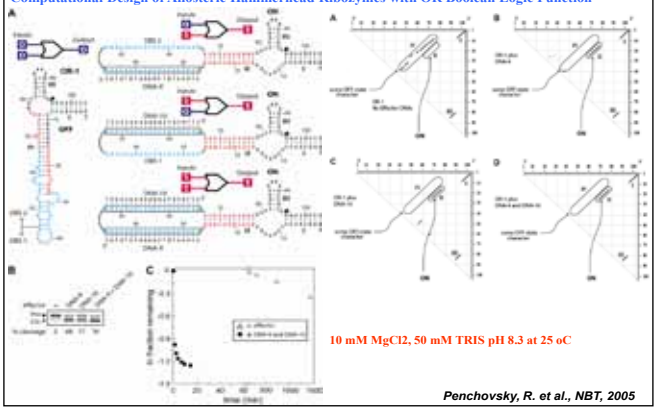
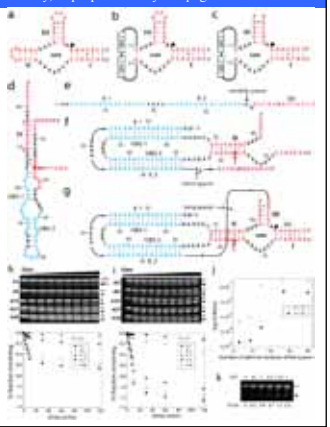


10 mM MgCl2, 50 mM TRIS pH 8.3 at 25 oC

Penchovsky, R. et al., NBT, 2005

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Computational Design of Allosteric Hammerhead Ribozymes that Sense the Length of Their Substrates



20 mM MgCl2, 50 mM TRIS pH 8.3 at 37 oC

Penchovsky R. ACS Synthetic Biology, 1, 471-482 (**2012**)

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

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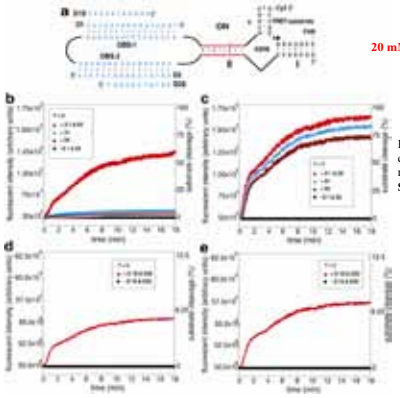
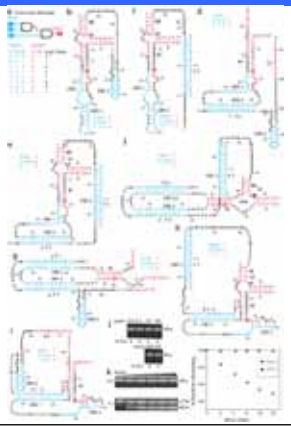


20 mM MgCl2, 50 mM TRIS pH 8.3 at 37 oC

Penchovsky R. Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics. **ACS Synthetic Biology**, 1, 471-482 (**2012**)

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Integrated Three-input Molecular AND Gate



20 mM MgCl2, 50 mM TRIS pH 8.3 at 37 oC

What about Huntington's disease causes by a CAG expansion in mRNA?

Penchovsky R. ACS Synthetic Biology, 1, 471-482 (**2012**)

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

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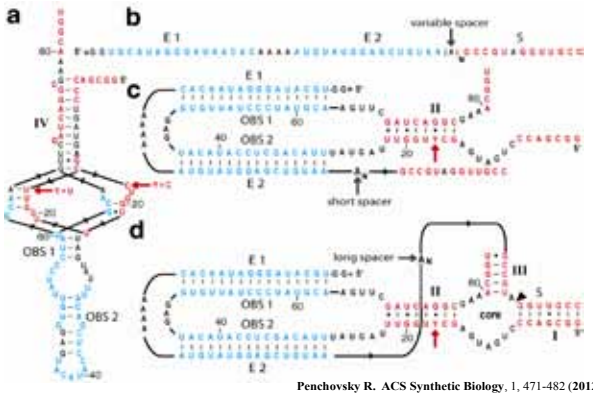

Penchovsky R. ACS Synthetic Biology, 1, 471-482 (**2012**)

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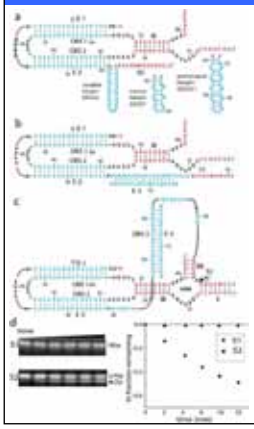

Ribozyme detection of RNAs with triplet repeats observed in the OPMD (oculopharyngeal muscular dystrophy disorder) . Two target RNAs were enzymatically synthesized and 5´-FAM-ApG labeled indicated by the star(*). Both targets have two effecter sites (E1 and E2, blue), a variable hairpin (blue), and a substrate domain (red). One RNA has a normal number of 7 GCG (Ala) repeats while the other has 11 triplet repeats observed in the OPMD disorder.

Penchovsky R. Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics. **ACS Synthetic Biology**, 1, 471-482 (**2012**)

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

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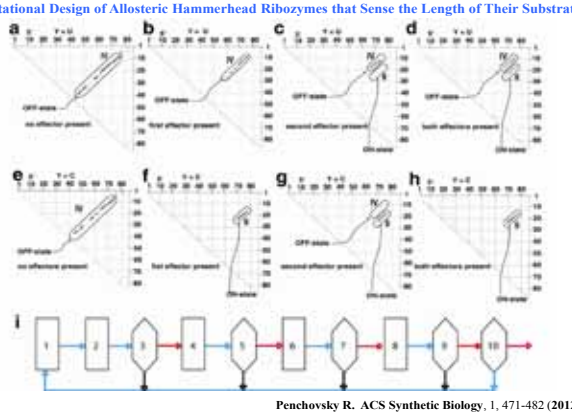
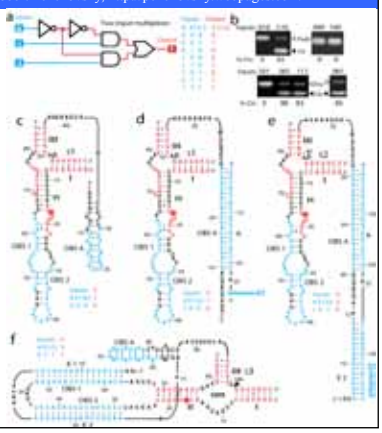


Penchovsky R. ACS Synthetic Biology, 1, 471-482 (**2012**)

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Integrated Two-input Molecular Multiplexer



20 mM MgCl2, 50 mM TRIS pH 8.3 at 37 oC

Penchovsky R. Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics. **ACS Synthetic Biology**, 1, 471-482 (**2012**)

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Two-input multiplexer

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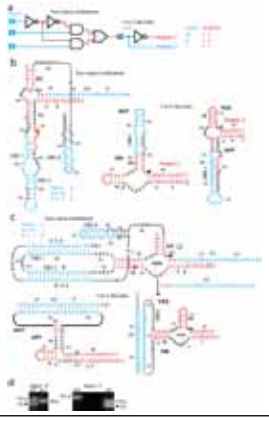


"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Summary

1. Fast, universal, and highly accurate allorithom for design of oligonuleotide-sensing logic gates

2. Design of allosteric ribozymes that sense the length of their substrates

3. We can apply allosteric ribozymes that sense the length of their RNA substrates for detection of TRE disorders

4. Buit integarted three-input AND gate and two-input multiplexer using lengh-sensing allotsric ribozymes

"Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics of rare diseases" by Robert Penchovsky, http://penchovsky.webpages.com/

Many thanks to Professor Ronald Breaker for inspiriting this particular piece of my research

Many thanks to the doctoral students in my laboratory. Thank you for your attention. Any questions?

Some publications of mine on the topic:

1. Penchovsky, R Computational design of allosteric ribozymes as Molecular Biosensors, Biotechnology Advances, 32, 1015-1027 (2014). – Impact factor (IF:11.08)

2. Penchovsky, R. Present and Future RNA-based Approaches to Medical Genomics, International Journal of Genomic Medicine, 10.4172/2332-0672.1000110, 1(2), 1-7 (2013).

3. Penchovsky R. Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics. ACS Synthetic Biology, 1, 471-482 (2012). – (IF: 5.5).

4. Penchovsky R. Chapter 5: Engineering Gene Control Circuits with Allosteric Ribozymes in Human Cells as a Medicine of the Future, in the book "Quality Assurance in Healthcare Service Delivery, Nursing and Personalized Medicine: Technologies and Processes", Publisher IGI Global, DOI: 10.4018/978-1-120-7, 71-96 (2012).

5. Penchovsky, R. & Breaker, R.R. Computational design and experimental validation of oligonucleotide-sensing allosteric ribozymes. Nature Biotechnology, 31, 1424-143 (2005) (IF: 45.2)

PLEVEN REGISTRY OF CONGENITAL ANOMALIES IN THE EUROCAT NETWORK

Katya Kovacheva

JRC - eurocat Central Registry

- The European Union (EU) funded the EUROCAT network since 1979
- The EUROPEAN Commission *Joint Research Centre* /JRC/ and Directorate General *Health and Food Safety* /DG SANTE/ are developing the **EU Platform on Rare Diseases Registration.**
- The ***EUROCAT Central Registry*** (Central Databases and European level coordination activities) was **transferred to the JRC** as part of **the EU Platform on Rare Diseases Registration.**

Today the **JRC - EUROCAT Central Registry** is located in European Commission Joint Research Centre (***Ispra, Italy***)

EUROCAT Overview

- Started in 1979 as an initiative of EUROPEAN Commission
- EUROCAT is a network of population-based registries for the epidemiologic surveillance of congenital anomalies (CAs)
- Covers over 1/3 of European birth (more than 1.7 million births surveyed per year)
- Over 750.000 CA cases
- 55 registries in 33 EU/EEA countries
- High quality multiple source registries, ascertaining CAs in livebirths, stillbirths as well as terminations of pregnancy, following prenatal diagnosis of CA
- EUROCAT acts as WHO Collaborating Centre for the Surveillance of Congenital Anomalies/ www.who.int/genomics

EUROCAT Member Registries / *Membership Criteria*

- **Full Member Registries** transmit *individual data* on all CA cases in their region
- **Associate Member Registries** transmit an *aggregate file* containing the total number of cases in each CA subgroup by type of birth
- **Affiliate Member Registries** *not obligatory but may transmit core data* that may be appended to the registry description on the EUROCAT website.
- ***"World Affiliate"* Registries** are non-European registries that benefit from close relation with the EUROCAT Network , do not transmit data to EUROCAT

** There is no membership fee required*
** The EUROCAT organization itself does not provide funding for the registries in the network*
** The financial support of the registries is provided by different sources: the relevant Ministry of health, Public health organizations, National health programs*

The Objectives of EUROCAT

- *To provide essential epidemiologic information on congenital anomalies* in Europe (prevalence of CAs, perinatal mortality data)
- To facilitate the early warning of new teratogenic exposures
- *To evaluate the effectiveness of primary prevention and develop recommendations* considered for primary prevention in the Rare Diseases National Plans for medicinal drugs, food/nutrition, lifestyle, health services, and environmental pollution.
- *To assess the impact of developments in prenatal screening and prenatal diagnosis of CA*

Pleven Registry of Congenital anomalies, Bulgaria – short description

History and Funding

- **1988 -** Pleven registry is established and has **started collecting data** on congenital anomalies (CAs)
- 1995 - using some of the EUROCAT criteria
- The registry is **not officially supported by the Ministry of Health** and is partially funded by the Medical University of Pleven (MUP).
- **November 2013** – **The Pleven registry is official member of EUROCAT** with initial status of **Affiliate Member**

The Objectives of EUROCAT

- *To act as an information and resource center* for patients, health professionals, policy makers and the general public, regarding clusters or exposures or risk factors
- *To provide a ready collaborative network and infrastructure for research* related to the causes and prevention of congenital anomalies and the treatment and care of affected children
- *To act as a catalyst for the setting up of registries throughout Europe collecting comparable, standardised data.*

Pleven Registry of Congenital anomalies in the EUROCAT network

Population Coverage

- Pleven region is a region in the North Bulgaria with about 248 000 inhabitants (3.4% of the country inhabitants).
- Up to 2006, the registry functions as population-based; the University hospital was the only hospital in the city, covering all deliveries of resident women
- Since 2007, along with University hospital two more delivery hospitals have opened doors in the city and reported data on CAs.
- Over the recent years, there is a trend of decreasing (with 5% for the last 5 yrs) the number of annual births both in Bulgaria and in Pleven region
- At the present time, the three hospitals in the city of Pleven cover about **2 400 annual births**, which is approximately 87% of all births in Pleven region and 3.7% of all births (65 500 for 2016) in Bulgaria. About 19% of all deliveries in these hospitals are of non-residents of the region.

Pleven Registry of Congenital anomalies in the EUROCAT network

Source of Ascertainment

- The registration is active. The ascertainment of the cases with CAs is manly (90% of the cases) based on an active searching / screening (conducted by neonatologists, obstetricians, clinical geneticists) of all births in the covered hospitals.

The registry records all cases of CA /according to EUROCAT criteria/ identified in:

- **Live births** (with birth weight ≥ 500 g)
- **Stillbirths** (with birth weight ≥ 500 g)

(*official statistics of fetal deaths ≥28 weeks gestation until 1992 and ≥24 weeks gestation after 1992), **including late miscarriages (fetal deaths of ≥20 weeks gestation***

- **Terminations of pregnancy**, following prenatal diagnosis of CA

Early fetal deaths before the 20th week of gestation were excluded

Calculation of Prevalence of CA /according to EUROCAT

- EUROCAT prevalence is always cited as per 10,000 births.
- The main types of prevalence that is calculated:
  - Total prevalence
  - Live birth prevalence
  - Fetal death prevalence
  - TOPFA prevalence

Pleven Registry of Congenital anomalies, Bulgaria – short description

Source of Ascertainment */according to EUROCAT criteria*

- Additional sources of information are used
  - hospital records (departments of OBGYN, neonatology and pediatrics),
  - records of pathological meetings,
  - medical genetics/cytogenetic records,
  - stillbirths register.
- In all cases of CAs and death (in perinatal period or later), **the data from pathological examinations were used** for diagnostic validity.
- Maximum age at diagnosis was as follows:
  - for the most cases - **up to the first week after delivery**;
  - for selected malformations (congenital heart defects, urogenital) - **up to the first year of life**;
  - in some (multiple anomalies) cases - **a follow-up was performed**

Pleven Registry of Congenital Anomalies

The main activities of the registry:

1. **Registration of CAs -** based on an active screening of all births in the covered city hospitals. The registration process is *organized* into the following stages:
   - *First stage* – Primary registration by a physician (neonatologist/obstetrician) in the first 7 days after delivery.
   - *Second stage* – Process of setting up the final diagnosis. Additional methods are applied to achieve a specific diagnosis.
   - *Third stage* – Final registration. The final registration is carried out by the clinical geneticist based on the final diagnosis.
2. **Genetic counseling -** provided to the families revealed through the registration of CA
3. **Families follow up** and **application of prenatal diagnosis** in future risk pregnancies.

Pleven Registry of Congenital anomalies in the EUROCAT network

Classification of CA and coding /according to EUROCAT

- **Isolated anomalies** - Cases with "isolated" anomalies are classified by organ system or body region into the following main anomaly subgroup:
  - Neural system
  - Eye
  - Ear, face and neck
  - Congenital heart defects
  - Respiratory
  - Oro-facial clefts
  - Digestive system
  - Abdominal wall defects
  - Urinary
  - Genital
  - Limb

Structure of the Pleven Registry - two sections

- **Registry of congenital anomalies** – database of all registered cases of CAs
  - provides reliable epidemiological information on CAs in Pleven region;
  - gives the opportunity to improve the diagnosis and to study the aetiology of CAs
- **Genetic family registry** - database of families with CAs revealed through the registration process
  - provides competent genetic information on congenital defects
  - ensures long-term support and follow-up of families with CAs
  - enables prenatal diagnosis and prevention of future high risk pregnancies

Pleven Registry of Congenital anomalies in the EUROCAT network

Classification of CA and coding /according to EUROCAT

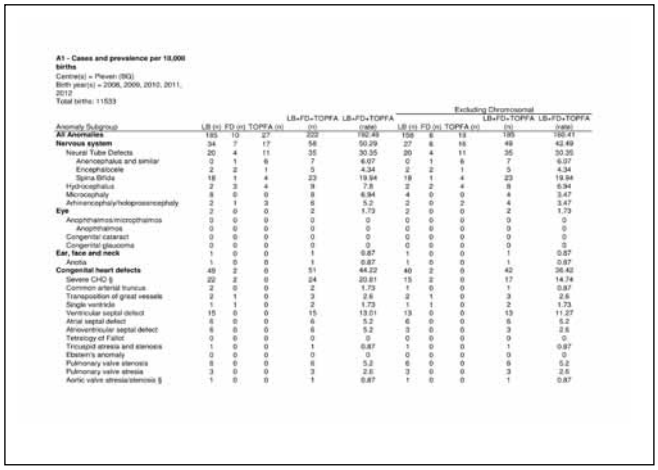
- **Multiple anomalies** – Cases with three or more CAs are classified as "multiple anomalies" into the following main subgroups:
  - **Other anomalies / syndromes:**
    - Skeletal dysplasias – osteochondrodysplasia with defects of growth of tubular bones and spine;
    - Craniosynostosis
    - Congenital constriction bands/amniotic band
    - Situs inversus
    - Conjoined twins
    - Congenital skin disorders
    - Teratogenic syndromes with malformations: Fetal alcohol, Valproate, Maternal infections
    - Genetic syndromes + microdeletions
    - Sequences
  - **Chromosomal**
    - Down syndrome
    - Patau syndrome / trisomy 13
    - Edwards syndrome / trisomy 18
    - Turner
    - Klinefelter
    - Other chromosomal abnormalities

Coding of cases - Guide 1.4 is used to code all cases of congenital anomaly and only ICD10 /with BPA extension/ codes are used .

The Pleven registry of Congenital anomalies - as an Affiliate Member of EUROCAT

- The cases of CA registered from the Pleven registry for the period from 2008 to 2015 year were transmitted to EUROCAT Central Registry
- The data for the period 2008-2012 have been already processed from the Central Registry and is appended to the Pleven registry description on the EUROCAT website

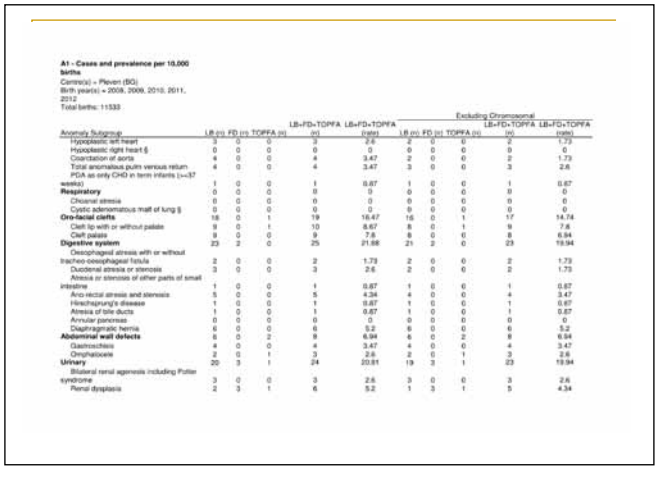
http://www.eurocat-network.eu/content/Reg-Des-Pleven-2008-2012-data.pdf



A1 - Cases and prevalence per 10,000 births

Centre(s) = Pleven (BG)
Birth year(s) = 2008, 2009, 2010, 2011, 2012
Total births: 11533

Anomaly Subgroup	LB (n)	FD (n)	TOPFA (n)	LB+FD+TOPFA (n)	LB+FD+TOPFA (rate)	Excluding Chromosomal LB (n)	FD (n)	TOPFA (n)	LB+FD+TOPFA (n)	LB+FD+TOPFA (rate)
All Anomalies	193	10	27	230	199.49	158	6	18	185	160.41
Nervous system	34	7	17	58	50.29	27	6	16	49	42.49
Neural Tube Defects	20	4	11	35	30.35	20	4	11	35	30.35
Anencephalus and similar	0	1	6	7	6.07	0	1	6	7	6.07
Encephalocele	2	2	1	5	4.34	2	2	1	5	4.34
Spina Bifida	18	1	4	23	19.94	18	1	4	23	19.94
Hydrocephalus	2	3	4	9	7.8	2	2	4	8	6.94
Microcephaly	3	0	0	3	2.6	4	0	0	4	3.47
Arhinencephaly/holoprosencephaly	2	1	3	6	5.2	2	0	2	4	3.47
Eye	2	0	0	2	1.73	2	0	0	2	1.73
Anophthalmos/microphthalmos	0	0	0	0	0	0	0	0	0	0
Anophthalmos	0	0	0	0	0	0	0	0	0	0
Congenital cataract	0	0	0	0	0	0	0	0	0	0
Congenital glaucoma	0	0	0	0	0	0	0	0	0	0
Ear, face and neck	1	0	0	1	0.87	1	0	0	1	0.87
Anotia	1	0	0	1	0.87	1	0	0	1	0.87
Congenital heart defects	49	2	0	51	44.22	40	2	0	42	36.42
Severe CHD §	22	2	0	24	20.81	15	2	0	17	14.74
Common arterial truncus	2	0	0	2	1.73	1	0	0	1	0.87
Transposition of great vessels	2	1	0	3	2.6	2	1	0	3	2.6
Single ventricle	1	1	0	2	1.73	1	1	0	2	1.73
Ventricular septal defect	15	0	0	15	13.01	13	0	0	13	11.27
Atrial septal defect	6	0	0	6	5.2	6	0	0	6	5.2
Atrioventricular septal defect	6	0	0	6	5.2	3	0	0	3	2.6
Tetralogy of Fallot	0	0	0	0	0	0	0	0	0	0
Tricuspid atresia and stenosis	1	0	0	1	0.87	1	0	0	1	0.87
Ebstein's anomaly	0	0	0	0	0	0	0	0	0	0
Pulmonary valve stenosis	6	0	0	6	5.2	6	0	0	6	5.2
Pulmonary valve atresia	3	0	0	3	2.6	3	0	0	3	2.6
Aortic valve atresia/stenosis §	1	0	0	1	0.87	1	0	0	1	0.87



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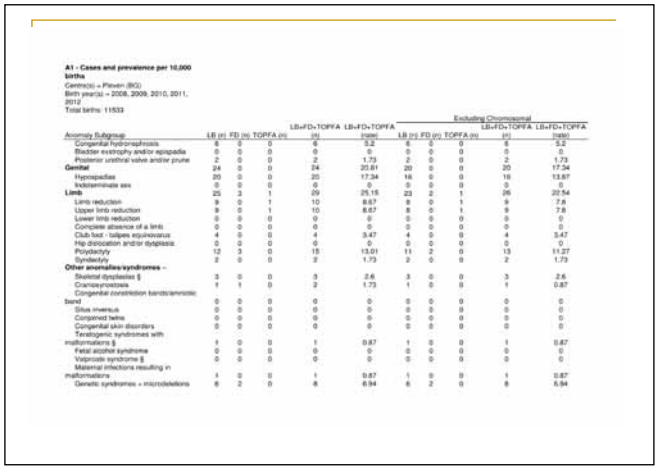
Hypoplastic left heart	3	0	0	3	2.6	2	0	0	2	1.73
Hypoplastic right heart §	0	0	0	0	0	0	0	0	0	0
Coarctation of aorta	4	0	0	4	3.47	2	0	0	2	1.73
Total anomalous pulm venous return	4	0	0	4	3.47	3	0	0	3	2.6
PGA as only CHD in term infants (>=37 weeks)	1	0	0	1	0.87	1	0	0	1	0.87
Respiratory	0	0	0	0	0	0	0	0	0	0
Choanal atresia	0	0	0	0	0	0	0	0	0	0
Cystic adenomatous malf of lung §	0	0	0	0	0	0	0	0	0	0
Oro-facial clefts	18	0	1	19	16.47	16	0	1	17	14.74
Cleft lip with or without palate	9	0	1	10	8.67	8	0	1	9	7.8
Cleft palate	8	0	0	9	7.8	8	0	0	8	6.94
Digestive system	23	2	0	25	21.68	21	2	0	23	19.94
Oesophageal atresia with or without tracheo-oesophageal fistula	2	0	0	2	1.73	2	0	0	2	1.73
Duodenal atresia or stenosis	3	0	0	3	2.6	2	0	0	2	1.73
Atresia or stenosis of other parts of small intestine	1	0	0	1	0.87	1	0	0	1	0.87
Ano-rectal atresia and stenosis	5	0	0	5	4.34	4	0	0	4	3.47
Hirschsprung's disease	1	0	0	1	0.87	1	0	0	1	0.87
Atresia of bile ducts	1	0	0	1	0.87	1	0	0	1	0.87
Annular pancreas	0	0	0	0	0	0	0	0	0	0
Diaphragmatic hernia	6	0	0	6	5.2	6	0	0	6	5.2
Abdominal wall defects	6	0	2	8	6.94	6	0	2	8	6.94
Gastroschisis	4	0	0	4	3.47	4	0	0	4	3.47
Omphalocele	2	0	1	3	2.6	2	0	1	3	2.6
Urinary	20	3	1	24	20.81	19	3	1	23	19.94
Bilateral renal agenesis including Potter syndrome	3	0	0	3	2.6	3	0	0	3	2.6
Renal dysplasia	2	3	1	6	5.2	1	3	1	5	4.34



A1 - Cases and prevalence per 10,000 births

Centre(s) = Pleven (BG)
Birth year(s) = 2008, 2009, 2010, 2011, 2012
Total births: 11533

Anomaly Subgroup	LB (n)	FD (n)	TOPFA (n)	LB+FD+TOPFA (n)	LB+FD+TOPFA (rate)	Excluding Chromosomal LB (n)	FD (n)	TOPFA (n)	LB+FD+TOPFA (n)	LB+FD+TOPFA (rate)
Congenital hydronephrosis	6	0	0	6	5.2	6	0	0	6	5.2
Bladder exstrophy and/or epispadia	0	0	0	0	0	0	0	0	0	0
Posterior urethral valve and/or prune	2	0	0	2	1.73	2	0	0	2	1.73
Genital	24	0	0	24	20.81	20	0	0	20	17.34
Hypospadias	20	0	0	20	17.34	16	0	0	16	13.87
Indeterminate sex	0	0	0	0	0	0	0	0	0	0
Limb	23	3	1	29	25.15	23	2	1	26	22.54
Limb reduction	9	0	1	10	8.67	8	0	1	9	7.8
Upper limb reduction	9	0	1	10	8.67	8	0	1	9	7.8
Lower limb reduction	0	0	0	0	0	0	0	0	0	0
Complete absence of a limb	0	0	0	0	0	0	0	0	0	0
Club foot - talipes equinovarus	4	0	0	4	3.47	4	0	0	4	3.47
Hip dislocation and/or dysplasia	0	0	0	0	0	0	0	0	0	0
Polydactyly	12	3	0	15	13.01	11	2	0	13	11.27
Syndactyly	2	0	0	2	1.73	2	0	0	2	1.73
Other anomalies/syndromes -										
Skeletal dysplasias §	3	0	0	3	2.6	3	0	0	3	2.6
Craniosynostosis	1	1	0	2	1.73	1	0	0	1	0.87
Congenital constriction bands/amniotic band	0	0	0	0	0	0	0	0	0	0
Situs inversus	0	0	0	0	0	0	0	0	0	0
Conjoined twins	0	0	0	0	0	0	0	0	0	0
Congenital skin disorders	0	0	0	0	0	0	0	0	0	0
Teratogenic syndromes with malformations §	1	0	0	1	0.87	1	0	0	1	0.87
Fetal alcohol syndrome	0	0	0	0	0	0	0	0	0	0
Valproate syndrome §	0	0	0	0	0	0	0	0	0	0
Maternal infections resulting in malformations	1	0	0	1	0.87	1	0	0	1	0.87
Genetic syndromes + microdeletions	6	2	0	8	6.94	6	2	0	8	6.94

Future challenges

- Improving of case ascertainment quality and attracting more specialists /neonatologists, obstetricians/ to engage in registration process
- Expanding of Pleven registry network, by inclusion of other delivery hospitals from Pleven region to participate in the registration of CA
- These actions will give the Pleven registry a better opportunity to become a population-based, as well as to change its member status and to candidate for an Associate member of EUROCAT

GENETIC STUDY OF ACHONDROPLASIA IN SERBIAN POPULATION

Sara Radunović, Valerija Dobričić, Ana Marjanović, Marija Branković, Ivana Joksić, Olivera Kontić, Goran Čuturilo, Ivana Novaković, Vladimir Kostić

GENETIC COUNSELING

- **AD** manner
- heterozygous with **100%** penetrance
- **80%** parents with average stature
- **20%** at least one parent with achondroplasia
- homozygous – **lethal**

- Parents with average stature: low risk
- Parents with average stature and one affected child: some reported cases presumably due to gonadal mosaicism
- One affected parent: 50% with each pregnancy
- Both affected: 25% of a child with average stature

Caesarean section in both affected mothers or mothers carrying an affected child

WHAT IS ACHONDROPLASIA?

- most common form of disproportionate small stature
- autosomal dominant
- **80% *de novo*** mutations

- heterozygous mutation p. G380R (c. 1138G>A) in FGFR3 gene

- pathogenic FGFR3 variant → phenotype
- short limbs, normal trunk and large head

- prevalence 1:26 000 – 1:28 000 live births

- several genetically related disorders
- mutations in FGFR3 → different types of skeletal dysplasia: hypochondroplasia, SADDAN syndrome, thanatrophic dysplasia

PATIENTS IN OUR STUDY

15 cases suspected of achondroplasia- like short stature
9 postnatally and **6** prenatal

Prenatal:

- 1 case of affected mother (c. 1138G>A confirmed)
- 5 cases suspected because of FGR or uncertainty in fetal growth seen by ultrasound

Postnatal:

- Clinically suspected of achondroplasia- like short stature

FGFR3 gene

- locus 4p16.3
- 19 exons
- FGFR family
- AA sequence highly conserved among divergent species

FGFR3 bind FGF's and slows down bone growth
Mutation in FGFR3 → **CONSTITUTIVELY ACTIVE**

FGFR3 mutations in achonroplasia:

- c. 1138G>A in 98% of cases
- c. 1138G>C in 1% of cases

Both result in amino acid change **p. G380R**

MATERIAL AND METHODS

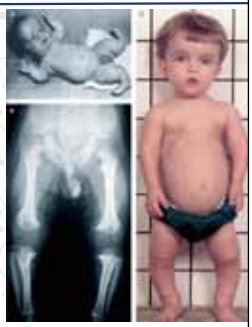
blood and amniotic fluid sampling

DNA extraction

direct Sanger sequencing (ABI3500 Genetic analyzer)

DIAGNOSIS

- based on clinical and x- ray findings
- molecular genetic testing

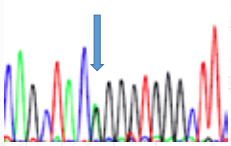


MOLECURAL GENETIC TESTING
Prenatal and postnatal

1. Targeted analysis of pathogenic variants
2. Sequence analysis of selected exons
3. Sequence analysis

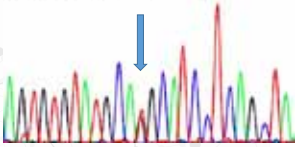
RESULTS

5/15 cases confirmed with FGFR3 mutation



c. 1138G>A (p. G380R)

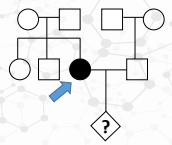
3 postnatal and 1 prenatal



c. 1123G>T (p. G375C)

1 prenatal

AFFECTED MOTHER



No mutation found in prenatal testing of affected mother – healthy child born

THANK YOU FOR YOUR ATTENTION

CONCLUSION

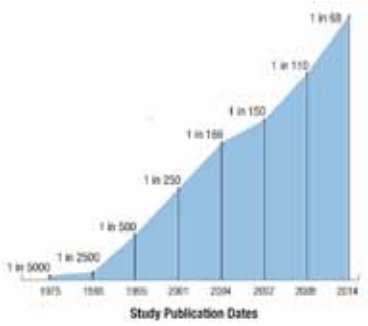
- **80%** of patients affected with achondroplasia **p. G380R**
- 1 case of very rare **p. G375C** mutation
- heterogenous genetic base of achondroplasia in Serbian population
- whole sequence analysis of the **exon 9** of the FGFR3 gene

DYSREGULATED PATHWAYS IN AUSTIM SPECTRUM DISORDER

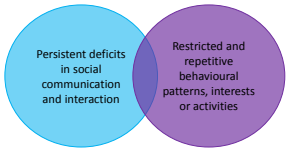
H. Ivanov, N. Popov, T. Vachev, I. Pacheva, R. Yordanova. I. Ivanov, V. Stoyanova

Epidemiology

ASD is diagnosed in all racial, ethnic and socioeconomic groups, five times more common in boys than in girls



DSM 5 - Autism Spectrum Disorder



Persistent deficits in social communication and interaction:
- deficits in social-emotional reciprocity
- deficits in nonverbal communicative behaviours
- deficits in developing/maintaining relationships

Restricted and repetitive behavioural patterns or interests
- excessive devotion to routines or extreme resistance to change
- highly fixated and/or abnormal interests.
- repetitive speech, movements or use of objects
- hyper- or hypo-reactive responses to sensory input or unusual interest in visual environmental cues

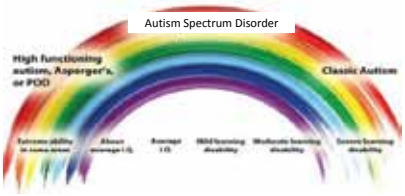
Etiology

- Using twin studies with homozygous twins, many researchers now believe the main cause of autism is genetic
- Hundreds of diverse ASD susceptibility genes have been identified, yet none of the mutations found account for more than a small subset of ASD cases.
- Environmental effects on the prenatal fetus including exposure to: valproic acid, thalidomide, alcohol, cocaine, toxic metals, air chemicals may also have effect by changing gene expression

It is hypothesized that different genetic variants (sequences or structural variations) or epigenetic modifications, each rare in the cases of ASD, may disrupt the function of a major group of genes, which in turn is sufficient to induce ASD.

DSM 5 - Autism Spectrum Disorder

- Onset during early childhood
- Signs and symptoms fall within a continuum ranging from severe to less severe impairments.
- Significant impairment
- Heterogeneous phenotypes (and many comorbidities)



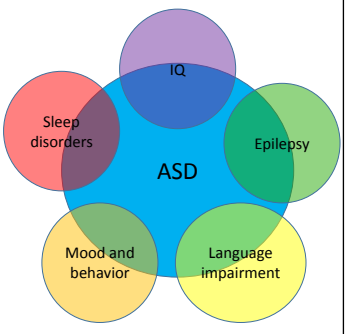
So... ASD has:

- many etiologies
- many comorbidities
- heterogeneous clinical picture
- different levels of severity

Could there be one or more mechanisms to explain these aspects of ASD?

.....and many comorbidities

- Intellectual deficiency from light to severe is observed in 70% of individuals with ASD
- Epilepsy in 10-30%; EEG changes at ~ 60%
- ~ 30% with minimal speech
- Depression, anxiety, irritability, ADHD
- ~ 80% insomnia



Although the number of studies attempting to integrate genetic discoveries in ASD is considerably smaller than studies aimed at looking for altered genes, we believe that through an integrative functional analysis of the genetic changes we can find common pathways and mechanisms underlying in the etiopathogenesis of ASD.

To identify biological pathways and gene profiles associated with ASD, we used differentially expressed genes sets from 16 expression studies after 2011 (one of which ours) and the targets of differentially expressed micro RNA from two our studies.

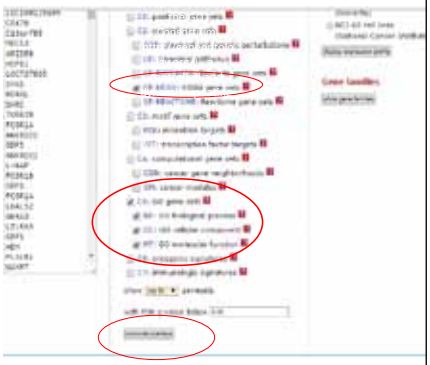
Tissue	Study	Number of samples	Dysregulated genes
Brain Tissue	James et al., 2014	13 ASD 13 Controls	7 genes
Brain Tissue	Anitha et al., 2012	9 ASD 8 Controls	36 genes
Brain Tissue	Chow et al., 2012	15 ASD 18 Controls	200 genes
Brain Tissue	Ginsberg et al., 2012	9 ASD 9 Controls	70 genes
Brain Tissue	Chana et al., 2015	27 ASD 30 Controls	3 genes
Brain Tissue	Khan et al., 2014	10 ASD 11 Controls	12 genes
Brain Tissue	Voineagu et al. 2011	19 ASD 17 Controls	444 genes

Tissue	Study	Number of samples	Dysregulated genes
Peripheral blood	Иванов и сътр. 2015	50 ASD 30 Controls	23 genes
Peripheral blood	Kong et al., 2013	20 ASD 20 unaffected sibs 18 Controls	145 genes
Peripheral blood	Kuwano et al., 2011	21 ASD 21 Controls	19 genes
Peripheral blood	Segura et al., 2015	21 adults with ASD 10 Controls	3 genes
Peripheral mononuclear cells	Glatt et al., 2012	60 ASD 68 Controls	154 genes
Lymphoblast cell lines	Chien et al., 2013	16 ASD 16 Controls	202 genes
Lymphoblast cell lines	Talebizadeh et al., 2014	5 ASD 5 Controls	57 genes
Nasal olfactory stem cells	Féron et al., 2016	11 ASD 11 Controls	156 genes
Hair follicles	Maekawa et al., 2015	18 ASD 24 Controls	1 genes

We applied the "Compute overlaps" function from the MSigDB website, an analysis that uses the hypergeometric distribution to indentify how the gene sets overlap

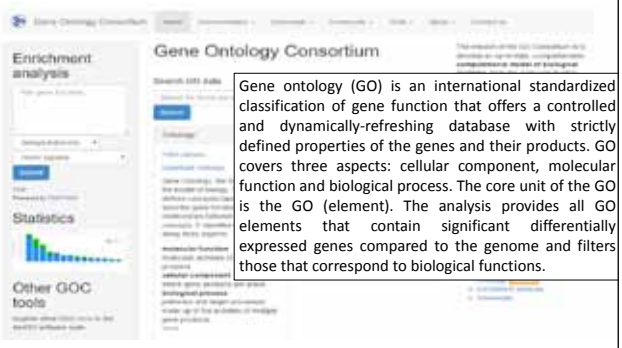
We used the MSigDB collections obtained from the KEGG Biological Road Database (http://www.genome.jp/kegg/pathway.html)

We also used and the GO Consortium (http://geneontology.org/) - a controlled and dynamic-updating database with strictly defined properties of the genes and their products.



The differentially expressed genes sets were analyzed by the freely available Molecular Signatures Database software, MSigDB v4.0 (http://www.broadinstitute.org/gsea/msigdb/index.jsp). MSigDB is a collection of annotated gene sets for use with the Gene Set Enrichment Analysis (GSEA) software.

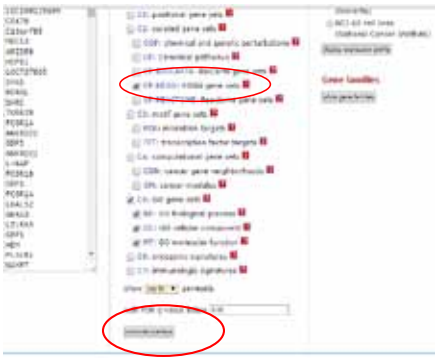
As an identifier for the analyzed genes we used gene symbol.



Gene ontology (GO) is an international standardized classification of gene function that offers a controlled and dynamically-refreshing database with strictly defined properties of the genes and their products. GO covers three aspects: cellular component, molecular function and biological process. The core unit of the GO is the GO (element). The analysis provides all GO elements that contain significant differentially expressed genes compared to the genome and filters those that correspond to biological functions.

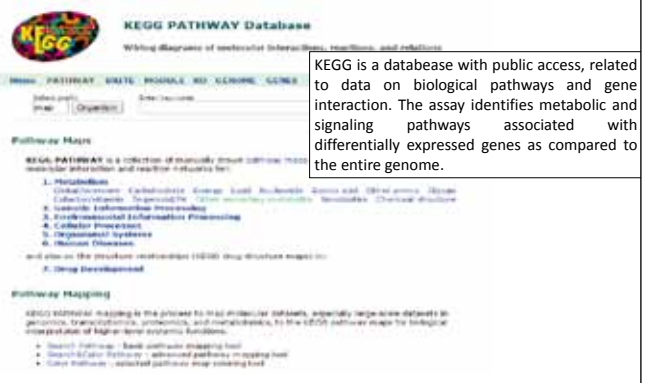
We applied the "Compute overlaps" function from the MSigDB website, an analysis that uses the hypergeometric distribution to indentify how the gene sets overlap

We used the MSigDB collections obtained from the KEGG Biological Road Database (http://www.genome.jp/kegg/pathway.html)



Gene Set Name	# Genes in Gene Set (K)	Description	# Genes in Overlap (k)	k/K	p-value	FDR q-value
KEGG_PATHWAYS_IN_CANCER	328	Pathways in cancer	73	0.2226	1.41E-31	2.62E-29
KEGG_MAPK_SIGNALING_PATHWAY	267	MAPK signaling pathway	59	0.2210	1.31E-25	1.22E-23
KEGG_FOCAL_ADHESION	201	Focal adhesion	50	0.2488	2.19E-24	1.36E-22
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	216	Regulation of actin cytos				
KEGG_CHEMOKINE_SIGNALING_PATHWAY	190	Chemokine signaling pat				
KEGG_HUNTINGTONS_DISEASE	185	Huntington's disease				
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	272	Neuroactive ligand-rece				
KEGG_OXIDATIVE_PHOSPHORYLATION	135	Oxidative phosphorylatio				
KEGG_PARKINSONS_DISEASE	133	Parkinson's disease				
KEGG_ALZHEIMERS_DISEASE	169	Alzheimer's disease				
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	267	Cytokine-cytokine receptor interaction	45	0.1685	3.75E-15	6.33E-14
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	97	Fc gamma R-mediated phagocytosis	26	0.2680	3.26E-14	5.05E-13
KEGG_CALCIUM_SIGNALING_PATHWAY	178	Calcium signaling pathway	34	0.1910	2.08E-13	2.97E-12
KEGG_WNT_SIGNALING_PATHWAY	151	Wnt signaling pathway	29	0.1921	1.03E-11	1.33E-10
KEGG_ERBB_SIGNALING_PATHWAY	87	ErbB signaling pathway	22	0.2529	1.07E-11	1.33E-10
KEGG_CELL_ADHESION_MOLECULES_CAMS	134	Cell adhesion molecules (CAMs)	27	0.2015	1.64E-11	1.91E-10
KEGG_SMALL_CELL_LUNG_CANCER	84	Small cell lung cancer	21	0.2500	1.98E-11	4.36E-10
KEGG_ENDOCYTOSIS	183	Endocytosis	31	0.1694	5.95E-11	6.15E-10
KEGG_TIGHT_JUNCTION	134	Tight junction	26	0.1940	8.38E-11	9.18E-10
KEGG_JAK_STAT_SIGNALING_PATHWAY	155	Jak-STAT signaling pathway	27	0.1742	5.27E-10	4.9E-9

Intriguing and surprising is that most of the overlapping genes are linked to cancer pathways. This may be due to the fact that these are multifunctional genes associated with both cancer and the development and diseases of the nervous system.



KEGG is a database with public access, related to data on biological pathways and gene interaction. The assay identifies metabolic and signaling pathways associated with differentially expressed genes as compared to the entire genome.

Cancer pathway

Like cancers, “autisms” are best conceptualized in the plural. ASD encompasses a broad range of putative causes, symptom presentations, and outcomes, including both macrocephaly and microcephaly, suggesting deficits in the cellular commitment to proliferation versus differentiation, similar to cancer. This difference may be in the life stage of cellular proliferation. Errors associated with genome maintenance during fetal life may occur at critical time periods for proliferation of neuronal precursors that affect prenatal brain development, resulting in neurodevelopmental disorders, whereas errors more commonly occur during adult life in cell types susceptible to tumors (Crawley 2016).

Cancer pathway

The functional overlap of genes and pathways between autism and cancer would suggest that individuals with autism may carry a higher cancer risk. While there is some epidemiological evidence of higher cancer risk in children, adolescents, and young adults with ASD, the absolute number of cases is low and more studies need to be conducted, particularly in adults, as cancer incidence is significantly correlated with age (Crawley, 2016).

MAPK signaling pathway

The RAS / MAPK (mitogen-activated protein kinase) pathway mediates the transmission of signals from cell surface receptors to their cytoplasmic and nuclear targets.

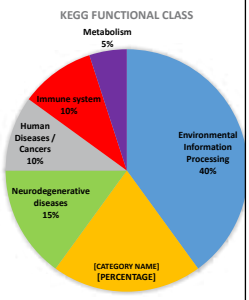
This pathway mediates various cellular functions such as proliferation, migration, differentiation and cell survival (Ebisuya 2005).

In the nervous system, this pathway is further involved in a diverse spectrum of activity-dependent neuronal activities such as synaptic plasticity, long-term potentiation and suppression (LTP and LTD), and memory formation (Satoh 1999).

Grouping of the enriched pathways

KEGG functional class

Environmental Information Processing
Environmental Information Processing; Signal transduction
MAPK signaling pathway; Calcium signaling pathway; Wnt signaling pathway; ErbB signaling pathway; Jak-STAT signaling pathway
Environmental Information Processing; Signaling molecules and interaction
Neuroactive ligand-receptor interaction; Cytokine-cytokine receptor interaction; Cell adhesion molecules (CAMs)
Cellular Processes/Transport
Focal adhesion; Regulation of actin cytoskeleton; Endocytosis; Tight junction
Neurodegenerative diseases
Huntington's disease; Parkinson's disease; Alzheimer's disease
Human Diseases / Cancers
Pathways in cancer; Small cell lung cancer
Immune system
Chemokine signaling pathway; Fc gamma R-mediated phagocytosis
Metabolism
Oxidative phosphorylation



MAPK signaling pathway

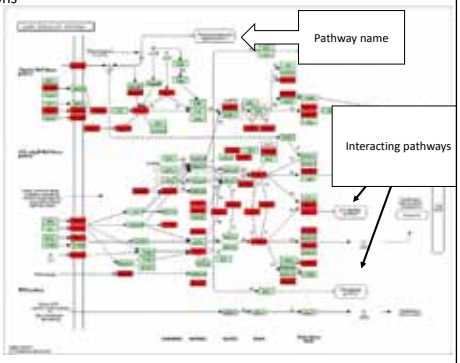
In several CNVs that are associated with ASDs - deletions 16p11.2 and duplications 7q11.23 and 22q11.2, are detected genes associated with Ras / MAPK-dependent signaling (Samuels, 2009).

Mutations in genes that have links to Ras / MAPK signaling are found in three of the syndromes most frequently accompanied by a diagnosis of autism - fragile X syndrome, tuberous sclerosis and Smith-Lemli-Opitz syndrome (Adviento, 2014).

These data indicate that dysregulation in Ras / MAPK signaling may play role in the pathophysiology of both "syndrome" and "non-syndromes" forms of ASD.

Pathway-pathway interactions

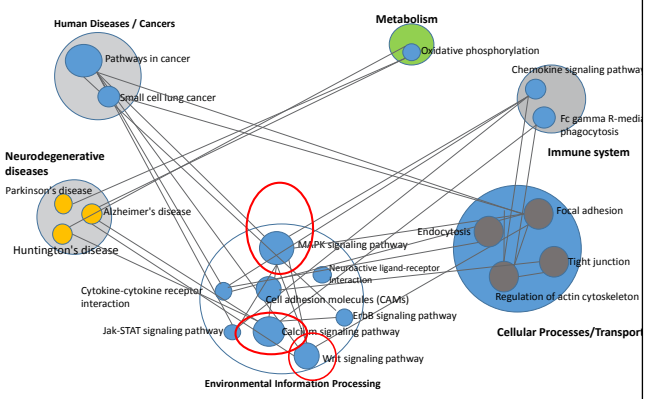
Pathway-Pathway interactions are presented in KEGG maps by displaying the name of the interacting paths in that map



MAPK signaling pathway

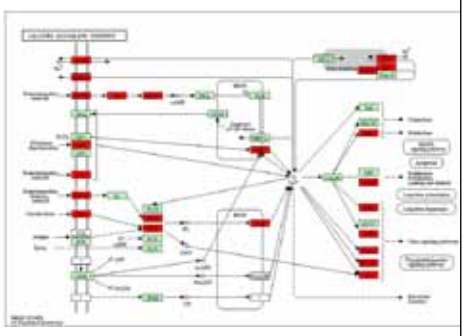
To investigate the role of the RAS / MAPK / ERK pathway in cell proliferation in neurons, Yang et al (Yang, 2012) used cultured cortical neurons overexpressing c-Raf. They observed damage to differentiation and maturation of nerve cells and unchanged apoptosis in up regulation of MAPK / Erk (Yang, 2012). Other studies, however, suggest that the down-regulation of the MAPK / Erk pathway may disrupt the proliferation and maturation of neuronal progenitor cells (Menard, 2002; Li, 2012; Pucilowska, 2012).It is possible that any change in the homeostatic balance in this path changes the cognition and behavior.

A study of Faridar et al. (Faridar, 2014) of a ASD mouse model indicate that the rate of activation of the MAPK / Erk pathway in the developing brain correlates directly by comparing brain tissue with peripheral lymphocytes. This suggests that measuring the activity of this pathway in peripheral lymphocytes can serve as a surrogate marker for MAPK / Erk activity in the central nervous system and possibly be used as a biomarker for ASD



Calcium signaling pathway

Calcium ions (Ca 2+) affect almost all processes in the cell. Calcium signaling is involved in various intracellular activities



Calcium signaling pathway

Disturbance of the Calcium signaling may contribute to the development of ASD. In neurons, voltage-dependent calcium channels regulate (through transcription) multiple cortical excitatory synapses (Shalizi, 2006). Changed calcium signaling may be a major factor in impaired synaptogenesis and thus leading to ASD. (Casanova, 2007; Pickett, 2005). Defects in the calcium signaling may also be responsible for disturbances in the excitatory / inhibition balance in the neuronal chains of GABA / glutamatergic synapses.

Wnt signaling pathway

Wnt signal proteins are present in hippocampal synapses and alter synaptic plasticity and long-term potentiation (activity dependent synaptic plasticity that is believed to be involved in memory formation) (Chen, 2006). β-catenin functions as a major effector molecule in the Wnt signal cascade and plays a role in the formation and stabilization of synapses. (Maguschak, 2012).In the nucleus, β-catenin interacts with transcription factors and activates the chain of target genes, including **FOSL1** (FRA-1), which are mainly related to the regulation of cell proliferation.

Given that the Wnt / β-katenin pathway is involved in wide range of processes its impaired regulation can have any number of negative effects on neuronal development and thus contribute to the pathogenesis of neurodevelopmental disorders such as ASD.

Calcium signaling pathway

Changed Ca2 + signaling in the pathogenesis of ASD is confirmed by several different studies. Functional mutation in the CACNA1C gene encoding L-type voltage-dependent Ca2 + channels - Cav1.2 causes Timothy's syndrome, with arrhythmias, webbing or fusion of the skin between some fingers, congenital heart abnormalities and autism spectrum symptoms (Splawski I). Similarly mutations in the L-type voltage-dependent Ca2 + channels - Cav1.4 (CACNA1F), induce an incomplete form of X-linked congenital stationary night blindness. Mutations with gain of function results in mental retardation and autism with or without epilepsy, while mutations with loss of function are not accompanied by similar symptoms (Hope CI, 2005).
All of these gain of function mutations result in the inability of inactivating Ca2 + -dependent channels and in excessive influx of Ca2 + into the cell.

Wnt signaling pathway

In a recent study of ASD mouse models induced by prenatal hypoxia, Wei et al. (Wei, 2016) detected reduced expression of FOSL1 and down-regulation of Wnt / β-catenin signaling in the hippocampus of adolescent mice with behavioral problems that have suffered prenatal hypoxia.

Their study shows how some environmental factors, by altering gene expression, are involved in the pathogenesis of ASD.

Calcium signaling pathway

Mutations indirectly, providing elevated calcium levels in the cell either by increased Ca2 + influx or by suppressing feedback mechanisms, have been found in patients with ASD (Laumonnier F, 2006).

Palmieri et al. report increased levels of calcium concentration in neocortical brain tissue (post mortem) in six patients with ASD compared to controls (Palmieri, 2010).

This evidence points to the role of calcium signaling in the pathogenesis of ASD, as well as a common link between "syndrome" and "non-syndromic" forms of ASD.

There is also a number of evidence that Ca2 + signaling directly and indirectly affects MAPK signaling pathways (White, 2010)

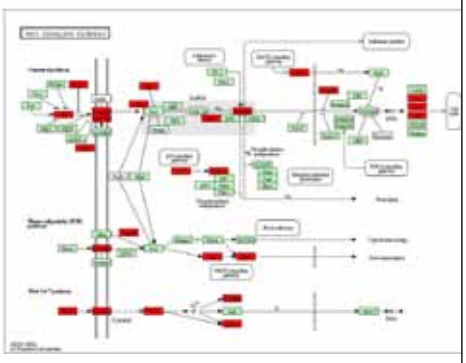
GO biological process

Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value
GO_CELLULAR_RESPONSE_TO_ORGANIC_SUBSTANCE	1848	332	0.1797	1.43E-115	8.45E-112
GO_RESPONSE_TO_EXTERNAL_STIMULUS	1821	318	0.1746	5.87E-107	1.74E-103
GO_REGULATION_OF_MULTICELLULAR_ORGANISMAL_DEVELOPMENT	1672	301	0.1800	1.8E-104	3.54E-101
GO_RESPONSE_TO_ENDOGENOUS_STIMULUS	1450				
GO_POSITIVE_REGULATION_OF_MULTICELLULAR_ORGANISMAL_PROCESS	1395				
GO_POSITIVE_REGULATION_OF_RESPONSE_TO_STIMULUS	1929				
GO_NEURON_PART	1265				
GO_REGULATION_OF_CELL_PROLIFERATION	1496				
GO_REGULATION_OF_CELL_DIFFERENTIATION	1492				
GO_REGULATION_OF_CELL_DEATH	1472				
GO_CELL_PROJECTION	1786				
GO_REGULATION_OF_TRANSPORT	1804				
GO_IMMUNE_SYSTEM_PROCESS	1984				
GO_CELL_JUNCTION	1151				
GO_ENZYME_BINDING	1737				
GO_PROTEIN_LOCALIZATION	1805				
GO_RESPONSE_TO_OXYGEN_CONTAINING_COMPOUND	1381	239	0.1731	1.25E-78	4.36E-76
GO_POSITIVE_REGULATION_OF_DEVELOPMENTAL_PROCESS	1142	216	0.1891	3.02E-78	9.93E-76
GO_NEUROGENESIS	1402	239	0.1705	2.87E-77	8.92E-75
GO_REGULATION_OF_IMMUNE_SYSTEM_PROCESS	1403	239	0.1703	3.32E-77	9.82E-75

Our analysis shows that differentially expressed genes participate in processes related to:
- response to various stimuli and cellular signaling;
- cellular proliferation, differentiation, and death;
- neurogenesis;
- the immune system and its regulation.

Wnt signaling pathway

It is concidered that the Wnt signaling pathway plays a critical role in the processes of cell proliferation, differentiation and apoptosis of the cells in the central nervous system during embryonic development (Ciani, 2005)



GO cellular component

Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value
GO_NEURON_PART	1265	255	0.2016	3.11E-99	1.8E-96
GO_CELL_PROJECTION	1786	289	0.1618	1.84E-88	5.35E-86
	1151	221	0.1920	2.13E-81	4.11E-79
	754	172	0.2281	4.38E-75	5.14E-73
	942	192	0.2038	4.43E-75	5.14E-73
	610	145	0.2377	9.08E-66	8.78E-64
	1259	207	0.1644	6.41E-64	5.31E-62
	1134	194	0.1711	1.04E-62	7.55E-61
	1376	211	0.1533	7.93E-60	5.11E-58
ASMA_MEMBRAN	1649	231	0.1401	2.83E-58	1.64E-56
GO_SOMATODENDRITIC_COMPARTMENT	650	139	0.2138	5.57E-57	2.94E-55
GO_MITOCHONDRION	1633	226	0.1384	4.83E-56	2.33E-54
GO_PLASMA_MEMBRANE_REGION	929	157	0.1690	5.54E-50	2.47E-48
GO_MEMBRANE_PROTEIN_COMPLEX	1020	165	0.1618	7.17E-50	2.97E-48
GO_CYTOSKELETON	1967	234	0.1190	2.14E-46	8.27E-45
GO_CELL_PROJECTION_PART	946	152	0.1607	1.4E-45	5.06E-44
GO_ENVELOPE	1090	164	0.1505	2.64E-45	9.00E-44
GO_POSTSYNAPSE	378	94	0.2487	1.13E-44	3.63E-43
GO_VACUOLE	1180	165	0.1398	2.2E-41	6.71E-40
GO_CELL_BODY	494	103	0.2085	2.35E-41	6.81E-40

Most of the differentially expressed genes are involved in cellular components associated with the:
- neuron,
- cellular projections,
- cellular connections
- and synapse

Summary

We evaluated the paths with the most overlapping differentially expressed genes, and then we generated a system (network) of biological pathways in which the gene products are involved.

Integrative analysis of genetic changes and networks of biological pathways helps to expand our understanding not only for individual genes but also for the function of the gene products and the network of their reactions and interactions.

Conclusion

In conclusion, the differentially expressed genes are involved in major kinase and / or signaling pathways. The results of the expression studies support the involvment of various genetic factors (heterogeneity) in the development of ASD that are participate in the same major pathways associated with environmental information processing and signal transduction, cell differentiation, cell survival, developmental processes, oxidative stress, immune processes and synaptic structure and plasticity. These results support the idea that in the genetic basis of ASD are pleiotropic (multifunctional) genes associated with important, key biological pathways.

From multiple genes to common biological processes

Genes do not work on their own, but in groups (pathways). We need to be aware that the phenotype is determined by the function of the gene products in the biological pathways. So we have to have a look at the whole picture, the whole "network map".

Biological pathways are like "streets in the city" (each cell is like a small town on its own).Molecules and other gene products move through the "city" in "streets". "Traffic jam" in key processes can easily affect many other processes, not just the products of one or several genes.

These processes can be affected when main pathways do not function well. In this way, disturbance of a major pathway can lead to a spectrum of symptoms. If we can overcome this "bottleneck", we can fix many problems, all at the same time.

Thank You for your attention

"Science is nothing but a series of questions that lead to more questions."
Terry Pratchett, The Long Earth

The results of the expression studies, including ours, confirm the hypothesis that, despite the wide variety of genetic changes detected in the ASD, they all eventually convert to major signaling pathways (Murdoch, 2013). It is worth noting that bioinformatic pathways analysis is still limited by current knowledge of signaling pathways and molecular interactions, and thus the identification of different pathways from different studies may partially reflect the still uncharacterized complexity of molecular signaling pathways (Voineagu, 2013).

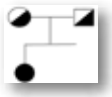
GENETICALLY PROVEN CASES OF WOLMAN DISEASE IN BULGARIA AND MUTATION SCREENING OF TWO PRESUMABLE ENDEMIC REGIONS

Angelina Mandadzhieva, Daniela Avdzhieva-Tzavella, Tihomir Todorov, Savina Tincheva, Vanya Sinigerska, Mariya Ivanova, Alexey Savov, Vanyo Mitev, Albena Todorova

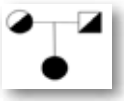
Materials and Methods

Two unrelated families:

Two unrelated families from Bulgarian origin were referred for genetic testing of the LIPA gene.



- DNA isolation
- PCR amplification
- Sanger sequencing



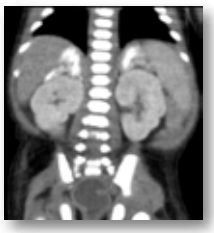
Selective screening:

- Direct PCR amplification on dry blood spots from Guthrie cards
- Sanger sequencing

Wolman disease

Clinical picture:

- Hepatosplenomegaly;
- Abdominal distention;
- Adrenal calcification;
- Hypotonia;
- Decreased body temperature



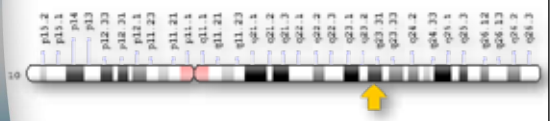
Clinical symptoms of the patients

- Anemia
- Muscle hypotrophy
- Low weight gain
- Hepatospenomegaly and hepatocytolysis
- Watery stools
- Adrenal glands calcification

The enzyme levels of acid lipase were reduced: 12.8 *nmolMU/h/mg* and 38 *nmolMU/h/mg*

LIPA gene

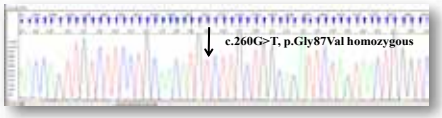
- Localized on chromosome 10 (10q23.31)
- 10 exons, 9 coding



Results

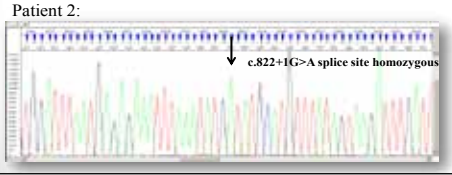
Missense c.260G>T, p.Gly87Val mutation in LIPA gene – exon 4

Patient 1:

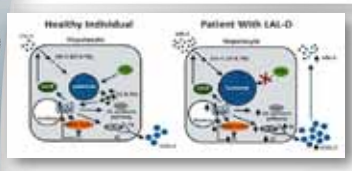


Splice site c.822+1G>A mutation in LIPA gene – intron 7

Patient 2:

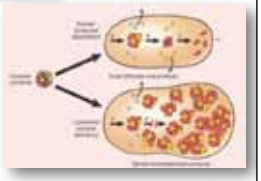


Lysosomal acid lipase



Reference values - 210-530 *nmolMU/h/mg*

Reduced content or total absence of enzyme activity - cholesterol esters and triglycerides do not degrade and accumulate in lysosomes.



Considering the high genetic heterogeneity of the Bulgarian population, it is extremely atypical to detect homozygous mutations in rare recessive conditions

Two possible explanation:

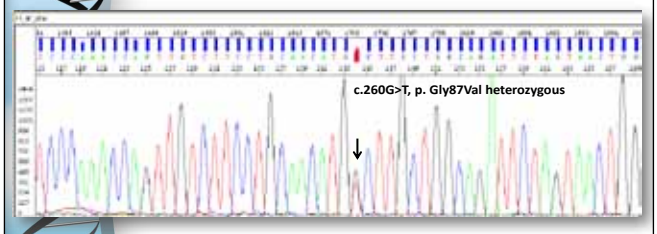
- Consanguineous marriages
- Higher mutation frequency in endemic regions –Kostenets and Bansko

Selective screening for these particular mutations newborn samples originating from the native regions of both patients

Selective screening

- **Sequencing**

Missense mutation c.260G>T – exon 4 of the LIPA gene



Kostenets region – One heterozygous carrier 1% (1/100)

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Conclusion

Region	LIPA gene mutation	Guthrie samples	Heterozygous carriers	Heterozygosity	Mutant allele frequency*
Kostenets	Exon 4 c.260G>T	100	1	1%	0.5%
Bansko	Intron 7 c.822+1G>A	100	2	2%	1%

*** Both alleles of the *LIPA* gene for each of the 100 samples (altogether 200 alleles) were considered in the calculations.**

- **Two targeted geographic regions – high carrier frequency of 1% and 2%**
- **High recurrence risk in these particular regions of about 1:10 000**

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Conclusion

- **These findings are from crucial importance for the inhabitants of the corresponding parts of Bulgaria!**
- **They may benefit from early genetic testing and adequate genetic counseling during family planning!**

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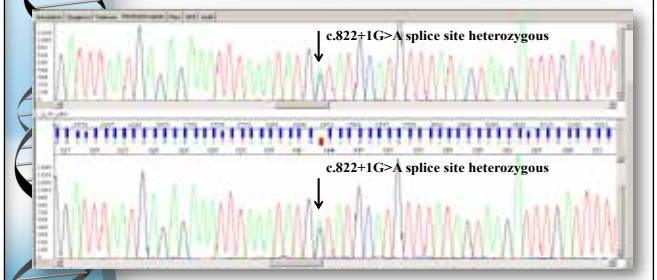
Selective screening

Splice site mutation c.822+1G>A – intron 7 of the LIPA gene



Bansko region - Two heterozygous carriers 2% (2/100)

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- I would like to thank the physicians for the clinical description;

- I am very grateful to the families for their cooperation;

- I would like to thank the National Genetic Laboratory for providing access to the Guthrie cards;

Thank you for your attention!

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NPC1 AND NPC2 GENE ANALYSIS IN SERBIAN PATIENTS WITH NIEMANN-PICK DISEASE TYPE C

Marija Branković, Valerija Dobričić, Nikola Kresojević, Ana Marjanović, Ivana Novaković, Vladimir Kostić

Material and methods

- 142 Serbian adult patients (with symptoms that meet the NP-C criteria, and asimptomatic relatives of positive patients)
- Analysis of 4 selected (mutation prone) exons of *NPC1* gene (exons 8, 19, 20 and 21)
- The other 21 exons of *NPC1* gene were further analyzed for patients with one mutant allele
- Sequencing of all five exons of ***NPC2* gene**
- Blood sampling
- DNA extraction
- Direct Sanger sequencing
- *In silico* analysis

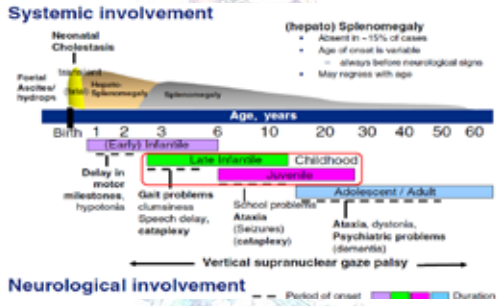
NIEMANN-PICK TYPE C (NPC)

- Lipid trafficking disorder
- Autosomal recessive
- 1:120 000-150 000
- Mutations in the *NPC1* (95%) or *NPC2* (5%) gene

Results

- 14 positive patients ★ compound heterozygotes (n=13)
  ★ homozygote (n=1) — *NPC1* gene
- Heterozygous mutations were identified in 22 relatives of patients
- One heterozygous mutation — *NPC2* gene

Clinical presentation of NPC



Modified from Vanier, 2010

Gene	Location	Mutations	Significance	Reference
NPC1	Exon 8	c.1204_1205 TT>GC, p.F402A	*0,999	novel
	Exon 8	c.1226T>C, p.I409T	*0,999	novel
	Exon 16	c.2486T>G, p.L829R	*0,999	novel
	Exon 19	c.2861C>T, p.S954L	pathogenic	Greer et al, 1999
	Exon 19	c.2819C>T, p.S940L	pathogenic	Greer et al, 1999
	Exon 20	c.3019C>G, p.P1007A	pathogenic	Greer et al, 1999
	Exon 20	c.3038A>G, p.K1013R	*0,999	novel
	Exon 23	c.3562G>C, p.E1188Q	pathogenic	Runz et al, 2008
	Intron18	c.2795+5 G>C	*1	novel
	Exon 21	c.3179_3180delTT, p.L1060Hfs*4	*1	novel
	Exon 24	c.3722T>A, L1241X	*1	novel
		*del. ex 6-9	#NA	novel
NPC2	Intron 4	c.441+1G>A	pathogenic	Bauer et al, 2013

* Significance for novel variants represented by Mutation Taster probability
*This variant was detected by MLPA method by Centogene (Rostock, Germany)
Not applicable

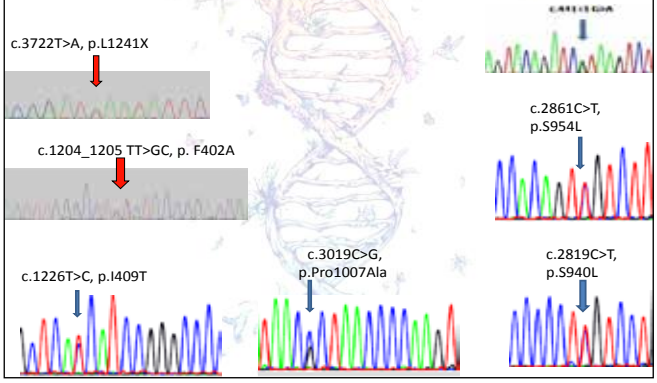
NPC1	*NPC2*
18q11-q12	14q24.3
25 exons	5 exons
Integral membrane glycoprotein	Soluble glycoprotein
1278 amino acid	151 amino acid
Over the 350 mutations described	Around 25 mutations described
70% missense mutations	Most common p.E20X

Davies & Ioannou, 2000

Partners in the same "business" Involved in the cellular postlysosomal/late endosomal transport of cholesterol and other cargo.

Friedland N et al., 2003

Results



Results

- Missense mutations constituted the majority of detected mutations (~ 85%)
- High frequency of novel mutations indicates unique mutation spectrum in this cohort of Serbian NP-C patients.

Conclusion

- These results confirm the importance of *NPC1* sequencing as standard genetic test in NP-C. Our observation is consistent with data from other ethnic groups that the majority of patients have affected *NPC1* gene, but in our cohort variants are predominantly in exons 8, 16, 19, 20 and 24.
- Our findings better characterize NP-C in Serbian population and facilitate future studies into genotype-phenotype correlations.

THANK YOU FOR ATENTION

SESSION 6-II

Moderators: Karin Writzl, Olga Boyanova

- **Genome structure of modern Bulgarians**
  S. Karachanak-Yankova
- **Medical legal challenges in genetic research**
  M. Petrova
- **Orphan drugs in Serbia: evaluation of market authorization, pricing, reimbursement and expenditure**
  A. Pejcic

Oral presentations:

- **Genetic research, family and family relations**
  V. Petrova-Tacheva
- **The era of precision medicine – a basic guide of how to navigate the field**
  R. Staneva
- **Genetic testing and insurance**
  Z. Gucev

GENOMIC STRUCTURE OF MODERN BULGARIANS

Sena Karachanak-Yankova, Desislava Nesheva, Angel Galabov, Draga Toncheva


PLOS ONE

Y-Chromosome Diversity in Modern Bulgarians: New Clues about Their Ancestry

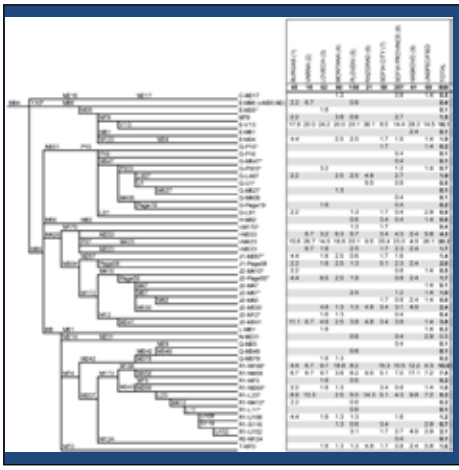
- 808 individuals genotyped
- 75 biallelic markers by DHPLC, RFLP and Sanger sequencing
- 247 individuals analyzed for 17 Y-STR loci

The uniparental genetic composition

Mitochondrial DNA and Y-chromosome haplogroups


Bulgarians vs the other European populations: a mitochondrial DNA perspective

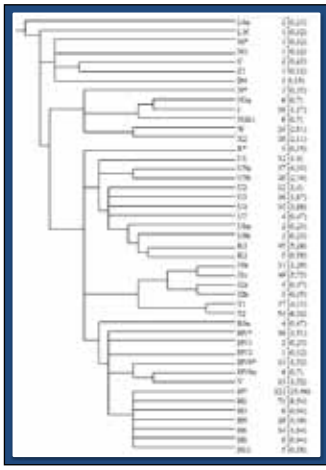
- 855 healthy, unrelated Bulgarian males from different regions of the country
- Haplogroup classification: Sanger sequencing of the mtDNA control region followed by genotyping of coding markers



Western Eurasian haplogroups

Most prevalent:
I-M423 (20.2%)
E-V13 (18.1%)
R-M17 (17.5%)

Contributions from Central Asia (C-M217), Northern Eurasia (N-M231), South West Asia (Q-M242, L-M61 and R-M124) have almost negligible frequencies

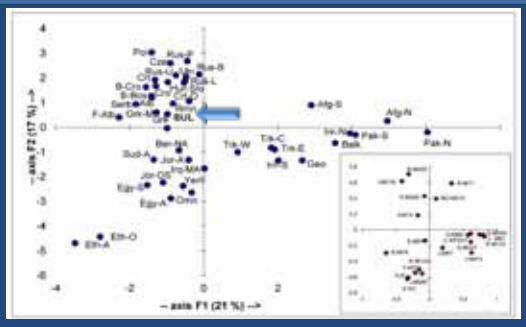


557 different control region haplotypes classified into 45 (sub-) haplogroups

Haplogroups typical of West Eurasian populations

Few exceptions (1.3%) belong to East Asian and African haplogroups

Comparison with African and Eurasian populations analyzed at a high level of phylogenetic resolution

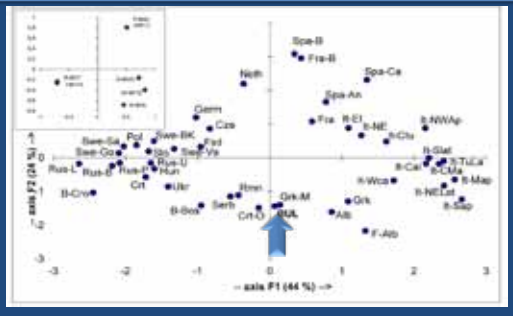


Bulgarians distribute within the European cluster

Inter-population comparison

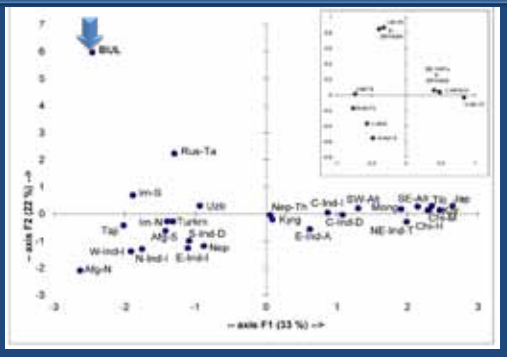
PCA: Bulgarians locate in an intermediate position between Eastern European and Mediterranean countries

Comparison of Bulgarians with European populations



Bulgarians are close to Northern Greeks and Slavic populations from the Western Balkans

Comparisons of Bulgarians with Asian populations



Bulgarians are clearly outliers

Original Paper

Human Heredity

Hum Hered 2010;70:141–149
DOI: 10.1159/000313854

Genetic Differences between Five European Populations

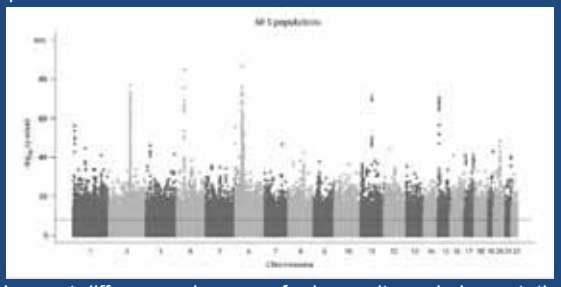
- Irish (n=1142), Scottish (n=656), Swedish (n=620), Bulgarian (n=1129) and Portuguese (n=347)
- GWAS using Affymetrix 6.0 and 5.0 arrays for 906,600 and 500,568 SNPs
- 40,593 SNPs stratified between the five populations
- The number of significant differences correlated with the distance between the countries
- For Bulgaria the largest differences were observed with Ireland, followed by Scotland, Sweden and Portugal

PLoS one

Genetic Structure of Europeans: A View from the North–East

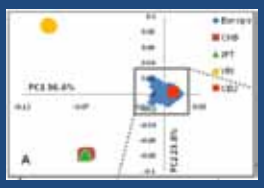
- 3,112 individuals across 16 European countries
- Genotyping of > 270,000 SNPs

Significance of population stratification between the five populations for each SNP versus its genomic position.



Largest differences in genes for immunity and pigmentation, the *LCT* gene, genes involved in NAD metabolism and *FOXP2* implicated in speech development.

Genetic homogeneity in Europe



CEU - Utah residents with ancestry from Northern and Western Europe, CHB – Han Chinese from Beijing, JPT - Japanese from Tokyo, YRI - Yoruba from Ibadan, Nigeria.

- genetic distances correlate considerably with geographic distances

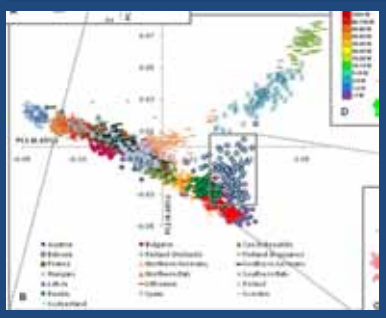
Science. Author manuscript; available in PMC 2015 September 14.

Published in final edited form as:
Science. 2015 September 11; 349(6253): aab3761. doi:10.1126/science.aab3761.

Global diversity, population stratification, and selection of human copy number variation

CNV discovery
WGS of 236 individuals from 125 populations
HiSeq 2000 followed by microarray validation

European genetic map



Genetic diversity corresponds to the NW-SE gradient and positions the populations according to their approximate geographic origin

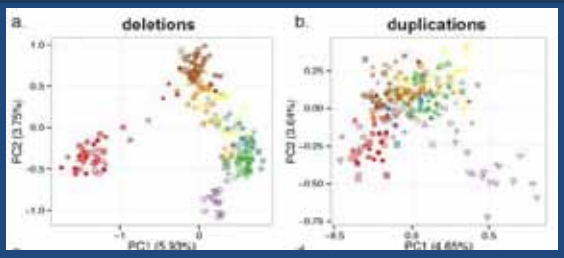
Distinct regions within Europe 1) Finland; 2) the Baltic region (Estonia, Latvia and Lithuania), Russia and Poland; 3) Central and Western Europe; and 4) Italy

Characteristics of human CNVs

Class	Autosomal (Mbp)	X chromosome (Mbp)	Exonic (Mbp)
Deletions	7,233 (78.99)	278 (6.61)	636 (0.32)
Duplications	7,234 (129.62)	267 (6.46)	2,093 (1.56)
Subtotal	14,467 (204.54)	545 (12.61)	2,729 (1.84)
SNVs	32,630,650 (32.63)	1,175,170 (1.18)	314,872 (0.31)
All	32,645,117 (237.17)	1,175,715 (13.79)	317,601 (2.15)

- 7.1% of the human genome is variable due to CNVs (deletions - 2.7%, duplications - 4.4%) as compared due to 1.1% SNP
- median CNV size is 7,396 bp (82.2% less than 25 kbp)

PCA for bi-allelic deletions (a) and duplications (b)



(a) African populations are broadly distinguished from non-African ones; West Eurasians, East Asians and Oceanic populations are distinguished
(b) similar trend albeit with far less definition.

Conclusions

Bulgarian genetic variation

- clusters the population among other Europeans
- fits within the south-east/north-west gradient of European SNPs diversity
- Bulgaria has been positioned on the European genetic map and on the global CNV map

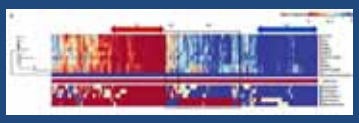
CNV diversity

Differences between deletions and duplications:
- genic deletions are significantly rarer than intergenic
-larger deletions are less frequent than small ones
-deletions recapitulate most properties of SNVs

Population stratification

•1,036 stratified CNVs
•21% of putative adaptive loci intersected with a CNV when compared to 6% of disease GWAS loci

The ancestral human genome

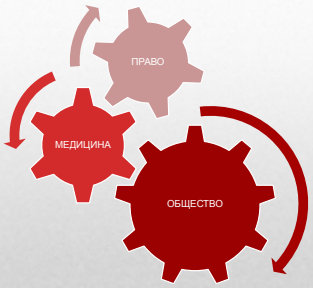


megabases of DNA lost in different human lineages

Thank you for your attention!!!

MEDICO-LEGAL CHALLENGES RELATED TO GENETICAL TESTING

Maria Petrova



- Law
- Medicine
- Society

- Адвокат в Софийска адвокатска колегия
- Изпълнителен директор на Европейския институт по медицнско право и здравен мениджмънт
- Медиатор към Министерство на правосъдието
- Експерт по здравеопазване в институцията на Омбудсмана на Република България
- Магистър по хуманна медицина
- Магистър по право
- Магистър по здравен мениджмънт

Адвокат д-р Мария Петрова

Правен режим на генетичните изследвания

- Закон за здравето
- Закон за защита на личните данни
- Закон за защита от дискриминация
- Наредба № 38- Стандарт по „Медицинска генетика“
- Наредба № 26 от 14 юни 2007 г. За предоставяне на акушерска помощ на здравно неосигурени жени и за извършване на изследвания извън обхвата на задължителното здравно осигуряване на деца и бременни жени

- Law of Health
- Personal Data Protection Law
- Law of Antidiscrimination
- Regulation No.38 – Standard of “Medical genetics”
- Regulation No.26 from 14 June 2007 for Medical Birth Assistance to Women Without Medical Insurance and for Medical Testing of Children and Pregnant Women Beyond the Obligatory Medical Insurance

- Attorney at Sofia Bar
- CEO of the European Institute for Medical Law and Health Management
- Certified mediator by Ministry of Justice
- Healthcare Expert at the Bulgarian Ombudsman Office

- MSc in Medicine
- MSc in Law
- MSc in Healthcare Management

Attorney Maria Petrova, MD

Предизвикателства

- Информирано съгласие
- Защита на личните данни
- Бързо развитие на науката и техниката

Challenges

- Informed consent
- Personal data protection
- Fast technological and science advancements

Информирано съгласие

- Законов процес
- Основни принципи
- Решение на ВКС 2011
- Строг режим при генетични изследвания

Informed consent

- Legal process
- Basic principles
- High Court decision of 2011
- Strict regulations on genetical testing

Право

- **„Ius est ars boni et aequi“**

Правото е изкуство на доброто и праведливото / Law is the art of the good and just

- **„Hominum causa ius constitutum est“**

Правото е създадено за хората / The Law is created for the people

Медицина

- **Est medicina triplex: servare, cavere**

Задачата на медицината е тройна: да пази, да предупреждава, да лекува / Medicine has a three-fold task: to protect, to warn, to treat

- **Salus aegroti suprema lex**

Благоденствието на пациента е най-висш закон / The wellbeing of the patient is a higher law

Имат ли общо правото и медицина?

Защита на лични данни

- Администрация на лични данни
- Степен на защита
- Нови европейски правила

Personal data protection

- Personal data administration
- Degree of protection
- New European regulations

Нови научни и технологични въведения

- Договори
- Липса на регулация за нови технологии
- Липса своевременни нормативни промени

- Agreements
- Lack of regulation on new technological advancements
- Lack of timely normative changes

Решения

- Повече информираност
- По-добър диалог
- Повишаване на правната култура
- Медиация

Solutions

- Increased awareness and information
- A better dialogue
- Increased legal knowledge / culture
- Mediation

Благодаря Ви за вниманието!
Thank you for your attention!

адв. д-р Мария Петрова www. lexmedicabg.com
mpetrova@lexmedicabg.com

ORPHAN DRUGS IN SERBIA: EVALUATION OF MARKET AUTHORIZATION, PRICING, REIMBURSEMENT AND EXPENDITURE

Ana Pejcic, Mihajlo Jakovljevic

Orphan drugs in Serbia

- Serbia, being a country outside of the EU, cannot directly benefit from the EU orphan drug legislation.
- Legislation specific to ODs doesn't exist in Serbia.

Serbia
EU candidate country
Upper-middle income economy
Total health expenditure as % of GDP (2014, WHO): **10%**
Total health expenditure per capita (2014, WHO): **US$ 633**
Predominant funder within the public healthcare sector is the National Health Insurance Fund.

Orphan drug

- **Formal regulatory term** used to describe a medicinal product that has been granted an orphan designation by a regulatory agency.

Market authorization

- ODs should **obtain market authorization** from the ***Medicines and Medical Devices Agency of Serbia*** in order to be marketed on a national level.
- Market authorization **procedure is *simplified*** for the ODs **authorized** through the ***centralized procedure in the EU***.
- ***Conditional*** market authorization is allowed for ODs.
- ODs are ***exempted of the market authorization fees***.
- ***23 ODs*** were authorized in June 2017 (of 95 drugs with active orphan designation and market authorization in the EU in this period).

Orphan drugs in the EU

- **Regulation (EC) No 141/2000** defines orphan drugs (ODs) as medicinal products:
  - (A) that are intended for the **diagnosis, prevention or treatment of a life-threatening or chronically debilitating condition** affecting **not more than five in 10 thousand persons in the Community**, or that are intended for the **diagnosis, prevention or treatment of a life-threatening, seriously debilitating or serious and chronic condition in the EU** and that **without incentives** it is **unlikely** that the marketing of the medicinal product **would generate sufficient return to justify the necessary investment**;
  - (B) and that there exists **no satisfactory method of diagnosis, prevention or treatment of the condition in question that has been authorised in the Community** or, if such method exists, that the medicinal product **will be of significant benefit to those affected by that condition**.

Import of unauthorized orphan drugs

- Special **policy regulates import** of drugs without market authorization in Serbia.
- There is a **list of 255 rare diseases** for which import may be authorized.
- **Medicines and Medical Devices Agency of Serbia** is responsible for authorization of import.

Orphan drugs in the EU

- Incentives for sponsors of ODs in the EU include:
  - Protocol assistance
  - Fee reductions
  - Access to the centralized authorization procedure
  - 10-year market exclusivity

- Orphan drugs **follow the same pricing and reimbursement procedures** as other pharmaceutical products.
- Pricing and reimbursement are set **nationally.**

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8-10 September 2017, Plovdiv, Bulgaria

Pricing

- **The Government sets** the pricing criteria and maximum wholesale prices for market authorized prescription drugs on a national level.
- **The pricing criteria:**
  1) The comparable wholesale price in reference countries;
  2) The average wholesale price of comparable drug in reference countries;
  3) Ratio of wholesale price in the Republic of Serbia and the average wholesale prices of comparable drug in reference countries;
  4) The current wholesale price of the drug;
  5) Pharmacoeconomic parameters;
  6) Wholesale costs.
- **Reference countries:** Slovenia, Croatia and Italy
- ***20 ODs*** were priced in June 2017 (of 95 drugs with active orphan designation and market authorization in the EU in this period).

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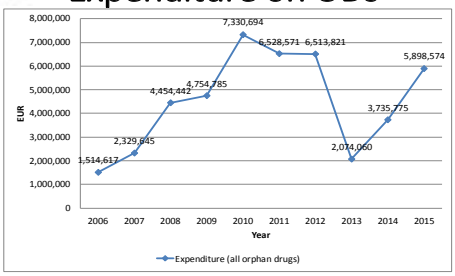
Expenditure on ODs



Source: Annual reports on turnover and consumption of pharmaceuticals in Serbia published by Medicines and Medical Devices Agency of Serbia 2006-2015

All orphan drugs – ODs with active orphan designation and market authoritation in respective years

Pejčić AV, Iskrov GG. Expenditure trends of orphan drugs in Serbia: 8-Year analysis of orphan drug market in Serbia. Hospital Pharmacology-International Multidisciplinary Journal. 2016;3(3):422-34.

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Pricing and reimbursement

- Application for inclusion of the drug in the reimbursement list can be made after the Government makes a decision on the maximum wholesale price of the drug.
- **If a drug is included** in the **reimbursement list NHIF again sets the price of the drug** .
  - **Reference countries**: Slovenia, Croatia and Italy or if drug is not priced there, Romania, Lithuania, Slovakia, Bulgaria, Hungary and Latvia
- At the end, if the drug gets placed on the reimbursement list, its **final price on the market** is the **price achieved in the process of centralized public procurement**.

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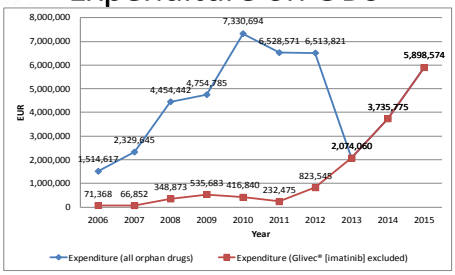
Expenditure on ODs



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Reimbursement

- Reimbursement decisions are made by the **National Health Insurance Fund (NHIF)**
- **Criteria** for inclusion of drugs in the reimbursement list:
  - **General** criteria:
    - pharmacotherapeutic justification of the drug
    - pharmacoeconomic justification of the drug
    - financial resources provided by the Financial plan of the NHIF
  - **Special** criteria:
    - special contracts
    - priority order

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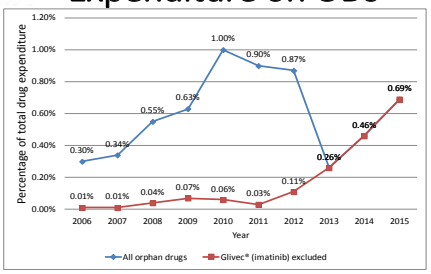
Expenditure on ODs



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Reimbursed ODs

- **Only 7 ODs** were on the reimbursement list in Serbia in June 2017 (of 95 drugs with active orphan designation and market authorization in the EU in this period):
  - B02BX04 **Nplate®** (romiplostim)
  - L01XC12 **Adcetris®** (brentuximab vedotin)
  - L01XE05 **Nexavar®** (sorafenib)
  - L01XE08 **Tasigna®** (nilotinib)
  - L03AX16 **Mozobil®** (plerixafor)
  - L04AX04 **Revlimid®** (lenalidomide)
  - N06BC01 **Peyona®** (caffeine)
- The **Government of the Republic of Serbia – Ministry of Health – annual budget** for the treatment of some of the patients with rare diseases whose expenditures cannot be reimbursed through National Health Insurance Fund.

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Conclusions

- Only a limited number of ODs is accessible to patients in Serbia.
- Although ODs are authorized faster through an expedited procedure, they still follow the same pricing and reimbursement procedures as all other drugs.
- Financial resources allocated to ODs are still limited, despite evident increase of expenditure in the recent years.
- Many patients still rely on donations.
- There is a need for further improvement in accessibility of ODs.

GENETIC RESEARCH, FAMILY AND FAMILY RELATIONS

V. Petrova-Tacheva, S. Alekova, B. Popov, V. Ivanov

Introduction



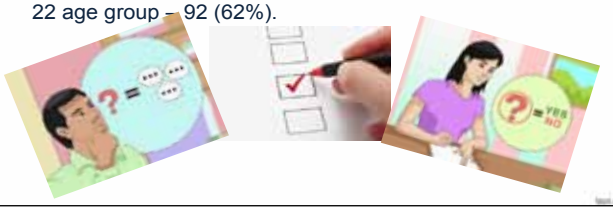
Genetics, molecular biology and biotechnology are some of the fastest growing sciences, whose achievements are entering faster and more massively into our day-to-day activities and lives. Various genetic tests are being developed, researched and conducted in many countries. However, the application of genetic research in practice often raises a number of ethical and moral dilemmas, discussions and problems affecting not only the individual but also his whole family.

Aim

We have set ourselves the goal of exploring and analyzing the personal position of young people of reproductive age about the influence importance of genetic research on family relationships.

Materials and methods

A sociological survey was conducted among 140 persons of reproductive age in the Stara Zagora region, with strict observance of the principles of voluntariness and anonymity. The sample includes 44 (31%) men and 96 (69%) women, the largest age group being within the 20-22 age group – 92 (62%).



Results and discussion

- *"The family takes a special place in every person's life. In it we grow, live, educate, adopt moral norms and values.*
- *The family reproduces biological life and social relations"*
- *The family is "the main building unit of society".*

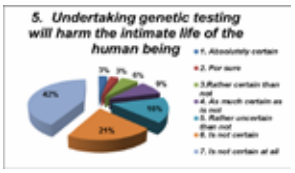
Results and discussion

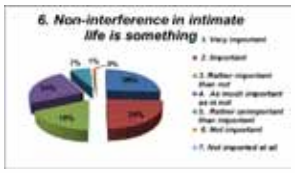
- Each family is created by two people, each of whom has his or her own personality and soul.
- In order to create and keep a family, an important condition is the existence of mutual love, honesty, responsibility and willingness to share and compromising between the two partners.
- Due to the difference between the two marital partners in their value systems, their expectations and feelings about what marital life and family are, positive results from genetic testing could have some negative consequences.

Results and discussion

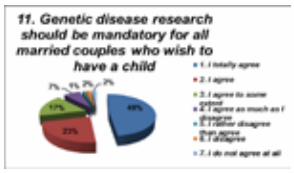
Results and discussion

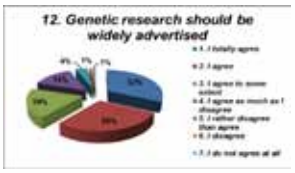
Results and discussion





Results and discussion



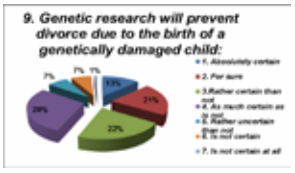


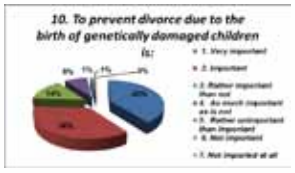
Results and discussion

Conclusions

- *A key point for the beneficial use of genetic research, both for the individual and for the whole family, is the professional and competently conducted medical-genetic counseling.*
- *For maximum effectiveness and coverage of the genetic tests themselves and of their application, an active health policy is required.*

Results and discussion





What is the family? A haven from the life storms. A return to yourself. A source of love and support. Memories. And dreams. And so many other things ...

Results and discussion

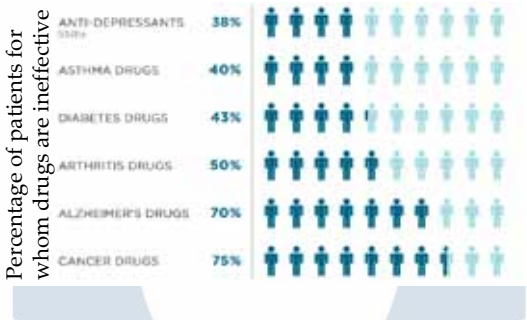
The key role of consultant in Medical Genetics

Thank you for your attention!

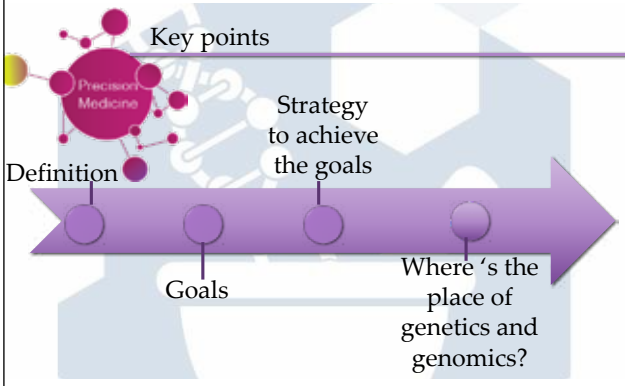
THE ERA OF PRECISION MEDICINE A BASIC GUIDE OF HOW TO NAVIGATE THE FIELD

Rada Staneva

Precision medicine – How is it different?



Key points



Precision medicine – What are we aiming at?

Finding the treatment that…

… is most likely to work

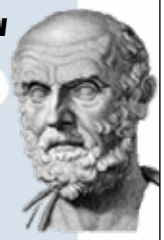
….will have less side effects

…will contain costs associated with the trial-and-error approach

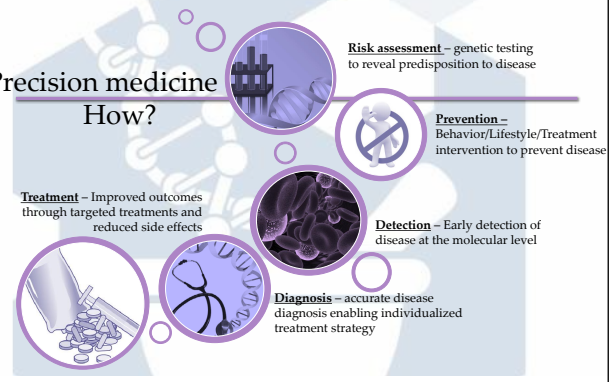
Precision medicine - definition

"It's far more important to know what person the disease has than what disease the person has. "

Hippocrates



Precision medicine How?



Precision medicine - definition

European Union
Providing the right treatment to the right patient, at the right dose at the right time.

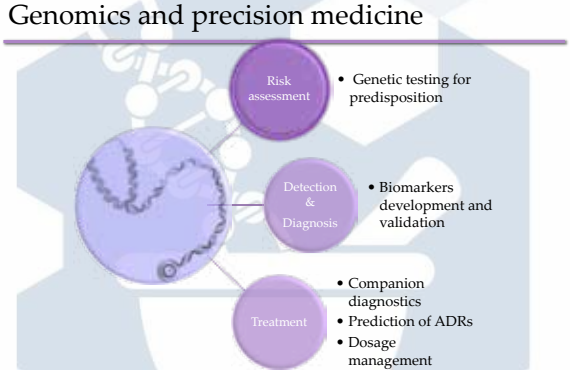
Personalized Medicine Coalition
The use of new methods of molecular analysis to better manage a patient's disease or predisposition to disease.

President's Council of Advisors on Science and Technology
The tailoring of medical treatment to the individual characteristics of each patient.

American Medical Association
Health care that is informed by each person's unique clinical, genetic, and environmental information.

National Institute of Health
A form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease.

Genomics and precision medicine



The example of breast cancer - BRCA1&2

✓Responsible for 2-6% of breast cancer cases

✓Narrow indications for testing

Clinical Biomarkers

„ Characteristic objectively measured and evaluated as indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention."

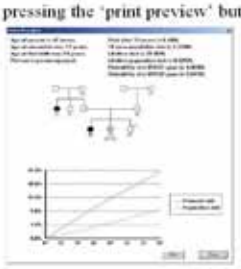
US National Institutes of Health Biomarkers Definition Working group

''Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. "

FDA U.S. FOOD & DRUG

The example of breast cancer – beyond BRCA1&2
Risk calculation software

After pressing the 'print preview' button



4. Report

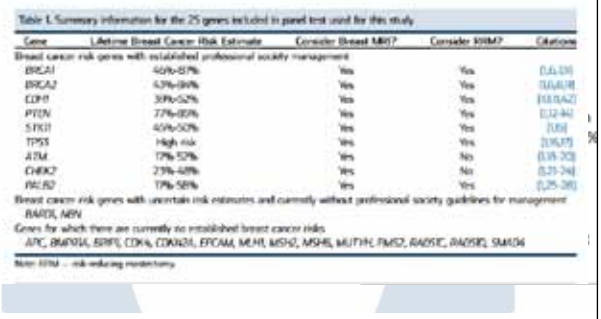
The example of cancer - biomarkers

PROGNOSTIC

PREDICTIVE

PHARMACODYNAMIC

The example of breast cancer – beyond BRCA1&2

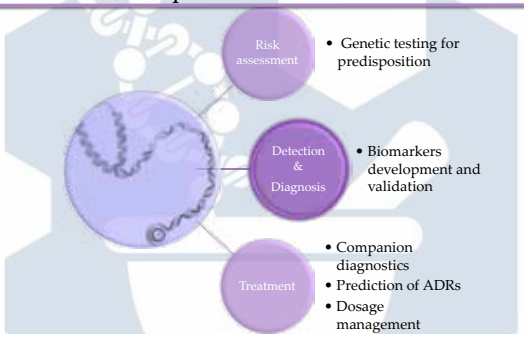


Predictive biomarkers

✓Correlate with probability of clinical response to treatment

HER-2 Amplification in breast cancer	• Sensitivity to anti-HER2 drugs
ER/PgR expression in breast cancer	• Hormone therapy
Philadelphia chromosome in CML	• Anti-BCR-ABL therapy
EGFR mutations in NSCLC	• EGFR inhibitors
KRAS wild type in CRC	• Anti-EGFR mabs
BRAF V600E in melanoma	• BRAF-inhibitors
EML4/ALK in NSCLC	• ALK-inhibitors

Genomics and precision medicine

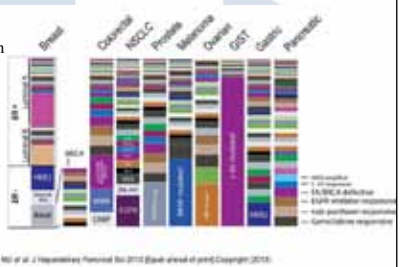


Genomics and predictive biomarkers

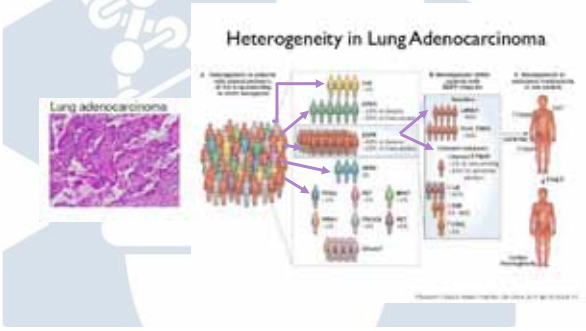
✓ NO cancer is without mutation

✓ Neoplasms from the same histological type differ by their molecular profile

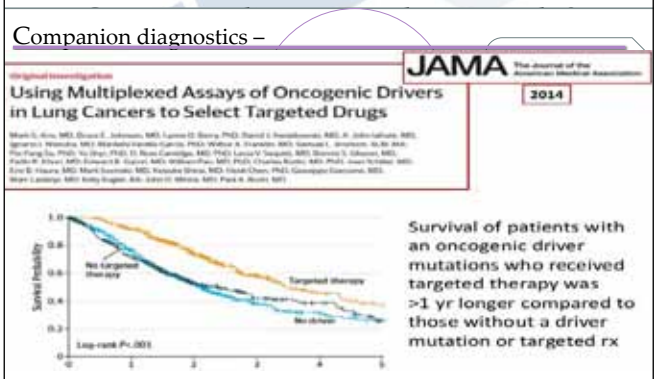
✓ Every common type of cancer is actually a mix of "rare cancers" from molecular prespective



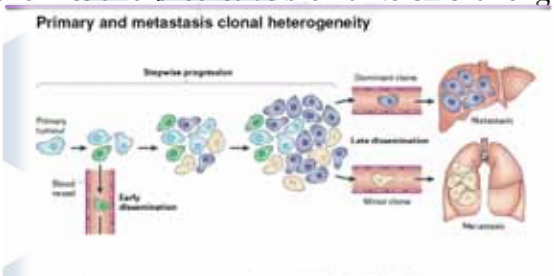
Genomics and predictive biomarkers



Companion diagnostics –



Genomics and predictive biomarkers - challenges



Cancer molecular heterogeneity and clonal evolution is an ongoing process

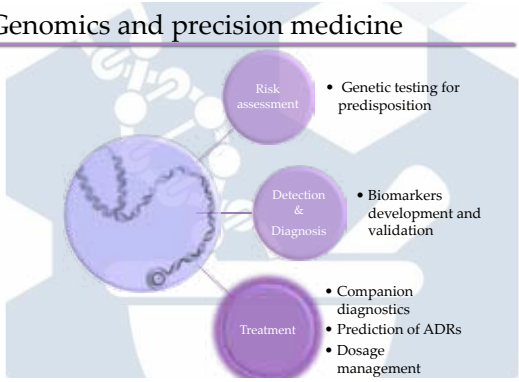
Prediction of ADRs

✓Adverse drug reactions can be prevented by genotyping

Multiorgan hypersensitivity reactions HLA B*5701

Steven-Johnson Syndrome
HLA-B*15:02

Genomics and precision medicine



Dosage management

✓For some medications genotyping is recommended before onset of therapy

CPIC

TPMT

CYP2C19

CYP2C9 and VKORC1

Companion diagnostics - definition

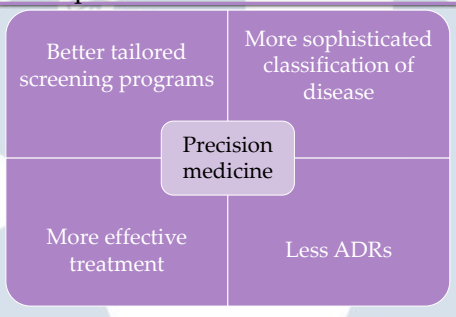
EU

A companion diagnostic is a device specifically intended to select patients with a previously diagnosed condition or predisposition as eligible for a targeted therapy

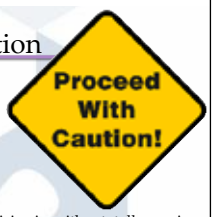
FDA

A companion diagnostic is a medical device, often an in vitro device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product. The test helps a health care professional determine whether a particular therapeutic product's benefits to patients will outweigh any potential serious side effects or risks.

To sum it up….



Precision medicine – words of caution



Prasad et al :

'… those oncologists who practice precision oncology are two steps ahead of the data—and the history of medicine has taught us that is an uncertain place to stand.' Lancet Oncology 2016

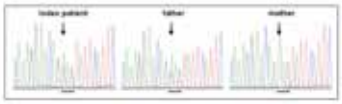
Bruce Chabner:

'Underlying this new effort is my conviction that genomic medicine is neither totally precise nor completely rational at this point in its development. collective sharing of findings and experiences among institutions will help improve the precision of precision medicine.We increasingly understand that the drugs are not "precisely" targeted. We have learned that targeted agents have multiple sites of actionand unanticipated side effects. New generations of drugs, such as the new series of PI3K and EGFR inhibitors, reduce side effects and narrow their spectrum of action. In addition, the multiplicity of mutations in a single tumor and clonal evolution of tumors at different metastatic sites lead to further imprecision in treatment planning.' The Oncologist 2016

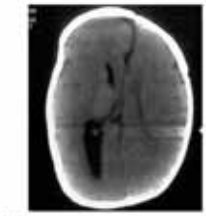
THANK YOU FOR YOUR ATTENTION!

RARE DISEASES: NEW GENES, MOLECULAR MECHANISMS, TREATMENTS

Zoran Gucev, Marko Kostovski,
Velibor Tasic, Momir Polenakovic



Human Genome Project:
State funded v. private = 2 billions v. 100m$
Francis Collins and Craig Venter: towards 1000$ human genome

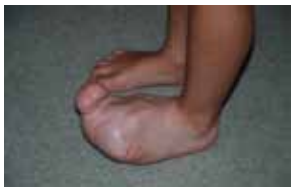


Alomari AI. Clin Dysmorphol. 2009;18:1–7.
Alomari AI. J Thorac Cardiovasc Surg. 2010;140:459–466.
Biesecker LG Am J Med Genet. 1998;79:311–319.

Few genes (~20 000 in human) encode all the proteins

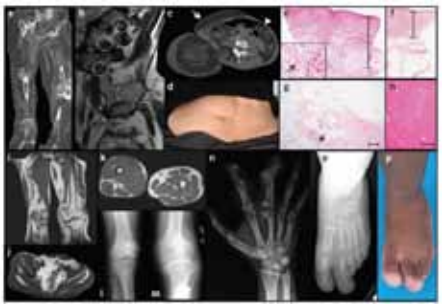
- These **protein-coding sequences** make up **1–2%** of the human genome. **"The rest is junk".**
- **Total number of proteins** is estimated to **be 5 million sequences.**
- The **Human Genome Project** maps the nucleotides contained in a **human haploid reference genome** (more than three billion). There are now **efforts to extend this to diploid human genomes**.

A 4.5 year old girl



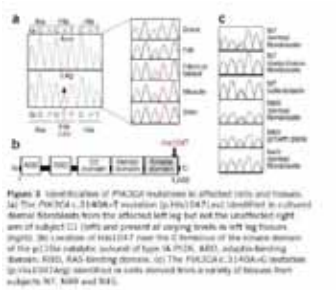
Lindhurst M, Gucev Z et al. Nature Genetics 2012;44(8):928-33.

Lindhurst M, Gucev Z et al. Nature Genetics 2012;44(8):928-33.

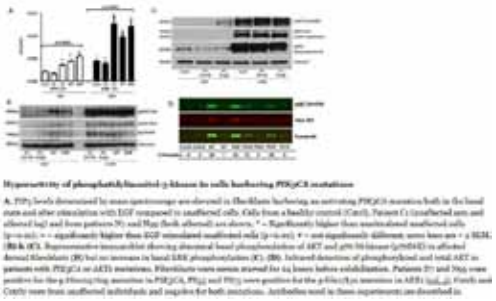


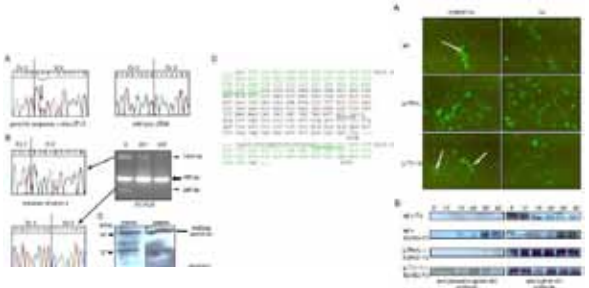
Unlike Proteus syndrome, 31/35 (89%) patients with PIK3CA mutations had congenital overgrowth, and in 35/35 patients this was asymmetric and disproportionate.

Lindhurst M, Gucev Z et al. Nature Genetics 2012;44(8):928-33.



Functional studies

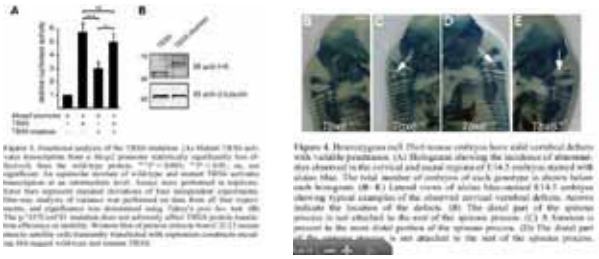




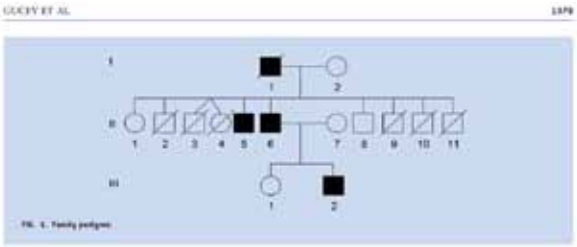
Also found in >130 cancers

- BREAST CANCER
- OVARIAN CANCER, EPITHELIAL
- COLORECTAL CANCER
  GASTRIC CANCER
- HEPATOCELLULAR CARCINOMA
- NONSMALL CELL LUNG CANCER

1. Functional Analysis of the *TBX6* mutations: *Mesp2* promotor affected
2. Heterozygous null TBX6 mouse embryos: vertebral defects with variable penetrance



Lorenz P, Rupprecht E. 1990. Spondylocostal dysostosis: Dominant type. Am J Med Genet 35:219–221.
Temple IK et al. 1988. Congenital spinal deformity in a three generation family. J Med Genet 25:831–834.



Other Spinal Defects

Table 2. Distribution of *TBX6*-associated CS patients and vertebral malformations.*

A) Distribution of *TBX6*-associated CS in two CS cohorts and one deletion cohort			
Cohort	n	Association of *TBX6* compound inheritance	% CS cases explained by *TBX6* compound inheritance
CS cohort 1	101	$P=1.8\times10^{-9}$	12.8%
CS cohort 2	76	$P=8.4\times10^{-7}$	7.9%
Deletion cohort 1	42	$P=1.00$	83.3%

B) Vertebral malformations in *TBX6*-associated CS patients		
CS patient	Morphology of vertebral malformations (n%)	
	Hemivertebra/hypoplasia	Other malformations
TBX6-associated	28 (82.4%)	6 (17.6%)
No *TBX6* mutation	345 (17.1%)	585 (82.9%)
P	<0.001	
OR (95% CI)	7.81 (3.24-19.30)	

* n,% match for number/percent respectively

Turnpenny PD, et al. 2007. 236:1456–1474.
Bulman MP et al. 2000.. Nat Genet 24:438–441.
Sparrow DB et al. 2006. Am J Hum Genet 78:28–37.
Sparrow DB et al . 2008. Hum Mol Genet 17:3761–3766.

CLINICAL REPORT

American Journal of Medical Genetics Part A

Autosomal Dominant Spondylocostal Dysostosis in Three Generations of a Macedonian Family: Negative Mutation Analysis of *DLL3*, *MESP2*, *HES7*, and *LFNG*

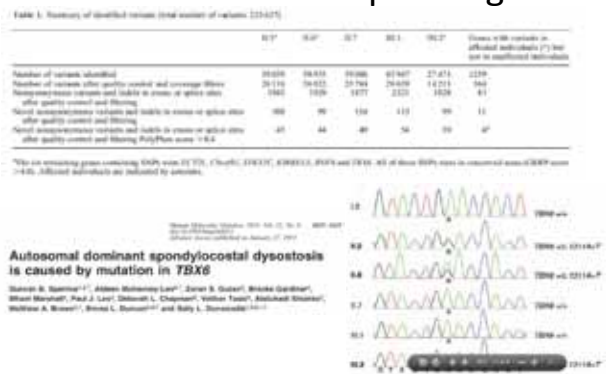
Zoran S. Gucev,[1]* Velibor Tasic,[1] Nada Pop-Jordanova,[1] Duncan B. Sparrow,[2,3] Sally L. Dunwoodie,[2,3] Sian Ellard,[4] Elizabeth Young,[4] and Peter D. Turnpenny[4,5]

A compound *TBX6* inheritance model with *TBX6* haplotype and *TBX6* null mutations

The NEW ENGLAND JOURNAL of MEDICINE

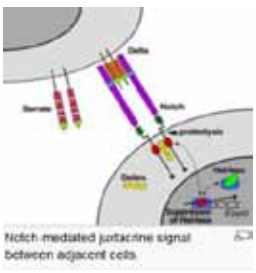
TBX6 Null Variants and a Common Hypomorphic Allele in Congenital Scoliosis

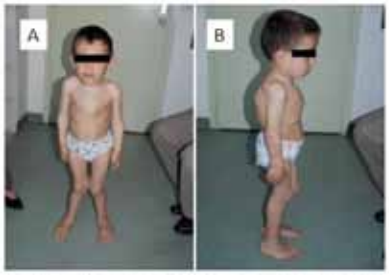
Whole Exome Sequencing



Autosomal dominant spondylocostal dysostosis is caused by mutation in *TBX6*

Turnpenny PD, et al. 2007. 236:1456–1474.
Bulman MP et al. 2000.. Nat Genet 24:438–441.
Sparrow DB et al. 2006. Am J Hum Genet 78:28–37.
Sparrow DB et al . 2008. Hum Mol Genet 17:3761–3766.





Clin Genet 2010: 78: 296–297
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CLINICAL GENETICS

Letter to the Editor

Lesch-Nyhan syndrome: a novel complex mutation with severe phenotype

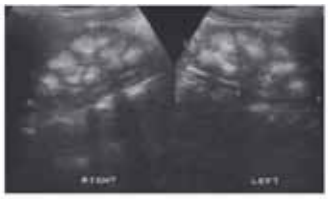
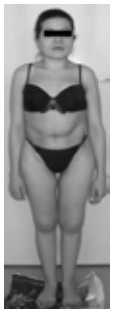
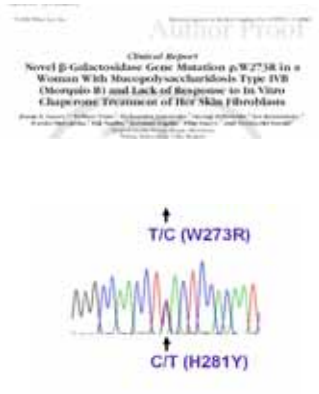
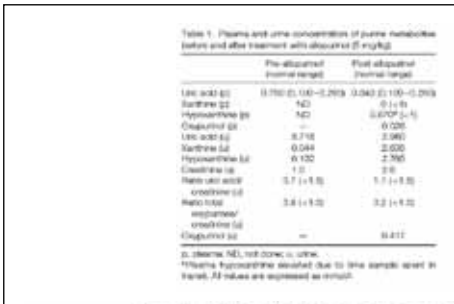


Fig. 1. Bilateral renodulary hyperechogenicity mimicking nephrocalcinosis.







Normal TCCTCATGGA CTAATTATGG ACA[intron 3 — exon 4]T (intron 3)

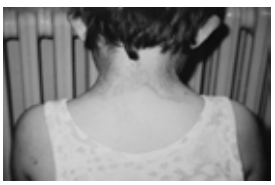
Variant TCCTCATGGACTAATTATGGACTAATTATGGACTAATTAAGATCT

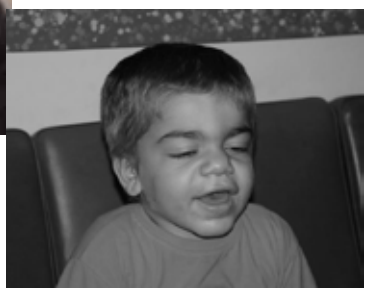
Fig. 2. A comparison between the normal and variant hypoxanthine guanine phosphoribosyltransferase 1 (HPRT1) exon 2 genomic sequences. The authentic intron 3 splice donor sequence is shown bound in lower case. The sequence motif GACTAATTATG is duplicated in the sequence variant, the intron 3 splice donor site is lost, and an additional 8 bases inserted.

Hunter syndrome (muccopolysaccharidosis type II; MPS II) in Macedonia and Bulgaria, novel alterations of the iduronate 2-sulfatase gene and prevalence rates. Z.Gucev, V.Tasic…J Inher Metab Dis, 2010 (submitted).

- Mucopolysaccharidosis II (MPS II) is caused by a deficiency of **iduronate-2-sulfatase** (IDS; EC 3.1.6.13).
- Two new mutations were discovered: p.K236N (c.708G>C) in a child with moderately severe phenotype, and p.Q80K (c.238C>A) which resulted in a severe phenotype and early death at the age of 11 years. Heterozygote carriers of the pathogenic allele were 29 female relatives. The calculated incidence rate for MPS II in Macedonia and 426,280) and Bulgaria are 0.36 and 0.46 respectively, while the calculated prevalence rate are 3.6 and 4.6 per 1,000,000 boys (aged 0-14 years).

Congenital erythropoietic porphyria (CEP) with two mutations of the uroporphyrinogen III synthase gene (UROS; Cys73Arg, Thr228Met). Gucev Z, Tasic V, et al. J Genet 2010; 15-17

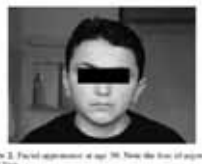




RESEARCH ARTICLE

A case of Silver–Russell syndrome (SRS): multiple pituitary hormone deficiency, lack of H19 hypomethylation and favourable growth hormone (GH) treatment response

ZORAN S. GUCEV*, VELIBOR TASIC, ALEKSANDRA JANCEVSKA and ILIJA KIROVSKI
Medical Faculty Skopje, 50 Divizija BB, 1000 Skopje, Republic of Macedonia



SESSION 7-I

Moderators: Dijana Plaseska Karanfilska, Ugur Ozbek

► **Monitoring of molecular response during the therapy with nilotinib as first-line tyrosine kinase inhibitor in patients with chronic myeloid leukemia**
G. Balatzenko

Oral presentations:

► **Pharmacogenetic studies in patients with cancer**
Z. Hammoudeh

► **Association of Fc gamma receptor polymorphisms with autoimmune hemolytic anaemia**
M. Pavkovic

► **Pjevic Analysis of the association between PAI-1 gene 4G/5G polymorphism and efficacy of thrombolytic therapy in patients with ischemic stroke**
M. Dusanovic

► **Association of ADORA2A gene rs2298383 polymorphism with efficacy/ toxicity of MTX**
M. Grk

► **Identification of CYP2C19*2 allelic variant in healthy Albanian population**
S. Mucaj

MONITORING OF MOLECULAR RESPONSE DURING THE THERAPY WITH NILOTINIB AS A FIRST-LINE TYROSINE KINASE INHIBITOR IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

Balatzenko G, Arnaudov G, Bogdanov L, Goranov S, Goranova-Marinova V, Grudeva-Popova J, Ivanova S, Ignatova K, Mihailov G, Spassov B, Stojanova J, Tumbeva D, Hadjiev E, Hristova I, Hrischev V, Tsvetkov N, Tsvetkova G, Guenova M.

INTRODUCTION (1)

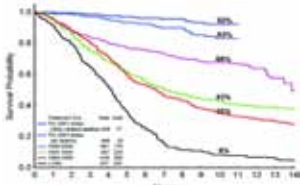
- Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by increased proliferation of myeloid cells with preserved capacity to differentiate, caused by a characteristic genetic abnormality - Philadelphia chromosome and *BCR-ABL1* fusion gene as a result of t(9;22)(q34;q11).
- The *BCR-ABL1* gene encodes a hybrid oncoprotein with an elevated tyrosine kinase activity , which plays a key role in CML pathogenesis. [Goldman & Melo. N Engl J Med. 2003; 349(15):1451-64.]

Glowacki et al., *Int. J. Mol. Sci.* 2013, *14*(8), 16348-16364

INTRODUCTION (2)

- The standard therapy for CML includes targeted inhibition of this elevated activity by tyrosine kinase inhibitors (TKIs), that not only prevents CML progression in most patients, but also spectacularly improves the disease burden and overall survival [Harrington P, et al., Curr Hematol Malig Rep. 2017;12(2):79-84].

SURVIVAL WITH CHRONIC MYELOID LEUKEMIA OVER TIME (1993–2013)



Tariq I. Mughal et al. Haematologica 2016;101:541-558

INTRODUCTION (3)

- The degree to which the bulk of disease is reduced is the most important prognostic marker in CML.
- The first noticeable indicator of leukemia burden reduction is the normalization of the peripheral blood cell counts. Then, the treatment response control continues by cytogenetics or RT-PCR.
- Molecular monitoring measures the dynamics of *BCR-ABL1* transcripts in the blood at pre-defined time points during therapy using standardized q-RT-PCR. The results are expressed as a ratio of *BCR-ABL1* to the number of control normal transcripts.
- This monitoring aims at detecting resistance or CML progression in order to identify patients at risk who require further evaluation, closer follow-up or early switch to 2nd-line therapy in order to achieve better long-term outcome [Shah J. J Community Support Oncol. 2014;12(5):179-87].

INTRODUCTION (4)

- Currently five different TKIs are approved for CML treatment: imatinib, nilotinib, dasatinib, bosutinib, ponatinib [Bauer S, et al. J Adv Pract Oncol. 2016; 7(1):42-54].
- Nilotinib is a second generation TKI which is more potent and effective than the first targeted agent imatinib [Abruzzese E, et al. BioDrugs. 2014; 28(1):17-26].
- Data from clinical trials indicate that the response to nilotinib as a front-line therapy is greater and faster than that to imatinib, resulting in a higher probability of achieving an optimal molecular response [Kantarjian HM et al., Lancet Oncology, 2011; 12:841-51].
- Real-life data in unselected patient cohorts is of significant practical value to confirm trial data and also to allow for the prediction of long-term outcome of the disease in individual patients, as well as for the selection of potential candidates for TKI discontinuation.

AIM OF THE STUDY:

TO EVALUATE THE MOLECULAR RESPONSE TO NILOTINIB AS A FIRST-LINE THERAPY IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

PATIENTS:

A total of 162 patients (pts) with a *BCR-ABL1*(+) CML, treated with a Nilotinib 2x300 mg daily as a front-line therapy were included in this study:

- Females (n=77) / Males (n=85)
- Mean age: 54,1±15,6 yrs / Median age: 56.0 (range 21-87) yrs

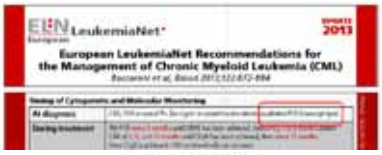
INITIAL DIAGNOSTIC WORK-UP:

- History & physical examination, including spleen size;
- CBC with differential and platelets
- Bone marrow aspiraion and/or biopsy for mophological evaluation;
- Cytogenetics;
- Molecular testing by RT-PCR

METHODS:

INITIAL BCR-ABL1 STATUS:

STANDARTIZED QUALITATIVE RT-PCR (BIOMED-1 Concerted Action)
[van Dongen JJ, et al., Leukemia. 1999;13(12):1901-28]



MOLECULAR FOLLOW-UP:

AUTOMATED RT-PCR BASED PLATFORM
[The GeneXpert IV system with Xpert® BCR-ABL Ultra cartridges]
RESULTS REPORTING: BCR-ABL1/ABL1% (IS)

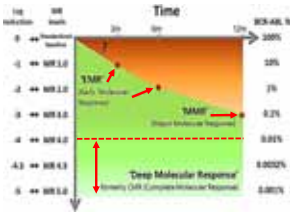
MOLECULAR RESPONSE MILESTONES:

EARLY MOLECULAR RESPONSE:
BCR-ABL1/ABL1 at the 3rd (≤10%) and 6th month (≤1.0%)

MAJOR MOLECULAR RESPONSE (MMR):
BCR-ABL1/ABL1 ≤0.1% (3-log reduction; MR3.0)

DEEP MOLECULAR RESPONSE:
BCR-ABL1/ABL1 ≤0.01%)

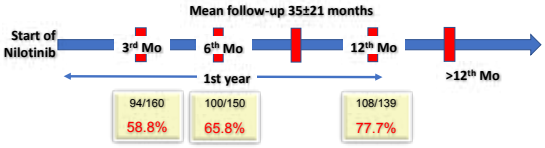
- MR4.0 (≥4-log reduction): **RT-PCR(+)** with ≤0.01% *BCR-ABL1/ABL1*[IS] or **RT-PCR(-)** in cDNA with ≥10000 *ABL1* transcripts.
- MR4.5 (≥4.5-log reduction): **RT-PCR(+)** with ≤0.0032% *BCR-ABL1/ABL1* [IS] or **RT-PCR(-)** in cDNA with ≥ 32000 *ABL1* transcripts
- MR5.0 (≥5-log reduction): **RT-PCR(+)** with ≤0.001% *BCR-ABL1/ABL1* [IS] or **RT-PCR(-)** in cDNA with ≥ 100000 *ABL1* transcripts.



Baccarani M & Soverini S. Blood 2014;124:469-471

Cross et al., Leukemia. 2015 May; 29(5): 999–1003

THE PROPORTION OF P210$^{BCR-ABL1}$(+) PTS WITH MOLECULAR MONITORING DURING THE 1st YEAR OF THERAPY



Timing of Cytogenetic and Molecular Monitoring

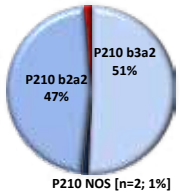
During treatment	RQ-PCR every 3 months until MMR has been achieved, then every 3 to 6 months and/or CBA at 3, 6, and 12 months until CCyR has been achieved, then every 12 months. Once CCyR is achieved, FISH on blood cells can be used.

Baccarani et al. J. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013, 8;122(6):872

INITIAL *BCR-ABL1* STATUS OF THE PATIENTS

Atypical BCR-ABL1 transcripts [n=2; 1%]

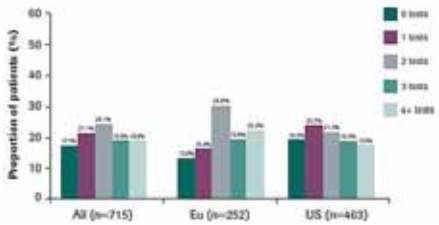
99% of the patients were positive for the typical for CML p210 *BCR-ABL1* transcripts



P210 NOS [n=2; 1%]

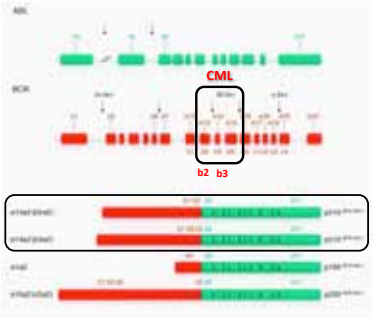
MOLECULAR MONITORING IN THE REAL WORLD

Outside clinical trials, <40% of patients undergo molecular testing 3-4 times during the 1st year after diagnosis as recommended by NCCN/ELN evidence-based guidelines.



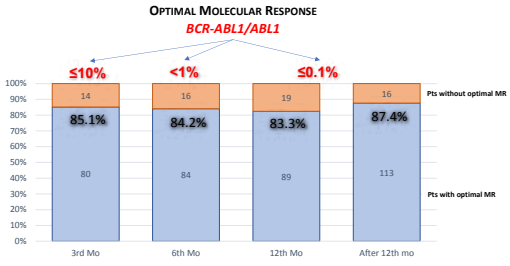
Goldberg SL. Monitoring Chronic Myeloid Leukemia in the Real World: Gaps and Opportunities. Clin Lymphoma Myeloma Leuk. 2015 Dec;15(12):711-4.

MOLECULAR DIVERSITY OF *BCR-ABL1* TRANSCRIPTS



Tariq I. Mughal et al. Haematologica 2016;101:541-558

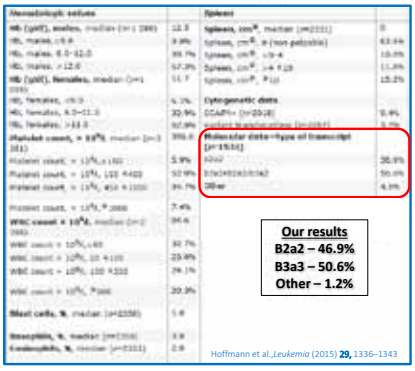
OPTIMAL MOLECULAR RESPONSE RATES DURING THE 1ST YEAR OF THERAPY



"Optimal molecular response": the treatment must be continued
"Failure" mandates for a change of treatment
"Warning" warns that the response must be monitored more frequently

Baccarani M et al., Blood 2013; 122:872-884

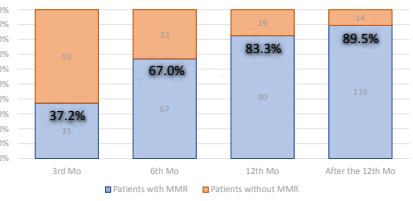
FREQUENCY OF *BCR-ABL1* TRANSCRIPTS IN COMPARISON TO THE EUTOS REGISTRY



Our results
B2a2 – 46.9%
B3a3 – 50.6%
Other – 1.2%

Hoffmann et al.,*Leukemia* (2015) **29**, 1336–1343

MAJOR MOLECULAR RESPONSE [*BCR-ABL1/ABL1* ≤0.1%] RATES DURING THE 1ST YEAR OF THERAPY



The MMR rates increased over time during the study period

Achievement of MMR is associated with improved survival

COMPARISSION OF MMR RATES IN DIFFERENT STUDIES WITH FRONTLINE THERAPY WITH NILOTINIB

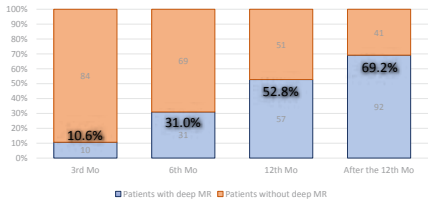
Study	Number of patients	Percentage of patients with an achieved MMR during the front-line therapy with Nilotinib		
		3rd Mo	6th Mo	12th Mo
This study	160	37.2%	67.0%	83.3%
Rosti et al., Blood. 2009, 114:4933-4938	73	52%	66%	85%
Cortes et al., J Clin Oncol, 2010,28(3):392-397	51	40%	71%	81%
Quintás-Cardama et al., Blood 2011 118:454	100	40%	35%	86%
Saglio et al., N Engl J Med 2010; 362: 2251-2259	282	9%	33%	44%
Wang et al.,Blood. 2015; 125(18): 2771–2778	134			52.2%
Hochhaus et al.,Leukemia, 2016, 30:57-64	1052	29.7%		56.3%
Saydam et al., Expert Opin Pharmacother, 2016,17(14):1851-8	112			66.1%

FATAL OUTCOMES DURING THE STUDY

Patient	Age	Gender	Last known BCR-ABL1 status	OS months	Death related to:
EMK	55	F	No MR	30	CML
ATA	54	M	No MR	32	CML
JJM	76	M	No MR	18	CML
PSN	69	M	No MR	51	CML
AMA	64	M	No MR	Lost of contact	Secondary malignancy
BAR	50	M	Deep MR	10	Not Known
RLK	63	M	Depp MR	31	Prostate Cancer
SME	41	M	Deep MR	21	Anaplastic Carcinoma
MAV	74	M	Deep MR	19	Lung Cancer

9/160 [5.6%]

DEEP MOLECULAR RESPONSE RATES [*BCR-ABL1/ABL1* ≤0.01%] DURING THE 1ST YEAR OF THERAPY



The deep MR rates increased over time during the study period

THE IMPACT OF INITIAL WBC COUNT ON THE OPTIMAL MOLECULAR RESPONSE RATES

WBC [x10⁹/l]	Optimal molecular response		
	3rd Mo *BCR-ABL1/ABL1* ≤10%	6th Mo *BCR-ABL1/ABL1* <1%	12th Mo *BCR-ABL1/ABL1* ≤0.1%
<50 (low)	16/18 88.9%	19/20 95.0%	16/17 94.1%
50-250 (medium/high)	36/41 87.8%	32/37 86.5%	33/39 84.6%
>250 (extremely high)	8/12 66.7%	10/18 55.6%	10/17 58.8%
P=	NS	0.004	0.022

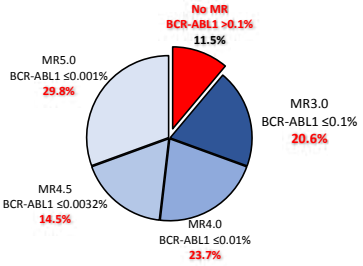
- No differences in the rates of OMR at the 3rd month among the 3 groups were observed.
- The rates of OMR achievement in patients with extremely high WBC count at the diagnosis were significantly lower at the 6th and 12th.
- The proportion of patients with an OMR achieved at the 3rd month remains stable during the course of treatment, regardless the initial WBC count

MOLECULAR RESPONSE RATES IN PATIENTS AFTER THE 12TH MONTH

Primary resistance (suboptimal response or failure): 15 (11.5%) pts

Secondary resistance (loss of previously achieved molecular response): 2 (1.8%) pts.

No molecular response was observed in 2 patients with p190BCR-ABL1-positive CML



Patients with major molecular response	88.5%
Patients with deep molecular response MR4.0	68%
Patients with deep molecular response MR4.5 / MR5.0	44.3%

THE IMPACT OF INITIAL WBC COUNT ON THE MAJOR MOLECULAR RESPONSE RATES

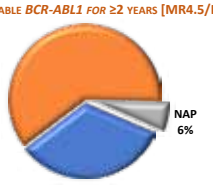
WBC [x10⁹/l]	Major molecular response [*BCR-ABL1/ABL1* ≤0.1%]		
	3rd Mo	6th Mo	12th Mo
<50	10/18 55.6%	18/20 90.0%	16/17 94.1%
50-250	15/41 36.6%	25/37 67.6%	33/39 84.65
>250	3/12 25.0%	7/18 38.8%	10/17 58.8%
P=	NS	0.004	0.022

- No differences in the MMR achievement on the 3rd month among the 3 groups were observed.
- The rates of MMR achievement in patients with extremely high WBC count at the diagnosis were significantly lower on the 6th and 12th.
- The proportion of patients with an MMR achieved on the increases in a time-dependent manner, regardless the initial WBC count

SUSTAINED UNDETECTABLE *BCR-ABL1* RATES AFTER THE 12TH MONTH

20 PTS WITH UNDETECTABLE *BCR-ABL1* FOR ≥2 YEARS [MR4.5/MR5.0] [17.7%]

33 (29.2%) PATIENTS ACHIEVED UNDETECTABLE *BCR-ABL1* STATUS



11 PTS WITH UNDETECTABLE *BCR-ABL1*, WHO MANIFESTED INTERMITTENTLY POSITIVE VALUES [9.7%]

Patients with a sustained deep molecular responses (at least MR4.5) with undetectable *BCR-ABL1* for over 2 years are considered to be candidates for stopping TKI-therapy.

THE IMPACT OF INITIAL WBC COUNT ON THE DEEP MOLECULAR RESPONSE RATES

WBC [x10⁹/l]	Deep molecular response [*BCR-ABL1/ABL1* ≤0.01%]		
	3rd Mo	6th Mo	12th Mo
<50	3/18 16.7%	10/20 50.0%	12/17 70.6%
50-250	5/41 12.2%	11/37 29.7%	19/38 50.0%
>250	0/12 0%	1/18 5.6%	3/17 17.6%
P=	NS	0.011	0.007

- No differences in the deep MR achievement on the 3rd month among the 3 groups were observed.
- The rates of deep MR achievement in patients with extremely high WBC count at the diagnosis were significantly lower on the 6th and 12th.
- The proportion of patients with an deep MR achieved on the increases in a time-dependent manner, regardless the initial WBC count

THE IMPACT OF EUTOS SCORE RISK GROUP ON THE MOLECULAR RESPONSE RATES

	Low Risk	High Risk	P=
Optimal MR at 3rd Mo	68/79 **[86.1%]**	5/8 **[62.5%]**	NS
Optimal MR at 6thMo	74/84 **[88.1%]**	1/5 **[20.0%]**	**0.002**
Major MR at 3rd Mo	30/79 **[38.0%]**	2/8 **[25.0%]**	NS
Major MR at 6th Mo	61/84 **[72.6%]**	1/5 **[20.0%]**	**0.028**
Major MR at 12thMo	79/90 **[87.8%]**	5/7 **[71.4%]**	NS
Deep MR at 3rd Mo	8/79 **[10.1%]**	1/8 **[25.0%]**	NS
Deep MR at 6th Mo	29/84 **[34.5%]**	1/5 **[20.0%]**	NS
Deep MR at 12thMo	45/90 **[50.0%]**	4/6 **[66.7%]**	NS
Undetectable *BCR-ABL1* at 6th Mo	13/84 **[15.4%]**	1/5 **[20.0%]**	NS
Undetectable *BCR-ABL1* at 12th Mo	18/89 **[20.2%]**	1/7 **[14.3%]**	NS
Undetectable *BCR-ABL1* after the 12th Mo	29/134 **[21.6%]**	2/8 **[25.0%]**	NS

ACHIEVEMENT OF DEEP MR4.5/MR5.0 RESPONSE DEPENDS ON THE WBC COUNT AND GENDER

Variable	Patients with deep (MR4.0/MR5.0) molecular response	P=
WBC <50x10^9/l	76.0% [19/25]	0.000
WBC 50-250x10^9/l	32.8% [20/61]	
WBC >250x10^9/l	8.3% [2/24]	
Low (EUTOS) risk	40.5% [53/131]	NS
High (EUTOS) risk	20.0% [2/10]	
B3a2 transcripts	41.2% [33/80]	NS
B2a2 transcripts	35.1% [26/74]	
Males	19.3% [16/83]	0.009
Females	24.0% [18/75]	
Age <65 years	35.0% [21/60]	NS
Age >65 years	43.3% [39/90]	

THE IMPACT OF GENDER ON THE MOLECULAR RESPONSE RATES

Gender	Optimal molecular response		
	3rd Mo *BCR-ABL1/ABL1* ≤10%	6th Mo *BCR-ABL1/ABL1* <1%	12th Mo *BCR-ABL1/ABL1* ≤0.1%
Females	36/43 83.7%	44/51 86.3%	45/48 94.0%
Males	44/51 86.3%	40/49 81.6%	45/60 75.0%
P=	NS	NS	0.01

Gender	Major molecular response [*BCR-ABL1/ABL1* ≤0.1%]		
	3rd Mo	6th Mo	12th Mo
Females	22/43 51.2%	37/51 72.5%	45/48 93.7%
Males	13/51 25.5%	30/49 61.2%	45/60 75.0%
P=	0.018	NS	0.01

ACHIEVEMENT OF DEEP MR4.5/MR5.0 RESPONSE DEPENDS ON THE PRESENSE OF EARLY MAJOR (OR BETTER) MOLECULAR RESPONSE

		Patients with undetectable BCR-ABL1	P=
3rd Month	Patient with optimal MR	65.7% [33/76]	0.07
	Patient without optimal MR	14.3% [2/14]	
	Patient with major MR	41.8% [23/35]	0.000
	Patient without major MR	21.8% [12/55]	
	Patient with deep MR	100.0% [10/10]	0.000
	Patient without deep MR	31.2% [25/80]	
6th Month	Patient with optimal MR	47.0% [39/83]	0.002
	Patient without optimal MR	6.2% [1/16]	
	Patient with major MR	50.3% [36/67]	0.000
	Patient without major MR	12.5% [4/32]	
	Patient with deep MR	80.6% [25/31]	0.000
	Patient without deep MR	22.1% [15/68]	

THE IMPACT OF THE *BCR-ABL1* TRANSCRIPT TYPE ON MOLECULAR RESPONSE AND PROGNOSIS

ACHIEVEMENT OF UNDETECTABLE *BCR-ABL1* mRNA DEPENDS ON BASELINE WBC COUNT

	Patients with undetectable BCR-ABL1	P=
WBC <50x10^9/l	33.3% [8/24]	0.034
WBC 50-250x10^9/l	20.3% [13/64]	
WBC >250x10^9/l	4.0% [1/25]	
Low (EUTOS) risk	21.8% [29/133]	NS
High (EUTOS) risk	20.0% [2/10]	
B3a2 transcripts	22.5% [18/80]	NS
B2a2 transcripts	21.5% [16/76]	
Males	19.3% [16/83]	NS
Females	24.0% [18/75]	
Age <65 years	16.7% [10/60]	NS
Age >65 years	25.0% [23/92]	

THE IMPACT OF THE *BCR-ABL1* TRANSCRIPT TYPE ON MOLECULAR RESPONSE RATES DURING 1ST YR

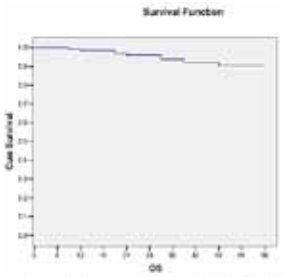
Transcripts	Optimal molecular response		
	3rd Mo *BCR-ABL1/ABL1* ≤10%	6th Mo *BCR-ABL1/ABL1* <1%	12th Mo *BCR-ABL1/ABL1* ≤0.1%
B3a2	42/49 85.7%	45/49 91.8%	52/57 91.2%
B2a2	37/44 84.1%	37/49 75.5%	37/50 74.0%
P=	NS	0.053	0.021

Transcripts	Major molecular response [*BCR-ABL1/ABL1* ≤0.1%]		
	3rd Mo	6th Mo	12th Mo
B3a2	22/49 44.9%	38/49 77.6%	52/57 91.2%
B2a2	12/44 27.3%	27/49 55.1%	37/50 74.0%
P=	0.089	0.032	0.021

ACHIEVEMENT OF UNDETECTABLE BCR-ABL1 mRNA DEPENDS ON THE PRESENCE OF EARLY MAJOR (OR BETTER) MOLECULAR RESPONSE

	Variable	Patients with undetectable BCR-ABL1	P=
3rd Month	Patient with optimal MR	18.8% [15/80]	NS
	Patient without optimal MR	7.7% [1/13]	
	Patient with major MR	34.3% [12/35]	0.001
	Patient without major MR	6.9% [4/58]	
	Patient with deep MR	70.0% [7/10]	0.000
	Patient without deep MR	10.8% [9/83]	
6th Month	Patient with optimal MR	25.3% [21/83]	NS
	Patient without optimal MR	6.3% [1/16]	
	Patient with major MR	28.8% [19/66]	0.039
	Patient without major MR	9.1% [3/33]	
	Patient with deep MR	40.0% [12/30]	0.008
	Patient without deep MR	14.5% [10/69]	

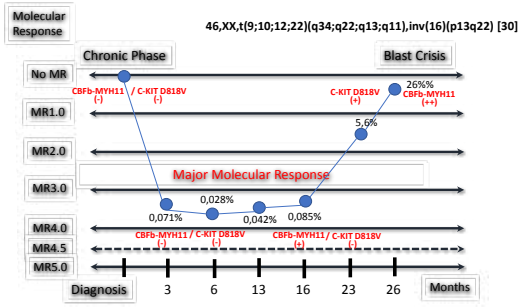
5-YEAR OVERALL SURVIVAL FOR THE WHOLE GROUP OF PATIENTS TREATED WITH NILOTINIB



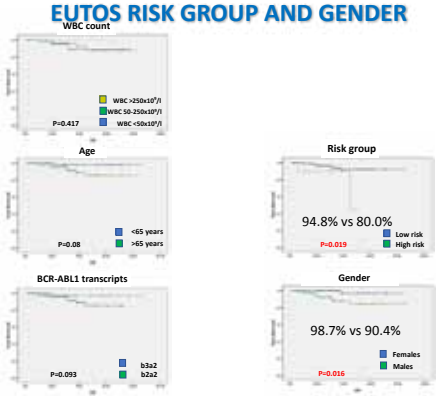
Cumulative 5-year OS: 91.0%

Case 1

SECONDARY ACQUISITION OF INV(16)/*CBFB-MYH11* & C-KIT mut IN A 55-YRS OLD WOMAN WITH A CML WITH A VARIANT t(9;10;12;22) (q34;q22;q13;q11) DURING MAJOR MR

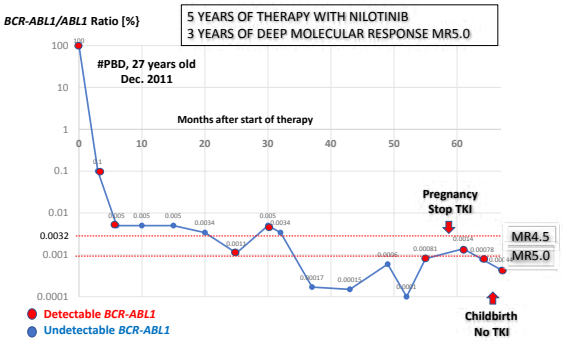


SURVIVAL WAS DIFFERENT ACCORDING TO THE EUTOS RISK GROUP AND GENDER

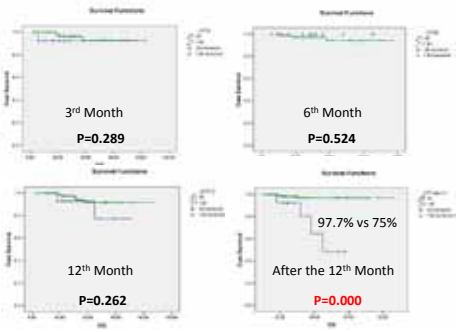


Case 2

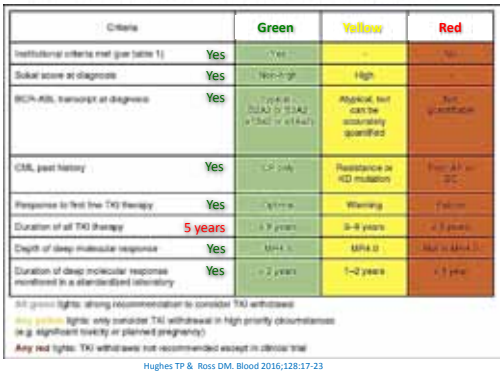
PERSISTANT DEEP MOLECULAR RESPONSE AFTER STOP OF NILOTINIB DUE TO PREGNANCY



SURVIVAL DEPENDS ON THE ACHIEVEMENT OF MAJOR MOLECULAR RESPONSE AFTER THE 12TH MONTH



CRITERIA TO GUIDE SELECTION OF PATIENTS SUITABLE FOR A TREATMENT DISCONTINUATION

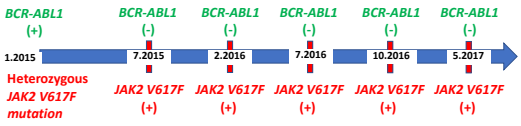


Hughes TP & Ross DM. Blood 2016;128:17-23

RARE CASES

Case 3

MOLECULAR RESPONSE TO NILOTINIB IN A PATIENT WITH *BCR-ABL1*(+) /*JAK2* V617F (+) MYELOPROLIFERATIVE NEOPLASM(S)



BCR-ABL1(+) / *JAK2-V617F*(+) CML
OR
CONCOMITANT *BCR-ABL1*(+) *CML* + *JAK2-V617F*(+) MPN
???

CONCLUSIONS

The results of our study confirm the previously reported data for the high efficiency of Nilotinib as a frontline therapy in regard to induction of early and deep molecular responses.

In concordance with several other reports, a better molecular response was observed in patients with lower WBC count at diagnosis, b3a2 type of BCR-ABL1 transcripts and in women.

THANK YOU FOR YOUR ATTENTION!

ANY QUESTIONS?

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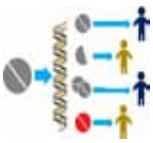
PHARMACOGENETIC STUDIES IN PATIENTS WITH CANCER

Hammoudeh Zora, Nikolova Dragomira, Balabanski Lubomir, Antonova Olga, Vazharova Radoslava, Weidner Sabine, Malinov Maxim, Toncheva Draga

Pharmacogenetic studies in patients with cancer

- DNA analysis on 28 patients with NSCLC (18 patients) and PTC (10 patients);
- NSCLC samples were extracted from formalin–fixed paraffin-embedded tissue (FFPET);
- PTC samples were extracted from blood;
- NGS technology
- Illumina platform, Cancer Sequencing Panel of 94 genes and 284 SNPs

Pharmacogenetic studies in patients with cancer



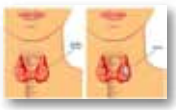
- ❖ Pharmacogenetic variants are inherited genetic differences that affect individual responses to drugs – therapeutic or adverse effects.
- ❖ Some single nucleotide polymorphisms (SNPs) could be related to individual variations in chemotherapy response.
- ❖ It is important to perform screening for pharmacogenetic variants before treatment of patients with chemotherapy or targeted therapy.

Pharmacogenetic studies in patients with cancer

DATA ANALYSIS

- Softgenetics NextGene Software (version 2.3.3);
- Mutation prediction software SIFT and PolyPhen-2;
- PharmGKB database (https://www.pharmgkb.org/) for known pharmacogenetic SNP variants associated with sensitivity to certain drugs.

Pharmacogenetic studies in patients with cancer



One of the most frequent types of thyroid malignancy are papillary thyroid cancer (PTC).

Non-small cell lung cancer (NSCLC) remains one of the major public health problems.

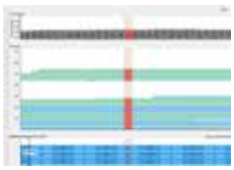
We need a personalized approaches to design a therapeutic strategy for cancer patients with any histology and most of all to clarify the role of pharmacogenetic alterations and overcome chemotherapy resistance in order to maximize the chances of patients for successful treatment.

Pharmacogenetic variants in cancer patients

Gene	SNP	Genotype	№ / %	Drug sensitivity
TP53	**rs1042522**	Heterozygote **GC**	4 (14%)	Increased risk of toxicity with cyclophosphamide and fluorouracil treatment
		Homozygote CC	18 (64%)	Decreased risk of toxicity with cyclophosphamide and fluorouracil treatment
ERCC2	**rs13181**	Heterozygote **TG**	11 (39%)	Higher survival when treated with platinum compounds
		Homozygote TT	1 (4%)	
		Homozygote **GG**	3 (11%)	Decreased survival when treated with platinum compounds
EGFR	**rs2227983**	Heterozygote **GA**	9 (32%)	Decreased risk of rash when treated with EGFR inhibitors
		Homozygote **AA**	3 (11%)	
XPC	**rs2228001**	Heterozygote **GT**	11 (39%)	Increased risk of toxicity with cisplatin treatment
		Homozygote TT	6 (21%)	Decreased risk of toxicity with cisplatin treatment
ERCC5	**rs17655**	Heterozygote **GC**	3 (11%)	Increased progression-free survival when treated with platinum-based chemotherapy

Pharmacogenetic studies in patients with cancer

- ➢ In this study, we use next generation sequencing (NGS) data for screening of variants known to be associated with drug sensitivity.
- ➢ We reveal SNPs which have been reported to be associated with sensitivity to chemotherapy and target therapy.



Pharmacogenetic studies in patients with cancer

Conclusions

We reveled five pharmacogenetic variants (rs13181, rs2227983, rs1042522, rs2228001, rs17655) in patients with NSCLC and PTC using cancer panel Illumina;

They are tolerated or benign and not associated with cancer development;

Type of polymorphism ⇒ Missense

Pharmacogenetic studies in patients with cancer

Conclusions

Two variants (rs2228001 and rs1042522) are associated with increased risk of toxicity with cisplatin, cyclophosphamide and fluorouracil treatment.

The cancer panel could be beneficial as a screening approach to detect pharmacogenetic variants associated with different therapeutic response in cancer patients.

12th Balkan Congress of Human Genetics

8th National Conference for Rare Diseases

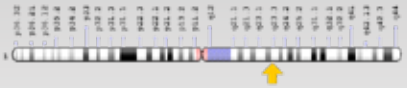
THANK YOU

ASSOCIATION OF Fc GAMMA RECEPTOR POLYMORPHISMS WITH AUTOIMMUNE HEMOLYTIC ANAEMIA

Marica Pavkovic, Rosica Angelovic, Tatjana Sotirova, Sonja Genadieva-Stavric, Aleksandar Petlickovski, Oliver Karanfilski, Lidija Cevreska, Aleksandar Stojanovic

INTRODUCTION

- **FCGR2A** is polymorphic and has two codominantly expressed alleles, FCGR2A-H131 and FCGR2A-R131. This polymorphic variation of FCGR2A is due to a single base substitution at nucleotide position 494. Nucleotide adenine (A) is substituted for guanine (G) and this results in a change of amino acid 131 histidine (H131) to arginine (R131). This polymorphism influences the affinity of the receptor.
- H131 (+494A) allele is characterized by a **high affinity** for human IgG2,
- R131 (+494G) arginine allele has a **low affinity** for human IgG2 [2] .



1. Van de Winkel et al. *Immunology Today* 1993 May; **14(5)**:215-22
2. Salmon JE, et al. *J Clin Invest* 1992 Apr; **89(4)**:1247-1281.

INTRODUCTION

- **Autoimmune hemolytic anemia(AIHA)** is the second most common autoimmune blood disorders with an estimated incidence in adults of 0.8–3 per 10^5/year, a prevalence of 17:100,000 and a mortality rate of 11%.
- AIHA is defined as increased destruction of red blood cells (RBC) due to autoantibodies specific for RBC autoantigens.
- AIHA may occur as primary (idiopathic) or secondary to other lymphoproliferative diseases like chronic lymphocytic leukemia (CLL) or autoimmune diseases.
- The etiology of AIHA remains unclear, but both genetic and environmental factors are thought to play role in the development of the disease.

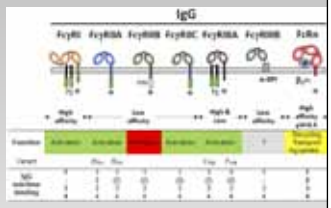
INTRODUCTION

- FCGR3A gene is mainly expressed on mononuclear phagocytes and has two polymorphic variant alleles: 158 valine (V158) and 158 phenylalanine (F158) due to a single base substitution of timidine to guanine (T to G) at nucleotide 559. This polymorphism is located in the proximal membrane domain (EC2) that influences ligand binding [1, 2] . **FCGR3A-158V allele variant has higher affinity for Fc fragment of IgG1 and IgG3 than 158F variant.**
- These Fc gamma receptor polymorphisms may influence ***antibody-mediated phagocytosis*** and ***antigen presentation activity***.
- These variations have been recently investigated in different autoimmune diseases like ***systemic lupus erythematosus (SLE), multiple sclerosis, Addison disease, heparin induced thrombocytopenia*** and they have been shown to be involved in disease susceptibility.

1. De Haas M, et al. *J Immunol* 1996; 156(8):2948-2955.
2. Tamm A & Schmidt RE. *J Immunol* 1996 ; 157(4): 1576-1581.

INTRODUCTION

- **Fc receptors** are glycoproteins and members of immunoglobulin superfamily of molecules. They are found on many different cells (neutrophils, macrophages, lymphocytes, platelets) and form a critical link between the humoral and cellular immune responses.
- Three different families of FcγR exist: FcγRI, FcγRII and FcγRIII and they are diverse in both their structure and function [1, 2] .



1. Van de Winkel et al. *Immunology Today* 1993 May; **14(5)**:215-22
2. Salmon JE, et al. *J Clin Invest* 1992 Apr; **89(4)**:1247-1281.

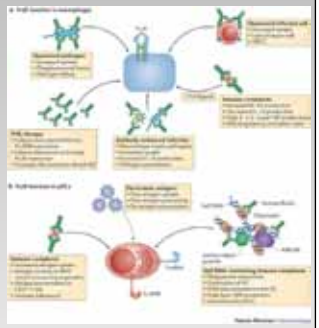
AIM

- The aim of this study was to examine the possible role and involvement of FCGR2A-131H/R and FCGR3A-158V/F polymorphisms in the etiology and development of autoimmune hemolytic anemia (AIHA) in Republic of Macedonia.

INTRODUCTION

Fcγ receptor function in macrophages are:
- Increased uptake of opsonized pathogen and pathogen killing
- Increased uptake of opsonized infected cell and ADCC
- Uptake of immune complexes with increased IL-10, IL-1, IL-6 and TNF production

Fcγ receptor function in Dendritic cells are:
- Increased antigen and immune complex uptake and antigen routing to MHCII processing organelles
- Antigen presentation to CD4+ cell
- Poor uptake, processing and presentation of particulate antigen can induce immune tolerance



METHODOLOGY

MATERIALS

- We have analyzed 70 adult patients with AIHA in total
- 35 patients with primary (idiopathic) AIHA and
- 35 patients with secondary AIHA and chronic lymphocytic leukemia (CLL)
- Controls were 120 healthy individuals

METHODS

- DNA was isolated from peripheral blood mononuclear cells and genotyping was performed by using PCR/RFLP methods already published[1, 2]. Written informed consent was obtained from all participants. The distribution of genotypes and allele frequencies were compared by using a chi-squared test or Fisher's exact test.

1. Foster CB, et al. *J Clin Invest* 1998; **102(12)**:2146-2155.
2. Carcao MD, et al. *Br J Haematol* 2003; **120(1)**:135-41.

RESULTS

Genotype distributions for FCGR2A-131H/R

Table 1. Genotype distributions for FCGR2A in patients with AIHA and control subjects.

FCGR2A-131H/R (+494A/G)	A/A	A/G	G/G	p value
AIHA patients (n=70)	45 (64.3%)	19 (27.1%)	6 (8.6%)	*0.048*
Controls (n=120)	55 (45.8%)	50 (41.6%)	15 (12.5%)	

RESULTS

Alelle frequencies for FCGR3A-158V/F

Table 4. Allele frequencies for FCGR3A in patients with AIHA and control subjects.

FCGR3A-158F/V (+559T/G)	T (F) allele (low affinity)	G (V) allele (high affinity)	p value
AIHA patients (n=70)	67 (47.9%)	73 (52.1%)	*0.007*
Controls (n=120)	150 (62.5%)	90 (37.5%)	

RESULTS

Allele frequencies for FCGR2A-131H/R

Table 2. Allele frequencies for FCGR2A in patients with AIHA and control subjects.

FCGR2A-131H/R (+494A/G)	A (H) allele (high affinity)	G (R) allele (low affinity)	p value
AIHA patients (n=70)	109 (**77.8%**)	31 (22.2%)	*0.028*
Controls (n=120)	160 (**66.6%**)	80 (33.3%)	

CONCLUSION

- Our results demonstrated significantly different genotype distribution for FCGR2A+494A/G in patients with AIHA and controls; ***p=0.048***.
- There was also significantly higher frequency of the high affinity FCGR2A-131H (+494A) allele in patients with AIHA compared with controls (77.8 versus 66.6%); ***p=0.028***.
- Statistical analysis of the genotype distribution for FCGR3A+559T/G showed significant difference between patients with AIHA and controls; ***p=0.025***.
- We also found significantly higher frequency of the high affinity FCGR3A-158V (+559G) allele in patients with AIHA compared with controls (52.1% versus 37.5%); ***p=0.007***.
- In conclusion our results, suggest possible role of both FCGR2A and FCGR3A polymorphism in the etiology and development of autoimmune hemolytic anemia, but further larger prospective studies are necessary to confirm these results.

RESULTS

Genotype distributions for FCGR3A-158V/F

Table 3. Genotype distributions for FCGR3A in patients with AIHA and control subjects.

FCGR3A-158F/V (+559T/G)	T/T	T/G	G/G	p value
AIHA patients (n=70)	22(31.4%)	23 (32.9%)	25 (35.7%)	*0.025*
Controls (n=120)	52(43.3%)	46 (38.3%)	22 (18.3%)	

Thank you for your attention!

ANALYSIS OF THE ASSOCIATION BETWEEN PAI-1 GENE 4G/5G POLYMORPHISM AND EFFICACY OF THROMBOLYTIC THERAPY IN PATIENTS WITH ISCHEMIC STROKE

Marija Dusanović Pjevic, Ljubica Vojvodic, Biljana Jekic, Gorica Maric, Milica Pesic, Milka Grk, Ljiljana Beslac Bumbasirevic, Katarina Kacar, Nela Maksimovic, Tatjana Damnjanovic

Aim

to determine whether 4G/5G polymorphism of the *PAI-1* gene modulates rt-PA therapy efficacy and occurrence of the hemorrhagic transformation

Introduction

Ischemic stroke (IS)

- sudden loss of blood circulation to an area of the brain.
- leading cause of death and morbidity worldwide.
- Common stroke signs and symptoms:
  - Sudden onset of hemiparesis, monoparesis
  - Hemisensory deficits
  - Dysarthria
  - Facial droop
  - Monocular or binocular visual loss

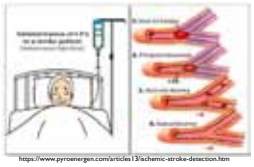
Methods

- 115 patients with IS who received rt-PA<3h
- The neurological outcome was measured with National Institutes of Health Stroke Scale (*NIHSS*) at hospital admission and a month after IS
- To evaluate functional recoveries after IS, modified Rankin scale (mRS) at hospital discharge and 3rd month was used
  - favorable outcome: 0-2
  - poor outcome: 3-6

Introduction

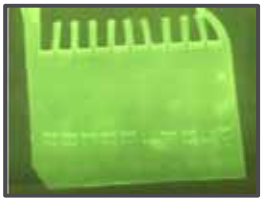
Thrombolytic therapy with recombinant tissue plasminogen activator (rt-PA)

- the only established acute thrombolytic treatment option in acute IS
- 2% to 5% of patients with acute IS receive rt-PA
- Hemorrhagic transformation(HT)



Methods

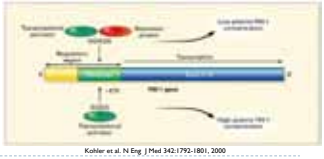
- Genotypisation was performed using PCR-RFLP method
- 100bp long PCR products were digested with restriction enzyme *BseI I*



Introduction

The plasminogen activator inhibitor-1 (PAI-1)

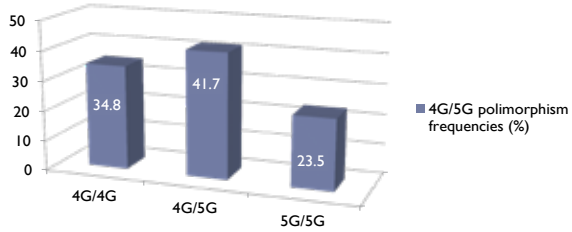
- main inhibitor of endogenously synthesized tPA
- *deletion/insertion polymorphism of a single guanine in the promoter region of PAI-1 (4G/5G) has been associated with plasma level of this molecule*



Kohler et al. N Eng J Med 342:1792-1801, 2000

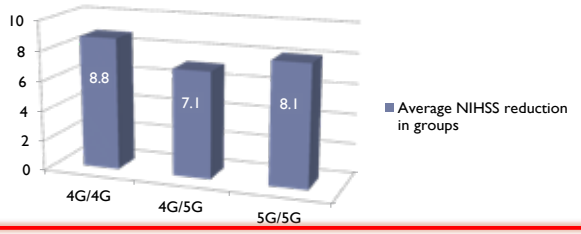
Results

Frequencies (%)



Results

Average NIHSS reduction



There was a significant reduction in NIHSS after thrombolytic therapy (p<0.001)

There was no significant difference between the groups with different genotypes

Results

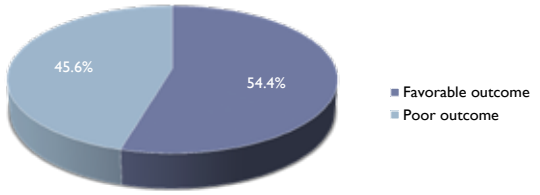
- The effect size (ES) analysis showed very large ES at one month after discharge for NIHSS, with the highest values for 4G/4G genotype

Table 1. Average values of NIHSS scores at hospital admission, 24h after rt-PA and at hospital discharge and the Effect size analysis in regard to genotype

NIHSS		At admission	After 24h	At discharge	After one month
Genotypes					
4G/4G	Score	13,5 (4,5)	8,5 (5,1)	6,2 (5,1)	4,7 (4,6)
	ES		1,07 (0,75-1,39) *	1,38 (1,07-1,69) *	1,89 (1,55-2,19) *
4G/5G	Score	12,2 (5,3)	9,3 (6,4)	7,3 (6,2)	4,1 (3,3)
	ES		0,63 (0,32-0,95) *	0,93 (0,62-1,23) *	1,51 (1,16-1,85) *
5G/5G	Score	12,3 (5,8)	8,9 (5,4)	5,8 (4,1)	3,7 (2,6)
	ES		0,74 (0,44-1,04) *	1,24 (0,89-1,60) *	1,80 (1,34-2,26) *

Results

modified Rankin scale (mRS)



There was no significant difference between the groups with different genotypes

Results

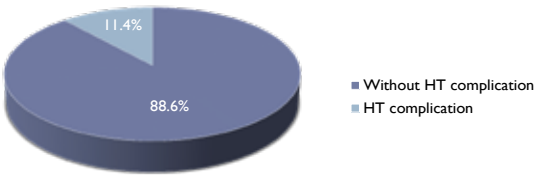
- The effect size (ES) analysis showed very large ES after three months for mRS, with the highest values for 4G/4G genotype

Table 1. Average values of mRS scores at hospital discharge, after one and three months after IS and the Effect size analysis in regard to genotype

mRS		At discharge	After one month	After 3 months
4G/4G	Score	2,9 (1,6)	2,3 (1,7)	2,0 (1,9)
	ES		0,73 (0,40-1,05) *	0,82 (0,46-1,18) *
4G/5G	Score	3,1 (1,8)	2,6 (1,9)	2,4 (1,9)
	ES		0,47 (0,20-0,73) *	0,54 (0,27-0,82) *
5G/5G	Score	2,8 (1,8)	2,7 (1,9)	2,7 (2,0)
	ES		0,37 (-0,11-0,85) *	0,40 (-0,05-0,84) *

Results

Hemorrhagic transformation (HT)



There was no significant difference between the groups with different genotypes

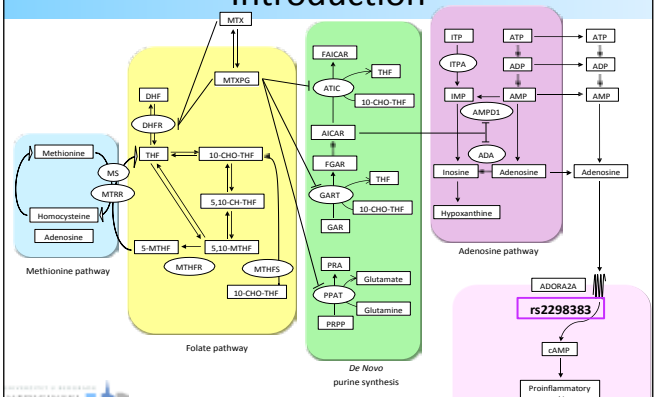
Conclusion

Our study did not show differences in outcome after rt-PA therapy between patients with different genotypes.

ASSOCIATION OF ADORA2A GENE RS2298383 POLYMORPHISM WITH EFFICACY/TOXICITY OF MTX

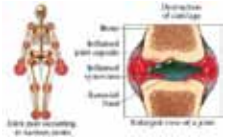
Milka Grk, Biljana Jekic, Vera Milic, Vita Dolzan, Marija Dusanovic Pjevic, Milica Pesic

Introduction



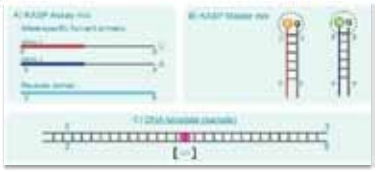
Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease characterized by joint swelling, tenderness and destruction of synovial joints, leading to severe disability and premature mortality.



Methods

- 126 RA patients
- rs2298383 polymorphism in *ADORA2A* gene
- KASP genotyping system



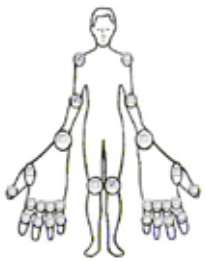
Introduction

A	Joint involvement	
	2-10 large joints	1
	1-3 small joints (with or without involvement of large joints)	2
	4-10 small joints (with or without involvement of large joints)	3
	10 joints (at least 1 small joint)	5
B	Serology	
	Negative RF and negative ACPA 0	0
	Low-positive RF or low-positive ACPA 2	2
	High-positive RF or high-positive ACPA 3	3
C	Acute-phase reactants	
	Normal CRP and normal ESR 0	0
	Abnormal CRP or abnormal ESR 1	1
D	Duration of symptoms	
	6 weeks 0	0
	6 weeks 1	1

2010 ACR/EULAR classification criteria

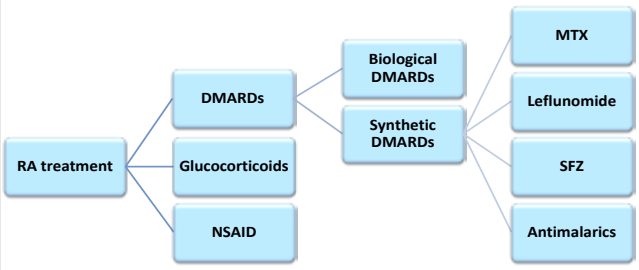
Methods

- ACR/EULAR criteria
- DAS28 = 0.56 * sqrt(tender28)
  + 0.28 * sqrt(swollen28)
  + 0.70 * ln(ESR)
  + 0.014 * GH
- At the begining
- After 6 months



http://www.4s-dawn.com/products/rheumatology/das28-calculator/

Introduction



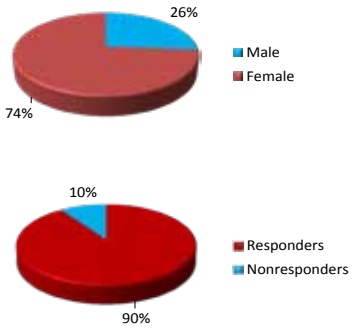
Methods

The efficacy of therapy was assessed after 6 months

EULAR Response Criteria			
Value of DAS28 at endpoint	Improvment in DAS28 from baseline		
	> 1,2	> 0,6 ≤ 1,2	≤ 0,6
≤3,2	Good	Moderte	None
> 3,2 ≤ 5,1			
> 5,1			

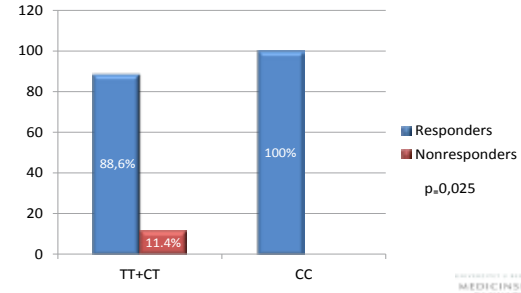
$rDAS=(rDAS_{beforeMTX} - rDAS_{after\ 6\ monts})/ rDAS_{beforeMTX}$

Results

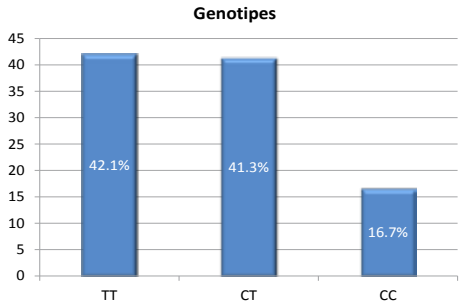


Results

Patients with CC genotype had significantly better response than T-allele carriers (p=0,025), when we used rDAS as a measure for MTX efficacy.



Results

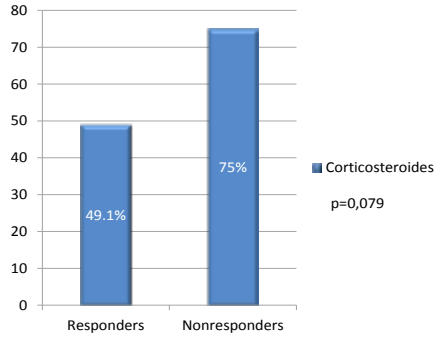


Conclusion

rs2298383 may influence efficacy of MTX treatment in RA patients.

There was no association between rs2298383 and adverse drug effects.

Results

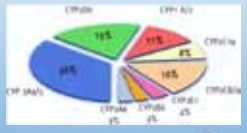


IDENTIFICATION OF CYP2C19*2 ALLELIC VARIANT IN HEALTHY ALBANIAN POPULATION

Suada Muçaj, Ethem Ruka, Grigor Zoraqi

PROPORTION OF DRUGS METABOLIZED BY CYP2C19 ENZYME

- CYP2C19 is important in the metabolism of many clinically relevant drugs, particularly for several pro drugs that require hepatic activation including clopidogrel. CYP2C19 protein metabolizes 8% of the prescription drugs in use today. Some of the commonly used and largest selling therapeutics for heart disease, peptidic ulcer disease, depression, hypertension treatments are all metabolized by CYP2C19 enzyme.



AIM OF THE STUDY

- This study aims to determine the frequency of genotypes CYP2C19*1/*1, CYP2C19*1/*2, CYP2C19*2/*2 and CYP2C19*1 and CYP2C19*2 allelic variants in healthy Albanian population.

SUBSTRATES OF CYP2C19 ENZYME

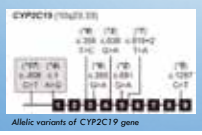
Proton Pump Inhibitors	Antiepileptics	Antidepressants	Anticancer	Antiplateled	Other
Lansoprazole/Dexlansoprazole	S-Mephenytoin	Amitriptyline	Nilutamide	Clopidogrel/Plavix	Progesterone
Omeprazole/Esomeprazole	Diazepam	Citalopram	Cyclophosphamide		Carisoprodol
Rabeprazole	Phenobarbital	Clomipramine	Neniposide		Indomethacin
Pantoprazole	Phenytoin	Moclobemide			Mephobarbital
	Primidone	Imipramine			Proguanil
		Desipramine			Hexobarbitol
		Sertraline			Nelfinavir
					Propranolol

CYP2C19 GENE

- The cytochrome P450, family 2, subfamily C, polypeptide 19 (CYP2C19) gene is located within a cluster of cytochrome P450 genes on chromosome 10q23.33.
- These gene is highly polymorphic as the other genes part of these cluster.
- Variation in drug plasma levels can either result in adverse effects and in on inappropriate therapeutic efficacy.

CYP2C19*2 ALELLIC VARIANT

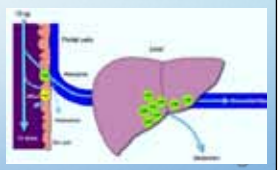
- The CYP2C19 gene has nine exons and is highly polymorphic, with over 35 variant star (*) alleles currently defined by the Human Cytochrome P450 Allele Nomenclature Committee.
- CYP2C19*2 and *3 are responsible for the majority of PM phenotypes in the metabolism of CYP2C19 substrate drugs.
- CYP2C19*2 allele has a single base pair mutation in exon 5 (681G>A), resulting an aberrant splice site. Creation of this splice site alters the reading frame of the mRNA beginning at amino acid 215 and produces a premature stop codon of 20 amino acids downstream resulting in a truncated and inactive CYP2C19 protein.
- CYP2C19*2 is the most common CYP2C19 loss-of-function allele. Its frequency among Caucasians varies from 8-16%.



Allelic variants of CYP2C19 gene

CYP2C19 ENZYME

- CYP2C19 enzyme is a member of Cytochrome P450 (CYP) family enzymes, a "super-family" of hem-containing enzymes involved in metabolization of different endogenous and exogenous substrates.
- CYP2C19 enzyme is located in hepatic endoplasmic reticulum. Generally involved in the metabolism of exogenous substances such as drugs. Play a critical role in determining the bioavailability of a wide range of important drugs
- These enzymes catalyzes many types of reactions, predominantly hydroxylations. CYP2C19 increase the polarity and aqueous solubility of a lipophilic xenobiotic usually via oxidation, thus facilitating its elimination from the body



RESPECTIVE GENOTYPES AND PHENOTYPES OF CYP2C19 GENE

Allelic variant of CP2C19 gene	Metabolizer phenotype	Genotypes
*1 homozygote	EM - extensive "normal" metabolizer	*1/*1
*2 carrier	IM - intermediate metabolizer	*1/*2
*2 homozygote	PM - poor metabolizer	*2/*2

Respective genotypes and phenotypes from the combination of CYP2C19 alleles

- The poor metabolizer phenotype which is inherited as an autosomal recessive trait represent 3-5% of Caucasians.

MATERIALS AND METHODS

1. Subjects:

- 100 healthy individuals from different regions of Albania.
- In our study we have male and female in equal proportion aged 37±14.48 years old.
- Blood samples were collected in K3/EDTA tubes.
- Genomic DNA was isolated from blood samples using DNA blood kit Qiagen, Hilden Germany and Invitrogen according to manufacturer instructions.

2. Genotyping of CYP2C19 gene :

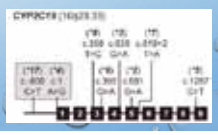
- PCR-RFLP protocol (Sviri et al.1999)

2a. PCR:

- PCR reaction of CYP2C19 *1 and CYP2C19 *2 was done in a final volume of 50 µl.
- 1 unit of Taq DNA polymerase (Life Technologies, Rockville, MD)
- 8.4 µl PCR mix buffer
- 34 µl H_2O
- 5 µl genomic DNA

EXPLAINING CYP2C19*1 ALLELE

- The supposed CYP2C19*1 allele comprise a heterogenous group of alleles that influence the metabolical activity of the enzyme.
- The CYP2C19*3 allele frequencies in most populations are below 1%
- Less frequent CYP2C19 alleles associated with absent or reduced enzyme activity are CYP2C19*4, *5, *6, *7, and *8. These variants typically have allele frequencies less than 1%.
- The CYP2C19*17 allele is associated with an increased CYP2C19 expression and activity. Its frequency is approximately 21% in Caucasians.
- Individuals who are classified as normal metabolizers from our study will be tested for the *17 allele a work that now is in progress.



•Primers

Forward Primer
5'-AATTACAACCAGAGCTTGGC-3'
Reverse Primer
5'-TATCACTTTCCATAAAAGCAAG-3'

•Amplification conditions

94 °C per 10 minutes
94 °C per 1 minute
60 °C per 1 minute } 35 cycles
72 °C per 1 minute

Last extension 72 °C per 10 minutes

2b. RFLP using SmaI restriction enzyme

*Schematic presentation of PCR-RFLP protocol for the determination of CYP2C19 *1/*1, *1/*2, *2/*2 genotypes*

FREQUENCY OF CYP2C19*2 ALLELES IN EUROPEAN POPULATIONS

Country	N	Frequency of CYP2C19*2 (%)
Albania	100	21.0
Greece	283	13.1
Italy	360	11.1
Slovenia	129	15.9
Turkey	404	12.0
Croatia	200	15.0
Germany	765	13.3
Slovakia	112	16.0
Romania	200	13.8
Macedonia	198	18.7
Hungary	221	12.9
Bosnia and Herzegovina	81	17.0
Bulgaria	142	16.2
France	359	17.8
Spain	570	14.9
Sweden	160	16.6

*Distribution of CYP2C19*2 allele among different ethnic groups*

RESULTS AND DISCUSSIONS

Identification of genotypes by gel electrophoresis

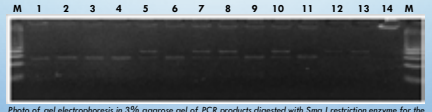
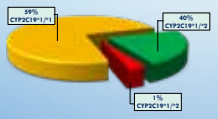


*Photo of gel electrophoresis in 3% agarose gel of PCR products digested with Sma I restriction enzyme for the identification of the genotypes CYP2C19*1/*1 and *1/*2*

CONCLUSIONS

- In this study was successfully applied the PCR-RFLP protocol for the identification of CYP2C19 genotypes: CYP2C19*1/*1, CYP2C19*1/*2 and CYP2C19*2/*2
- The frequencies of CYP2C19*1/*1, CYP2C19*1/*2 and CYP2C19*2/*2 genotype was respectively 59%, 40% and 1%
- The frequency of CYP2C19*1 allele was found to be 79% while the frequency of CYP2C19*2 allele was found to be 21%
- The frequency of CYP2C19*2 allele (21%) found in Albanian population is the highest found in Europe.
- More than 1/3 of Albanian population are intermediate metabolizers. Identification of genetic variants of CYP2C19 gene could give useful indications about the correct use of drugs in patients therapy according to CYP2C19 individual genotype.

FREQUENCIES OF CYP2C19 GENOTYPES AND THE FREQUENCIES OF CYP2C19*1 AND CYP2C19*2 ALLELES IN ALBANIAN POPULATION



The frequencies of CYP2C19*1/*1, *1/*2, *2/*2 genotypes

The frequencies of CYP2C19*1 and CYP2C19*2 alleles

SESSION 7-II

Moderators: Ivaylo Tournev, Radostina Simeonova

Oral presentations:

- **Autosomal recessive neurologic disorders among Bulgarian Muslims**
  T. Chamova
- **Clinical and genetic study of Huntington's disease: the 9 years of experience of our team**
  S. Zhelyazkova
- **Genetic forms of amyotrophic lateral sclerosis in Bulgaria**
  S. Sarafov
- **Charcot-Marie-Tooth: ethnic differences, genetic and clinical spectrum in Bulgaria**
  K. Kastreva
- **The genetic epidemiology of hereditary spastic paraplegias in Bulgaria**
  J. Samuel
- **NGS approach in cases with congenital neuromuscular disorders**
  T. Todorov
- **Screening for SNPs in a set of drug-metabolizing enzymes in Bulgarian population**
  O. Antonova
- **NGS sequencing in service of neurogenetics in Bulgaria**
  D. Kachakova

AUTOSOMAL RECESSIVE NEUROLOGIC DISORDERS AMONG BULGARIAN MUSLIMS

Teodora Chamova, Ivaylo Tournev

TCAP associated phenotypes

LGMD

Onset- second to third decade of life
Proximal weakness in lower limbs, accompanied or followed by weakness of tibialis anterior muscles
Calf hypertrophy, scapular winging, and joint contractures

Congenital muscular dystrophy

Delayed motor development
Predominant proximal muscle weakness
Calf pseudohypertrophy
Mild joint hyperlaxity
Rigidity of the lumbar spine
Achille tendon contractures

Dilated and hypertrophic cardiomyopathy

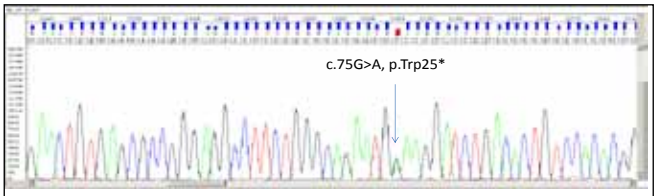
AD trait of inheritance
Missense mutations

Autosomal-recessive disorders

- More frequent among
  - Ethnic minorities
  - Religious minorities

In Bulgaria- more than 15 AR disorders among Roma/Gypsy population , caused either new genes or private mutations

- Hereditary sensory and motor polyneuropathies: CMT 4D (*NDRG1*), CMT 4G (*HK1*), CCFDN (*CTDP1*)
- Myopathies: GNE- myopathy, LGMD 2C (*SGCG*)
- Congenital myasthenic syndromes: CMS 1A (*1267delG*)
- Hereditary ataxias: GRM1, ANO10, VLDLR, POLR3A
- Hereditary spastic paraparesis- *SPG7*
- Metabolic disorders- PDHX, NPB (*SMPD1*), GALK1
- Congenital glaucoma- CYP1B1, LTBP2



LGMD 2G in Bulgaria

Autosomal-recessive disorders among Bulgarian Muslims

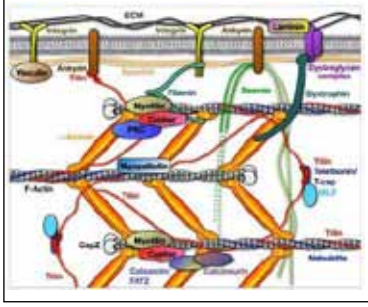
- Limb-girdle muscular dystrophy 2G (LGMD 2G)
- Variant of ataxia-telangiectasia, Louis-Bar disease
- Hereditary sensory and motor neuropathy Lom type (CMT 4D)

Clinical phenotype of the Bulgarian LGMD 2G patients

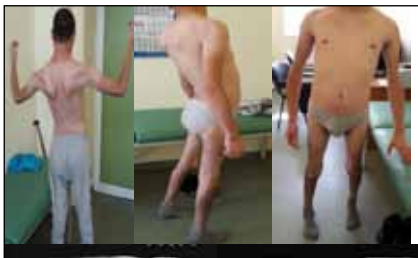
- 17 affected (11 male and 6 female) from 11 pedigrees
- Mean age at onset 18.26 ± 4.04 y, (10-25 y)
- Initial manifestations:
  - Proximal muscle weakness in the lower limbs
  - Accompanied or followed by difficulties in ankle dorsiflexion
- Proximal muscles of the upper limbs- involved 5-9 years after the disease onset

Limb-girdle muscular dystrophy 2G (LGMD 2G)

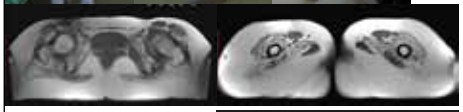
- AR disorder
- Mutations in *TCAP* gene on chromosome 17q12



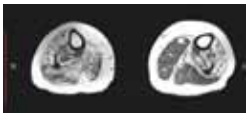
- Telethonin- highly expressed in skeletal and cardiac muscles
- Located at the Z-disc
- It provides binding sites to link titin and other Z-disc associated proteins during sarcomere assembly



- Hypotrophies of thigh muscles and tibialis anterior muscle
- Bilateral pes equinovarus, more pronounced on the right
- Scapular winging
- Calf hypotrophies



- Relative preservation of sartorius and gracilis muscles



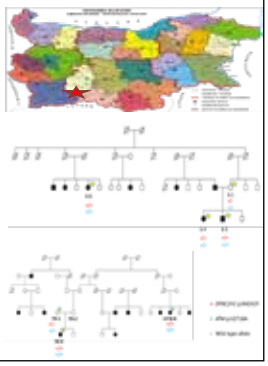
- Heavy fibro-fatty changes of glutei maximi muscles, tensor fasciae latae muscles and obturator internus muscles
- Heavy fibro-fatty replacement of anterior compartment and soleus muscles bilaterally

When to suspect LGMD 2G?

- Bulgarian Muslims with LGMD with predominant proximal and distal weakness in the lower limbs
- AR inheritance or sporadic cases
- Onset in second-third decade
- Increased CK

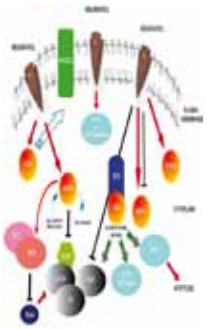
Variant of A-T with primary appearing tremor-dystonia

- 14 patients, belonging to two big Bulgarian muslim pedigrees, with data of more than 25 affected from four consequent generations
- Evaluations- neurological examination, brain MRI was performed in 7, PET-CT- in 1, neuroophthalmological assessment- in 9
- Exome sequencing was performed in 8- 5 affected and three healthy relatives



Ataxia–telangiectasia (A-T)

- Autosomal recessive disorder due to mutations in the *ATM*-gene
- ATM protein, a nuclear serine/threonine protein kinase
- Crucial in the cellular response to DNA damage such as double-strand breaks
- Classic A-T- by two truncating ATM mutations with total loss of ATM protein
- Missense mutations- associated with a less severe disease course- variant A-T

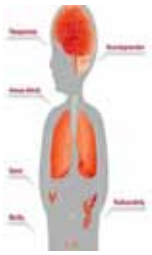


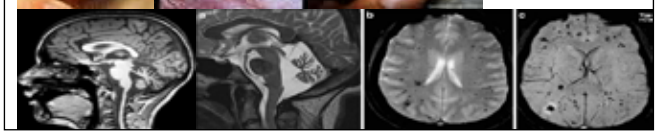
Variant of A-T with primary appearing tremor-dystonia

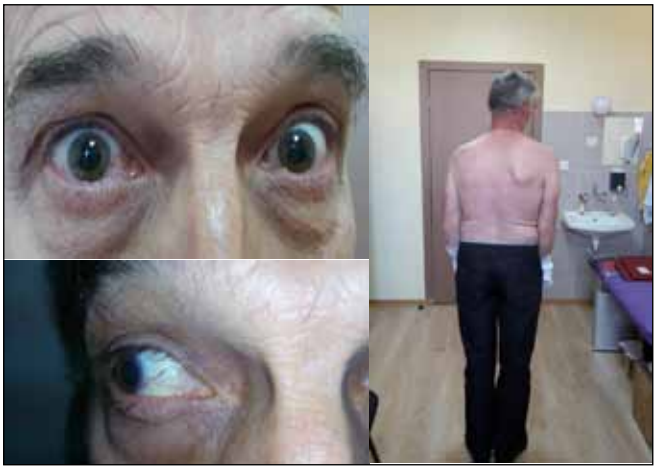
- Age at onset in our group 14 days and 20 years
- Main symptoms:
  - Dystonic and choreic hyperkinesias, more prominent in the upper limbs and the neck
  - Dystonic disarthria and dysphagia
  - Slowly progressive clinical course
- Brain imaging- normal
- Increased AFP
- Homozygous mutation p.V2716A in *ATM*-gene

Classic ataxia–telangiectasia (A-T)

- Progressive childhood-onset cerebellar ataxia
- Oculomotor apraxia
- Telangiectasias of the conjunctivae
- Hypersensitivity to ionizing radiation
- Immunodeficiency
- Increased alpha-fetoprotein







Classical A-T		Variant A-T
1-5 y	Onset	0-34 y
Ataxia of stance and gait	Initial symptoms	Chorea, atetosis, tremor
+	Ataxia	+/-
+	Cerebellar dysarthria	+/-
+	Oculomotor apraxia	+
+	Nystagmus	+/-
+/-	Choreic and dystonic hyperkinesia	+
+/- (25%)	Myoclonus	+/-
+	Tremor	+
8-10 y	Loss of ambulation	Broad variation
+ around 6 y	Ocular and cutaneous teleangiectasias	Rare
Growth delay Delay in sexual development Diabetes melitus	Endocrine dysfunction	Rarely delayed sexual development
Lymphomas, leukemia, breast, ovarian, stomach cancers, melanimas	Malignancies	Lymphomas, leukemia, breast, ovarian, stomach cancers, melanimas
Deficiency of IgA, IgG2 and IgG4	Immune deficiency	Decreased IgG2 and IgG4
+	Restrictive respiratory insufficiency	-
+	Increased AFP	+
Axonal sensory-motor polyneuropathy	NCS	Axonal sensory-motor polyneuropathy
Cerebellar atrophy	Brain MRI	Normal or cerebellar atrophy

Variant of A-T with primary appearing tremor-dystonia

- Mutations in *ATM* gene- very broad phenotype
- The disease may appear as dystonia, especially of early onset, without frank cerebellar involvement and also normal cerebral imaging
- A-T should be considered in all patients with unexplained, even mild movement disorders
- AFP levels to be measured

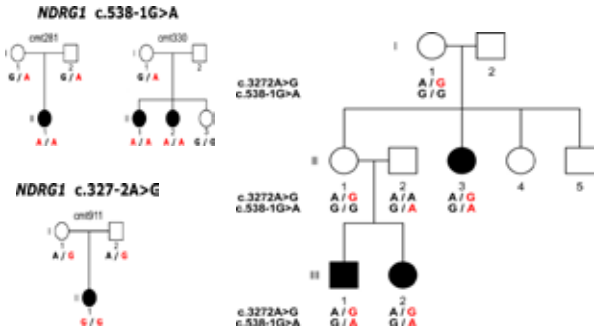
Hereditary sensory and motor neuropathy- Lom (CMT 4D)

- Charcot–Marie–Tooth (CMT) disease- a genetically heterogeneous group of disorders of the peripheral nervous system, with a quite homogeneous clinical phenotype (progressive distal muscle weakness and atrophy, foot deformities, distal sensory loss and usually decreased tendon reflexes)
- Around 10% of the cases with AR trait of inheritance
- HSMN Lom (CMT 4D)- identified for the first time in Bulgarian Roma/Gypsy from Lom, due to homozygous mutation with a founder effect R148X in *NDRG1*

N-myc Downstream-Regulated Gene 1 Is Mutated in Hereditary Motor and Sensory Neuropathy–Lom

Luba Kalaydjieva, David Gresham, Rebecca Gooding, Lisa Heather, Frank Baas, Rosalein de Jonge, Karin Blechschmidt, Dora Angelicheva, David Chandler, Penelope Worsley, Andre Rosenthal, Rosalind H. M. King, and P. K. Thomas

CMT 4D among Bulgarian Muslims



Hereditary sensory and motor neuropathy- Lom (CMT 4D)

- *NDRG1* (N-myc downstream regulated gene 1)
- NDRG1 is expressed at particularly high levels in the Schwann cell
- Role in the peripheral nervous system, possibly in Schwann cell signaling necessary for axonal survival, lipid synthesis and myelination
- Cell functions:
  - Embriogenesis
  - Cell growth
  - Differentiation
  - Cell migration
  - Stress response
  - Immunity
  - Tumorogenesis

CMT 4D among Bulgarian Muslims

- c.327-2 A>G
  - leads to the skipping of exon 6
  - alternative transcript starting at c.352 (25bp further in exon 6)
- c.538-1 G>A
  - leads to the skipping of exon 9
  - alternative transcript starting at c.545 (7bp further in exon 9) leading to a frameshift

Hereditary motor and sensory neuropathy—Lom, a novel demyelinating neuropathy associated with deafness in gypsies
Clinical, electrophysiological and nerve biopsy findings

Luba Kalaydjieva, Amelia Nikolova, Ivo Turnev, Julia Petrova, Anna Hristova, Boryana Ishpekova, Iva Petkova, Alexander Shmarov, Stella Stancheva, L. Middleton, Luciano Merlini, A. Tregu, J. R. Muddle, R. H. M. King and P. K. Thomas

- Weakness in the distal parts of the lower limbs and gait disorder in the first decade of life
- Upper limb involvement in the second decade
- Motor involvement is greater than sensory
- All sensory modalities are affected
- Foot deformity (pes cavus and equinovarus), scoliosis
- Sensorineural deafness in the third decade
- NCS with severe reduction of CV velocity and prolonged DL

CMT 4D among Bulgarian Muslims

- IVS8-1 G>A и IVS6-2 T>G mutations in *NDGR1*-gene – causing demyelinating CMT with onset in the first decade in Bulgarian Muslims
- Early clinical diagnosis and genetic verifications are important for genetic counseling
- The carrier rate of both mutations to be elucidated

CMT 4D among Bulgarian Muslims

- 8 patients from 5 pedigrees
- Age at onset between 3 and 9 y with weakness in the distal parts of the lower limbs, impaired gait
- Weakness in the distal muscles of the upper limbs- between 10-16 y
- Different degree of hearing impairment in 6/8 on audiometry
- Increased latencies of BAEP's

Conclusion

- Only 2% of the Bulgarian ethnicity belong to the Muslim religious minority
- The carrier rate of
  - c.75G>A, p.Trp25* in *TCAP*
  - p.V2716A *in ATM*
  - IVS8-1 G>A и IVS6-2 T>G *in NDGR1*

  can be high and is to be measured
- Probably due to cumulative effects of historical endogamy in this closed religious minority

CLINICAL AND GENETIC STUDY OF HUNTINGTON'S DISEASE: THE 9 YEARS OF EXPERIENCE OF OUR TEAM

Zhelyazkova S, Chamova T, Sarafov S, Cherninkova S, Bichev S, Savov A, Todorova A, Tournev I

- DNA testing for Huntington's disease was performed at the National Genetic laboratory and Genica laboratories.

The local database contains demographic (age at onset, age at referral, place of residence and ethnic origin), clinical and genetic HD patient data.

Figure 1. Different regional distribution of the patients

Huntington's disease is a devastating neurodegenerative disorder with an incidence of 0.38 per 100,000 per year and a prevalence of 5.70 per 100,000 in Europe.

- It is characterized by chorea, psychiatric disturbances and dementia.
- Huntington disease is caused by the expansion of repeating CAG triplet series (>35 CAG) in the huntington gene on chromosome 4, resulting in a protein with an abnormally long polyglutamine sequence.
- It is inherited in an autosomal dominant manner.
- Increasing CAG repeats accelerate age at disease onset, hence directly relating to disease severity.
- The clinical course of the disease is relentlessly progressive, the average survival period after diagnosis being 10-20 years.

RESULTS

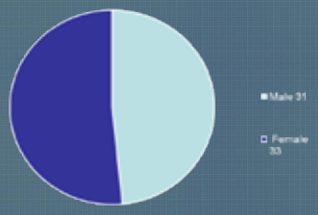
- A total of 80 symptomatic individuals from 60 families were evaluated.
- Their origins varied from different regions in Bulgaria, belonging to two large ethnic groups – Bulgarian and Turkish.
- There are no patients in our database from the third largest ethnic group in Bulgaria- the Roma.
- According to the family history, 60 additional members of these families also have signs of HD.
- The diagnosis was confirmed with genetic testing in 54 symptomatic and 10 asymptomatic individuals.
- Maternal inheritance was observed in 28 patients and paternal inheritance in 18 patients.

Objectives

Our aim is to determine the demographic, clinical, and molecular genetic features of HD in Bulgaria and to create a local database of patients with the disease.

- In 4 affected there was no known family history, while 14 others had insufficient ancestral information.

The sex distribution of the 64 patients is shown in Figure 2:



Methods

- Medical history, clinical and neurological evaluations were performed on patients during hospital admission.
- The stage of the disease was determined using the Total Functional Capacity Rating scale (Shoulson and Fahn Staging Scale)
- Formal neuropsychological assessments were performed using the Mini-Mental State Examination (MMSE) and Standard Raven progressive Matrices. Patient performance scores were determined based on standard procedure outlines from published test manuals.
- Magnetic resonance/ computed tomography imaging of brain

The age at onset of the clinical symptoms and age at referral to our Clinic for diagnosis of the 54 affected individuals are shown on Table 1:

	Number of patients
Age at onset (years)	
0-19	0
20-39	14
40-59	33
>59	7
Age at referral (years)	
0-19	0
20-39	12
40-59	32
>59	10

- All affected had CAG repeats >35.
- The age at disease onset varied from 22 to 66 years.
- Motor onset was the most common (with chorea affecting the four extremities and facial muscles, and gait disturbances), followed by mixed onset (motor and cognitive signs) and neuropsychiatric symptoms (depression and anxiety).
- Cognitive decline, which vary between mild cognitive impairment and severe dementia, was observed in all 54 symptomatic patients.
- In most patients, the Huntington disease staging based on the Total Functional Capacity Rating scale, varied between II and III at the time of evaluation in our Clinic. Only one patient was at stage V, ten years after disease onset.

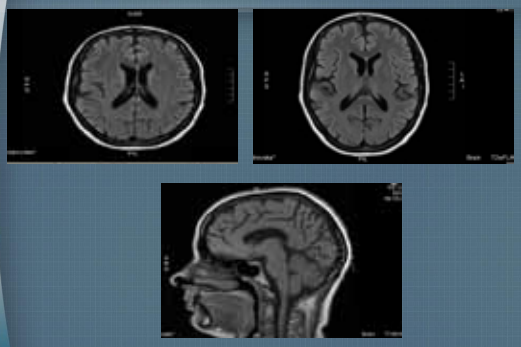
Conclusions

- The HD phenotype and genotype of our patients are similar to those reported in literature.
- We also observed both anticipation and genome imprinting.
- Creating a local database for patients with the disease will enable systematic patient monitoring, as well as help in implementation of new therapeutic strategies aimed at improving the quality of life in our patients.

- Brain MRI/ CT scans were performed in a total of 29 patients. Abnormal findings of cerebral atrophy with lateral venricular enlargement, dilatation of the subarachnoid spaces and T2-hyperintense lesions in the frontal cortex (**Fig.3**) were reported in 23 affected.
- Most patients were on treatment for hyperkinesis with Haloperidol.

Thank you for your attention!

Figure 3. Axial FLAIR and sagital T1 MRI images of a 44 yr female patient, showing diffuse brain atrophy and hyperintense lesions in the frontal cortex.



GENETIC FORMS OF AMYOTROPHIC LATERAL SCLEROSIS IN BULARIA

Stayko Sarafov, Julie van der Zee, Lubina Dilen, Stojan Bichev, Andrey Kirov, Tihomir Todorov, Albena Todorova, Albena Jordanova, Christine Van Broeckhoven, Ivailo Tournev

BACKGROUND

Amyotrophic Lateral Sclerosis (ALS) – a neurodegenerative disease of the nervous system, Dominantly sporadic

About 7% are familial genetic forms with mutations.

More than thirty different genes have become known around the world.

In Bulgarian literature the first three ALS families (not DNA verified) were described in 1963 by Prof.I.Georgiev.

OBJECTIVES

Since 1990 we have established a hospital register for ALS patients so far with more than 1000 sporadic and 10 familial cases with proven mutations, three other familial cases did have DNA analysis.

This study aimed at performing extensive mutation analysis of 300 patients from a cohort collected since 2004 and subsequent genotype-phenotype correlations. We have amassed DNA sequencing of all newly diagnosed patients since 2011.

METHODS

All patients were clinically and electrophysiological diagnosed according to the El Escorial revisited criteria. Genetic testing of seven ALS-associated genes (*SOD1, FUS, ANG, TAR-43, UBQLN1, C9orf72, TBK1*), was performed by Sanger sequencing or repeat analysis.

RESULTS

1. Three families have proven mutations in the *SOD1* gene: Leu106Val, Leu144Phe and Leu38Val. One patient carried the Cys146X mutation.
2. Three families have a G4C2-repeat expansion in the C9orf72 gene. One sporadic case carried the same expansion C9orf72 – G4C2.
3. One family has mutation in *TBK1* gene.
4. One patients was with a polymorphism (Pro440Leu) in the *UBQLN2* gene.

Clinical-genetic correlations for SOD1

Every one of the affected families has an own clinical phenotype different from the others in regard to:

Clinical-genetic correlations for SOD1

1. Age of onset of the affected members.

Could be the same / approximately the same (Leu144Phe, Leu38Val) or different (Leu106Val) for the next generations – usually lower due an anticipation. In the family with Leu106Val there are 3 strong cases of anticipation with different age of onset between the parents and kids, respectively 11,4 / 21,7 & 38,3 years. In the last case the first affected was the son, 38,3 years latter his mother was affected. Father and son (Leu144Phe) affected simultaneously. The sequence of the disease onset is mixed in the different members (Leu106Val) and from few sibs the first affected could be the youngest.

Clinical-genetic correlations for SOD1

2. Average age of onset of the affected patient's generation (Leu144Phe, Leu38Val) and next generations (Leu38Val). For Leu106Val it is highest for the first affected generation, lower for the second, third and forth due to anticipation: 50,9; 49,5; 42,6; 30,3 years.
3. Onset region.

For the most patients it is the lower limbs, but for some of them the disease started from the upper limbs or the brain steam.

Clinical-genetic correlations for SOD1

4. First symptoms from the onset region.
For the most patients with onset from the limbs it is muscles weakness progressing in palsy. Two members from different generations (uncle and nephew) with Leu106Val had fascilulations more than 20 years before the muscles weakness.
5. Survival
Approximately the same for patients from the same and from different generations (Leu144Phe – 4 years; Leu38Val – 12 months). For patients with Leu106Val it varies between 7 and 15 months.

Clinical-genetic correlations for C9orf72

5. Survival
Could be the same or varying from 1 to 5 years between the patients from the same or different generations
6. Patients were with the same or different gender.
7. Different clinical manifestations of the disease in patients from the same or different generations: one case of aunt and nephew. His mother was free of symptoms. The nephew was with brain stem onset and FTD, the aunt was with injury of the lower motor neuron only.

Clinical-genetic correlations for SOD1

6. For Leu106Val, crossing the gender between a sick parent and a sick child, when the parent has two children with different gender: sick father – sick daughter, sick mother – sick son.
7. Patients with Leu144Phe were males only.
8. Patients with Leu38Val are brother and sister
9. In those three mutations in SOD1 gene, Leu participates at different positions.

Genetic clinical correlations for TBK1

One family of brother and sister.
Sick is the brother, onset at age of 47,5.
Mother with the similar symptoms and age of onset.
Clinical events of lower motor neuron only.
Survival – more than 8 years, still alive.
Sister – with slightly pronounced psychiatric symptoms and mental decline, no motor symptoms.
First cousin (female) on maternal line with psychiatric symptoms – no DNA test performed yet.

Clinical-genetic correlations for C9orf72

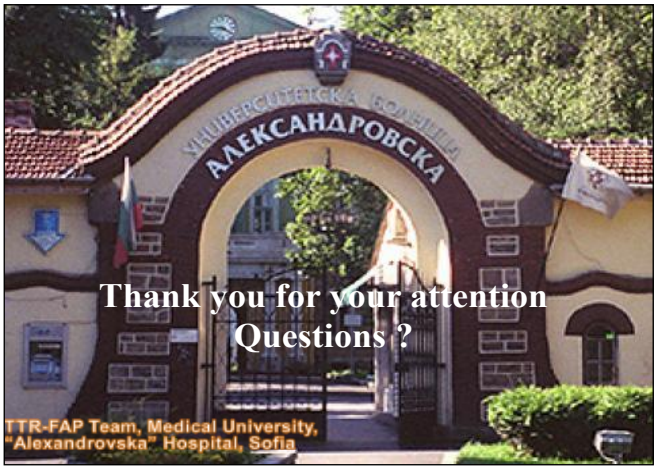
1. Age of onset of the affected members.
The same of lowest in the next generation due to anticipation. A case with firstly affected daughter (32,2 age), 4,6 years after her death, mother (59,8 age) was affected.
A similar case of simultaneously affected mother and daughter was described in the Bulgarian scientific literature in 1963.
One case of affected father, his sons reveled the disease one by one.

CONCLUSION

The variations in the clinical presentations, onset and survival for patients with C9orf72 and TBK1 are more expressed than for the patients with mutations in SOD1.
Families with SOD1 present clinically only with motor symptoms. The onset is often from the legs.
Patients with C9orf72 have combination of motor symptoms and FTD
For TBK1 the combination is – motor and psychiatric symptoms.
For C9orf72 and TBK1 there are changed clinical phenotype in different members from the same or different generations – motor symptoms in the one, non-motor symptoms in the other.

Clinical-genetic correlations for C9orf72

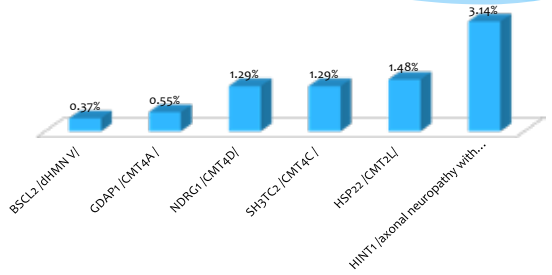
2. Varying age of onset for the affected members of the same or different generations
3. Onset region.
For the most patients it is the brain stem, followed by the lower limbs, but for some of them.
4. First symptoms from the onset region.
Speech disturbances and muscles weakness progressing in palsy. Often event is Fronto-Temporal Dementia varying from slightly to moderate cognitive disturbances and behavioral disturbance with hallucinations.



CARCOT-MARIE-TOOTH DISEASE: ETHNIC DIFFERENCES, GENETIC AND CLINICAL SPECTRUM IN BULGARIA

Kristina Kastreva, Teodora Chamova, Boryana Ishpekova, Velina Guergueltcheva, Stoyan Bichev, Ivan Litvinenko, Lyudmila Angelova, Veneta Bojinova, Luba Kalaydjieva, Albena Jordanova, Ivailo Tournev

Bulgarian ethnic group – other less common forms of CMT



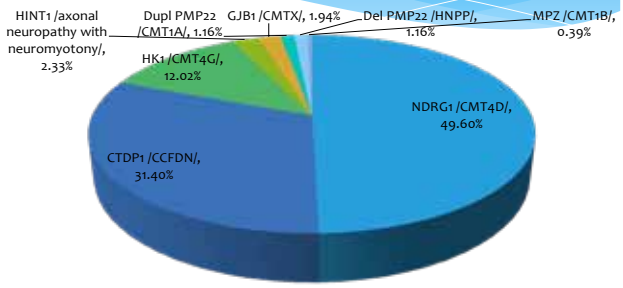
* **Background and purpose:**

Bulgarian population consists of three major ethnicities: Bulgarians, Turks and Roma. The purpose of the study is to determine the genetic and clinical spectrum of CMT in the different ethnic groups in our country.

* **Methods:**

The survey includes all of the patients with diagnosis of CMT in our **neuromuscular center in the University Hospital "Alexandrovska" in Sofia** and patients data that was collected in **National Genetic Laboratory**, which is a referral center for CMT genetic testing for Bulgaria. Patients underwent neurological, neurophysiological, clinical genealogical and genetic testing. In total data from 831 patients with genetically confirmed diagnosis of CMT was analyzed.

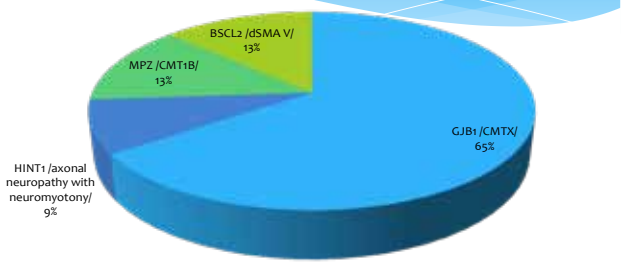
Roma ethnic group (n=258)



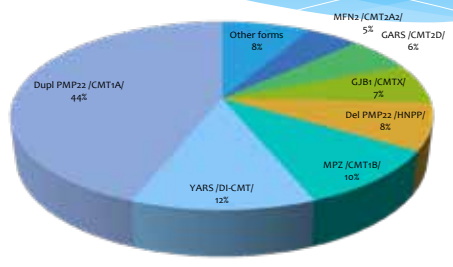
Results:

* Ethnic distribution of genetically confirmed patients: **542 Bulgarians, 258 Roma, and 31 Turks.**
* **16 different forms of CMT, 15 different genes**
  * 1) AD demyelinating HPN - CMT1A, HNPP, CMT1B;
  * 2) AR demyelinating neuropathies – CMT4D, CCFDN, CMT4G, CMT4C, CMT4A;
  * 3) AD axonal polyneuropathies - CMT2A;
  * 4) AR axonal polyneuropathy with neuromyotonia with defects in HINT1 gene;
  * 5) X-linked polyneuropathies – CMTX1;
  * 6) Dominant intermediate CMT, caused by defects in YARS gene – DI-CMTC;
  * 7) Hereditary motor neuropathies, related to mutations in GARS (CMT2D), HSP22 (CMT2L), BSCL2 (CMT5B), IGHMBP2 (CMT2S);

Turk ethnic group (n=31)



Bulgarian ethnic group (n=542)

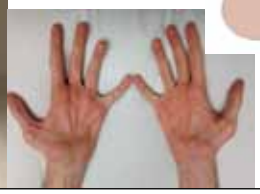


Inheritance patterns

* AD CMT – 55%
* AR CMT – 33%
* X-linked forms of CMT– 6 %
* Sporadic cases (no family history of the disease) – 6%

Clinical spectrum

- sensory-motor neuropathies
- predominantly motor neuropathies
- neuropathies with neuromyotonia



Conclusions:

- Duplication in **PMP22** gene is not only the most common mutation in studied clinic population, but also was identified in all three ethnic groups, as well as mutations in **MPZ**, **GJB1** and **HINT1** genes.
- Several **rare forms of CMT**, caused by mutations in GDAP1, YARS, BSCL2, IGHMBP2, NDRG1 and HSP22 were found in patients of Bulgarian ethnicity (mutations in BSCL2 were also found in 3 Turkish patients).

Conclusions:

- In our cohort:
  - AD CMT are more common among Bulgarians (86.3%)
  - AR CMT are more common among Romani (95.3%)
  - Despite the cohort of Turks is very small, CMT1X was distinguished as the most common form in this ethnic group

Thank you for your attention!

THE GENETIC EPIDEMIOLOGY OF HEREDITARY SPASTIC PARAPLEGIAS IN BULGARIA

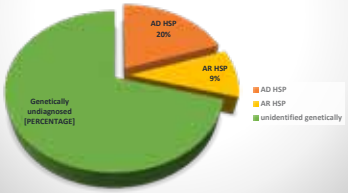
Jean Samuel, Albena Andreeva, Nevyana Ivanova, Teodora Chamova, D. Kancheva, Ivan Litvinenko, Ara Kaprelyan, Margarita Grudkova, Luba Kalaydjieva, Albena Jordanova, Ivailo Tournev

HSP worldwide	HSP Bulgaria
• Most common ADHSP- SPG4 (50%) & SPG3A (40%) • Most common ARHSP-SPG11 (21%) & SPG15 • Families without genetic diagnosis- ADHSP 45-67% • ARHSP 71-82%	• Most common ADHSP- SPG4 (40% of genetically confirmed cases) • Most common ARHSP-SPG7 (24% of genetically confirmed cases) • Families without genetic diagnosis (71% of all cases)

- The hereditary spastic paraplegias (HSP)s are a group of clinically and genetically heterogenous neurodegenerative disorders, that present with progressive lower limb spasticity and weakness, causing gait impairment which eventually leads to permanent disability
- This is mainly due to retrograde axonal degeneration of the corticospinal tracts
- HSP affects males and females of all ages and ethnic groups from around the world, with more than 80 genetic types
- In Europe, the prevalence of HSP is estimated to be 1-9:100,000
- Genetic penetrance is high (estimated at 90%)

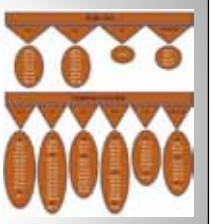
HSP in Bulgaria

- From 1997 - 2017, genetic testing has been performed on 269 patients and their family members (a total of 495 individuals) from 198 families belonging to Bulgarian, Turkish and Roma ethnic groups with spastic paraplegia syndrome
- Genetic studies confirmed the diagnosis in 101 patients from 51 families



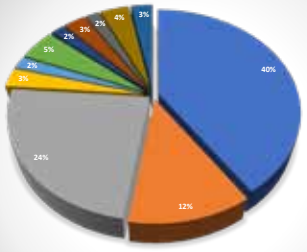
Classification of HSP

Inheritance pattern	• Autosomal Dominant (AD) • Autosomal Recessive (AR) • X-linked forms
Clinically	• Uncomplicated or pure • Complicated



AD-HSP:31 families

- Spastin: 41 from 27 families
- Atlastin: 12 from 4 families



AR-HSP: 20 families

- Paraplegin: 24 from 11 families
- ZFYVE26: 3 from 2 families
- Spatascin: 2 from 1 family
- TUBB4A: 5 from 1 family
- Alsin: 2 from 1 family
- NT5C2: 3 from 1 family
- GBA2: 2 from 1 family
- AP4S1: 4 from 1 family
- ATP13A2: 3 from 1 family

"Pure"/ "Uncomplicated"

- Progressive lower extremity spastic weakness
- Hypertonic urinary bladder disturbance
- Mildly decreased vibration sensation in the lower extremities

"Complicated"

- Impairments present in pure form
- +
- Ataxia
- Seizures
- Intellectual disability
- Dementia
- Amyotrophy
- Extrapyramidal disturbance
- Peripheral neuropathy

AD-HSP in Bulgaria

	SPG4	SPG3A
Patient count	41 from 27 families	12 from 4 families
Gene	spastin (SPAST)	atlastin (ATL1)
Gene localization	2p22.3	14q22.1
Gene function	Microtubule dynamics and organization	Formation of the tubular endoplasmic reticulum (ER) network and axon elongation
Mutations	Missense, splice, deletions, duplication, single nucleotide substitution	Missense, deletion
Inheritance pattern	AD, sporadic	AD, pseudo AR (*de novo* mutation as a mosaicism in one of the unaffected parents
Patient gender	Both sexes, m>f	Both sexes, m=f
Patient ethnicity	Bulgarian, Turkish, Roma, Bulgarian Muslims	Bulgarian, Roma
Average age at onset	30.2 (2-65y)	4.3 (1.5-9y)
Average age at loss of ambulation	43.4 (34-59y)	16 (10-19y)
Clinical type	Uncomplicated 90% Complicated10%	Uncomplicated 50% Complicated 50%
Clinical severity	Mild to moderate	Mild to moderate, 1 family with severe phenotype
MRI findings	Mild cerebral atrophy 15% Non specific white matter lesions 18%	Normal

- In relation to disease onset, late onset was observed in *spastin* gene mutations and early onset in *atlastin* gene mutations
- *Spastin* mutations showed slow disease progression while *atlastin* mutations showed faster progression of the disease
- *Spastin* is found to be more frequent in the Bulgarian ethnic population (60%) while *ATL1* is distributed equally among all populations
- Anticipation observed is 45% in *spastin* gene mutations and in *atlastin* is 50%
- In *spastin* mutations, complicated forms of the disease showed additional symptoms of cognitive decline and epilepsy
- 14 novel *spastin* mutations were observed
- A novel in-frame heterozygous c.1220_1222del mutation in the *atlastin gene* was found in a gypsy family with 2 affected siblings resembling AR inheritance pattern.
  - The c.1220_1222del variant was absent in both parents, so we assume that it originated as a *de novo* germline and/or somatic mosaicism in one of the parents and was transmitted to the affected children
  - The clinical presentation included neonatal onset with delayed developmental milestones, severe spastic quadriparesis, distal muscle wasting, sensorimotor neuropathy, dysphonia, dysmasesis, dysarthria and oro-facial dysmorphism

- Our study demonstrates that p.L78X mutation in SPG7 has a founder effect among Bulgarian Roma patients. It is responsible for late onset pure or complex phenotypes with predominant ataxia and cerebellar atrophy. Heterozygotes may display slight features of upper neuron involvement but not overt weakness or spasticity
- Mutations in TUBB4A have been associated with dystonia type 4 and hypomyelination with basal ganglia and cerebellar atrophy.
  - A novel c.568C>T TUBB4A mutation has been found in a consanguineous Roma family affected by early-onset complicated HSP with regional hypomyelination, mild cerebellar atrophy, and no dystonia, basal ganglia atrophy, or cognitive dysfunction.
  - The mutation was also found in the mosaic state in the unaffected mother, hence making the mode of inheritance AD despite the high level of inbreeding and ratio of affected children
- Loss of ATP13A2 function has been linked to Kufor-Rakeb Syndrome which is an early-onset atypical form of Parkinson's disease with dementia
  - A novel mutation was found in the ATP13A2 gene in a consanguineous Roma family. The disease presentation in our patients was dominated by an adult-onset lower-limb spastic paraparesis. Very mild cognitive impairment was observed along with cerebellar ataxia and axonal neuropathy

AR-HSP in Bulgaria: Genotype

	SPG7	SPG15	SPG11	SPG45	SPG46	SPG52	SPG78	Infantile ascending HSP	TUBB4A
Patient count	24 from 11 families	3 from 2 families	2 from 1 family	3 from 1 family	2 from 1 family	4 from 1 family (2 of them died at ages 22 and 24)	3 from 1 family	2 from 1 family	5 from 1 family
Gene	Paraplegin	ZFYVE26	spatacsin	NTSC2	GBA2	AP4S1	ATP13A2/ PARK9	Alsin ALS2	TUBB4A
Gene localization	16q24.3	14q24.1	15q21.1	10q24.32-q24.33	9p13.3	14q12	1p36.13	2q33.1	19p13.3
Gene function	It is part of the mitochondrial inner membrane that degrades misfolded proteins and regulates ribosome assembly	It is found in endosomes and take part in membrane transport and programmed cell death	Neuronal axonal growth and intracellular cargo trafficking	Maintenance of a constant composition of intracellular purine/pyrimidine nucleotides	It is an enzyme of sphingolipid metabolism, helps in cell signaling responses and structural components of the plasma membrane	AP complexes function in endosome membrane trafficking, vesicle formation and selective inclusion of specific proteins for these vesicles	ATP13A2 offers cellular protection to heavy metal ions (Mn2+, Fe3+, Zn2+) and mitochondrial toxicity	Intracellular endosomal trafficking	Encodes a brain-specific member of the beta-tubulin family that is most highly expressed in the cerebellum, putamen, and white matter
Mutations	Nonsense mutation p.L78X	Nonsense mutation c.2639T>C, p.L880P	Splice site mutation p.Arg945hr	c.1449+2T>C, splice site mutation	c.451+2T>C	Deletion(c137, c138+2delAAG T)	p.Thr512Ile (c.1535C 4T)	R921X	c.568C>T
Inheritance pattern	AR	AR	AR	AR	AR	AR	AR	AR	AD (mosaicism in the mother)

Conclusion

- The most common form of HSP in Bulgaria is SPG4 (40%) and is distributed among the various ethnicities
- SPG7 being the second most common form in Bulgaria (24%), has been found only in the Roma population
- SPG4 accounts for 87% of all AD-HSP presenting mainly with uncomplicated phenotypes
- The second most common form of AD-HSP in Bulgaria is SPG3A presenting with both pure and complicated phenotypes
- Nine complicated AR-HSP forms have been found, of which SPG7 is the most prevalent, followed by SPG15. The observed SPG7, SPG15, SPG45, SPG46, SPG52, SPG78 and *TUBB4A* gene cases belong to the Roma gypsy population while the family with SPG11 belongs to the Bulgarian Muslim minority
- It is to be noted that, despite advances in genetic research, most families (71% of all cases) remain without identified genetic mutation after extensive testing

AR-HSP in Bulgaria: Phenotype

	SPG7 (paraplegin)	SPG15 (ZFYVE26)	SPG11 (spatacsin)	SPG45 (NTSC2)	SPG46 (GBA2)	SPG52 (AP4S1)	SPG78 (ATP13A2)	IAHSP (alsin)	TUBB4A
Patient ethnicity	Roma	Roma	Bulgarian Muslims	Roma	Roma	Roma	Roma	Bulgarian	Roma
Consanguinity	yes	in 1 family	No	yes	no	Yes	Yes	No	Yes
Average age at onset	37 (17-52y)	16 (14-19y)	19y	1.5y	16y	1.5 y	32y	1.5 y	2.3 (2-3y)
Average age at loss of ambulation	18 (15-23)	3rd decade	21y	Still ambulatory in the IInd decade	36	17y	48y	7.5y	15.5 (14-17y)
Clinical type	Complicated- cerebellar ataxia, optic atrophy, mild cognitive impairment, myopathy	Complicated- mild cerebellar signs, axonal polyneuropathy	Complicated- cerebellar ataxia, Polyneuropathy, Cognitive decline, distal muscle atrophy in UL	Complicated- motor delay, Cerebellar ataxia, mild intellectual disability, foot and spine deformities	Complicated- cerebellar ataxia, dysarthria	Complicated- motor delay, neonatal epilepsy, severe intellectual disability, poor speech development, oro-facial dysmorphism	Complicated- mild cognitive impairment, cerebellar ataxia, axonal sensorimotor neuropathy	Complicated- Motor delay, dysphonia, dysarthria to anarthria, dysmanesis, dysphagia, spine deformities, urinary retention	Complicated- delayed motor milestones, cerebellar symptoms and foot deformities
Clinical severity	Mild to moderate	Mild to moderate	Moderate	Mild to moderate	Moderate	Severe	Moderate	Severe	Moderate to severe
MRI findings	Cerebellar atrophy, mild spinal atrophy, non specific white matter lesions	Thin corpus callosum, periventricular leukoencephalopathy, cerebral and cerebellar atrophy	Corpus Callosum hypoplasia, cervical spine hypotrophy, non specific white matter lesions	NA	NA	Enlarged lateral, third and fourth ventricles	progressive vermian and hemispheric cerebellar atrophy, mild cortical atrophy, mild periventricular white matter changes	Normal	Bilateral hyperintense confluent lesions on T2 and FLAIR AQs sequences in the periventricular white matter and mild cerebellar atrophy.

Thank you for your attention!

NGS APPROACH IN CASES WITH CONGENITAL NEUROMUSCULAR DISORDERS

Tihomir Todorov, Bilyana Georgieva, Andrey Kirov, Ivan Litvinenko, Velina Guergueltcheva, Ales Maver, Borut Peterlin, Ivailo Tournev, Albena Todorova

Disclosures

The study was supported by the Medical University Sofia, Grant number Д-58/2017

Neuromuscular disorders

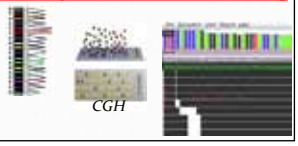
- Enormous group of clinically overlapping disorders
- Sometimes difficult to make a differential diagnosis

Genetically heterogeneous group: many different genes, chromosomal regions and genetic loci are involved

Inheritance:
Autosomal recessive, autosomal dominant and X-linked

Molecular-genetic approaches to clarify the disease-causing mutation:

- Gene-by-gene sequencing
- MLPA and array-CGH technologies
- **Next generation sequencing (NGS)**



AIM

to clarify at molecular level clinically ambiguous cases of congenital neuromuscular disorders

- Study design:
- NGS approach and targeted evaluation of genes associated with the clinical symptoms of the patients (**filtering by phenotype**)
- Sanger sequencing verification of pathogenic variants
- Segregation analysis within the family

RESULTS

Case 1: Congenital myasthenic syndrome

Clinical symptoms: generalized hypotonia, ankle contractures, delayed psychomotor development, dolichocephaly, myopathy, myasthenic syndrome, ataxia, dystonia, craniosynostosis, and neuropathy. The symptoms were presented right after birth. There is no familial consanguinity and no other affected siblings.

Genetic tests:
1. Gene-by-gene approach
Sanger sequencing of the following genes: CHRNE, COLQ, GMPPB

Negative results

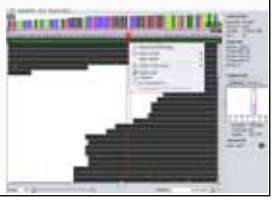
Genetic tests:
2. MLPA analyses for copy number variations

Negative results

Genetic tests:
3. NGS approach
Clinical exome (Illumina TruSight One):

Negative results

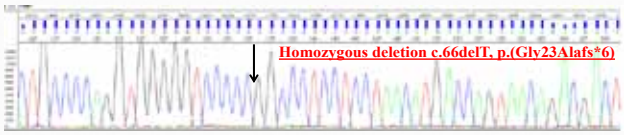
Genetic tests:
4. NGS approach
Whole exome sequencing of trio (WES):



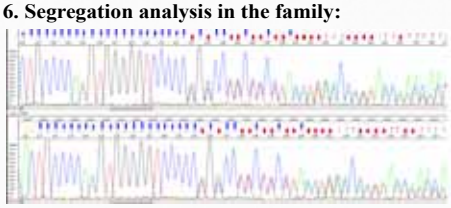
c.66delT, p.(Gly23Alafs*6)

novel homozygous variant in the *VAMP1* gene

Genetic tests:
5. Sanger sequencing verification:



Genetic tests:
6. Segregation analysis in the family:



Both parents are heterozygous carriers

CASE SOLVED

RESULTS

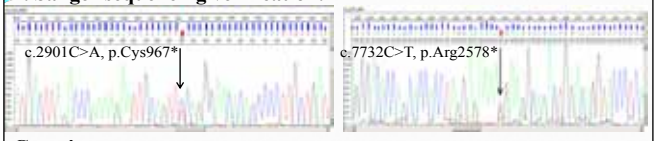
Case 2: Congenital muscular dystrophy

Clinical symptoms: the patient, since birth, presents with low muscle tone, passive movements, contracture of knee joints, weak head control, limited dorsal flexion of the joints, curved foot, CPK abnormal (4349 UI), delayed motor development.
Asymptomatic parents are not related. There is no affected sibling.

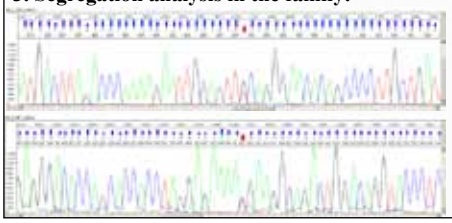
Genetic tests:
1. NGS approach
Whole exome sequencing of trio (WES) (CentoXome GOLD®):

Heterozygous c.2901C>A p.(Cys967*) Heterozygous c.7732C>T p.(Arg2578*)

Genetic tests:
2. Sanger sequencing verification:



Genetic tests:
3. Segregation analysis in the family:

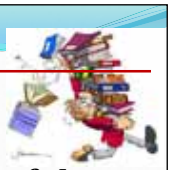


Both parents are heterozygous carriers

CASE SOLVED

as merosindeficient congenital muscular dystrophy type 1A

Take home messages

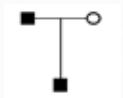


Verifying the genetic cause of the congenital neuromuscular disorders allows not only determining the accurate diagnosis, prevention and therapy but also the accurate genetic counseling and family planning.

RESULTS

Case 3: Congenital myopathy

Clinical picture: conflicting clinical presentation and different clinical diagnoses like congenital myopathy and hereditary motor sensory neuropathy have been considered.



Autosomal dominant familial inheritance pattern

Genetic tests:
1. NGS approach
Clinical exome (Illumina TruSight One):

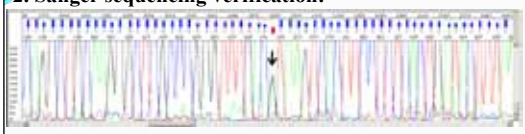
Heterozygous splice-site variant c.1056+1G>A in *COL6A1* gene

Thank you for your attention

ZGENICA CENTOGENE THE RARE DISEASE COMPANY

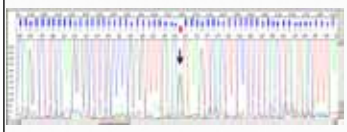
Genome Center Bulgaria

Genetic tests:
2. Sanger sequencing verification:



c.1056+1G>A

Genetic tests:
3. Segregation analysis in the family:



c.1056+1G>A

The mutation is inherited from the affected father

CASE SOLVED

as *COL6A1* associated Bethlem myopathy type 1

SCREENING FOR SNPs IN A SET OF DRUG-METABOLIZING ENZYMES IN BULGARIAN POPULATION

Antonova O., Mihaylova V., Hammoudeh Z., Staneva R., Kuncheva B., Mihaylova M., Toncheva D., Gerasimova B.

Aim of the study

- PGs variants are carried by healthy people and do not occur until the certain drug is received.
- Their actual incidence is not known.

To determine the genotype frequency of certain SNPs in a set of genes, involved in drug metabolism in our population.

12th Balkan Congress of human genetics, 8-10 September 2017, Plovdiv, Bulgaria

Pharmacogenetics & Pharmacogenomics

12th Balkan Congress of human genetics, 8-10 September 2017, Plovdiv, Bulgaria

Materials, methods and working strategy

Informed consent ← *103 Healthy volunteers* → *Clinical interview*

Non-invasive biosample collection by buccal swab → *DNA isolation*

Genotyping with QuantStudio 12K Flex Real-Time PCR for 25 SNPs in 23 genes

Data analysis by PharmGKB (http://www.pharmgkb.org) database for SNPs variants contributed to differences in reactions to drugs and GraphPad Prism 5 graphing and statistics software.

12th Balkan Congress of human genetics, 8-10 September 2017, Plovdiv, Bulgaria

Pharmacogenetics & Pharmacogenomics

- Gene – drug interaction
- The study of heritable variability in drug response, *Friedrich Vogel, 1959*, in "*Moderne Probleme der Humangenetik*".
- The role of genetics in the interindividual variability to drug response, "*Drug Reactions, Enzymes and Biochemical Genetics*", *Arno Motulsky, 1957.*

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Studied SNPs and related drugs

Gene	SNPs	Type	Effect	Drugs
LPL	rs328	Stop Codon	Efficacy	Pravastatin
CETP	rs708272	Intronic	Efficacy	Pravastatin
APOC3	rs5128	3' UTR	Toxicity	Ritonavir
APOE	rs429358	Missense	Toxicity	Ritonavir
	rs7412	Missense	Efficacy	Atorvastatin, Ritonavir
MTHFR	rs1801133	Missense	Toxicity	Cyclophosphamide, Carboplatin, Methotrexate, Pemetrexed, Benazepril, FOLFOX therapy, Mercaptourine, Cisplatin, NO, FA and vit B-complex, Pravastatin, Disulfiram, Antipsychotics
	rs1801131	Missense	Toxicity / ADR	Clozapine, Olanzapine, Methotrexate, Pemetrexed, NO, Fluorouracil, Leucovorin, Oxaliplatin
MTR	rs1805087	Missense	Efficacy, Toxicity	Methotrexate, Benazepril, Cisplatin, cyclophosphamide, dactinomycin, doxorubicin, methotrexate and vincristine
MTRR	rs1801394	Missense	Toxicity	Methotrexate, Folic acid and Vitamin B-complex
COMT	rs4680	Missense	Efficacy	Nicotine replacement therapy, Fluvoxamine, Analgesics (such as opioids, NSAIDs, triptans), Antipsychotics, Haloperidol, Entacapone, Paroxetine, Morphine, Venlafaxine, Clozapine, Opioids
CYP1A1	rs1048943	Missense	Efficacy	Capecitabine and docetaxel
GSTP1	rs1695	Missense	Toxicity / ADR	Platinum-based drugs, Fluorouracil, Oxaliplatin and platinum-based therapy, Cyclophosphamide, Epirubicin, Cisplatin-based regimens, Methotrexate, Fumaric acid esters, Isoniazid, rifampin,
NQ01	rs1800566	Missense	Efficacy	Platinum compounds, anthracyclines and nucleoside inhibitors
IL-6	rs1800795	5' Flanking	Efficacy	Fenofibrate, Adalimumab, Etanercept, Infliximab, Tumor necrosis factor alpha (TNF-alpha) inhibitors, Peginterferon alfa-2b and Ribavirin

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Pharmacogenetics & Pharmacogenomics

- The study of how drugs interact with the total genome, to influence biological pathways and processes (*Daxinger, L. et all, 2010, "Transgenerational Epigenetic Inheritance: More Questions Than Answers").*
- A direct by-product of The Human Genome Project.

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Studied SNPs and related drugs

Gene	SNPs	Type	Effect	Drugs
TNFA	rs1800629	5' Flanking	Efficacy	Anti-TNF therapies, Carbamazepine , Atoevastatin, Sorafenib, Cyclosporine, Ethambutol,Iisoniazid, Pyrazinamide and Rifampin
eNOS	rs1799983	Missense	Efficacy	Cyclophosphamide-based regimens, Sorafenib, Aspirin, Beta Blocking Agents, clopidogrel and hmg coa reductase inhibitors, Anthracyclines and related substances, platinum compounds, nucleoside inhibitors or folate analog metabolite inhibitors, Salvianolate, Daunorubicin, Ace Inhibitors, Plain, Angiotensin II Antagonists, Beta Blocking Agents, Digoxin, Spironolactone
MnSOD/ SOD2	rs4880	Missense	Efficacy	Cyclophosphamide, Methotrexate, Valproic acid, Asparaginase, Paclitaxel
VDR	rs1544410	Intronic	Efficacy	Bisphosphonates, Midazolam, Hormone replacement therapy, Peginterferon alfa-2a and ribavirin
COL1A1	rs1800012	Intronic	Dosage	Recombinant human growth hormone, Valproic acid
PPARG	rs1801282	Intronic	Toxicity	Olanzapine
TCF7L2	rs7903146	Intronic	Efficacy	Sulfonamides, Urea derivatives, Tacrolimus, Sirolimus, Cyclosporine
FTO	rs9939609	Intronic	Efficacy	Pegylated-interferon-alpha plus ribavirin therapy
CYP1A2	rs762551	Intronic	Efficacy	Olanzapine, Deferasirox, Carbamazepine, Clopidogrel, Antipsychotics, chlorpromazine, fluphenazine, thioridazine and trifluoperazine, increased coffee consumption, Leflunomide, Paroxetine
ACE	rs4646994	unknown	Efficacy	Salvianolate
AGT	rs699	Missense	Efficacy	Aatenolol, Irbesartan, Antiinflammatory agents, NSAIDs, Quinapril and other ACE inhibitors

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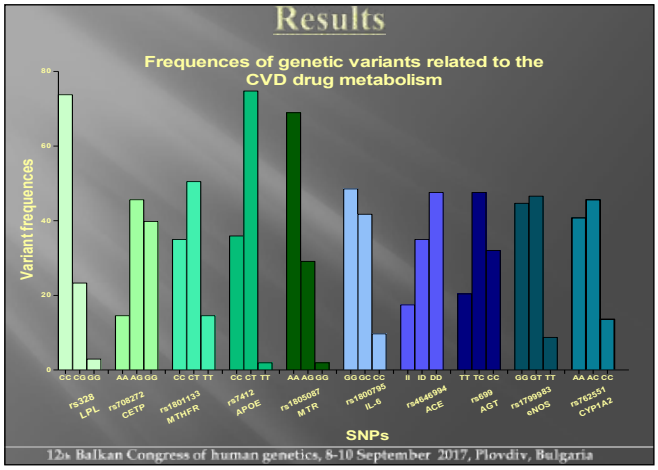
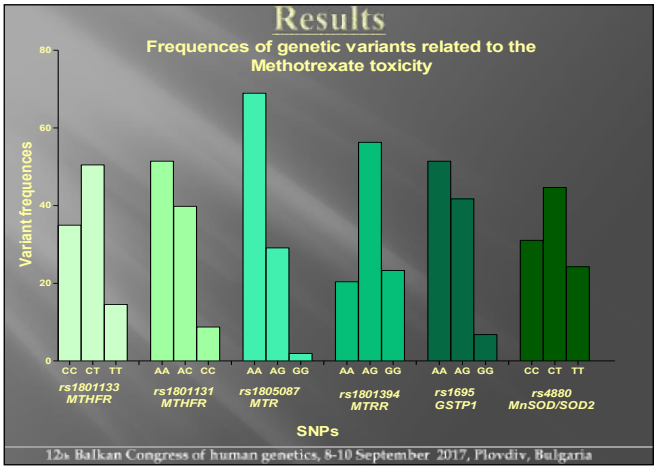
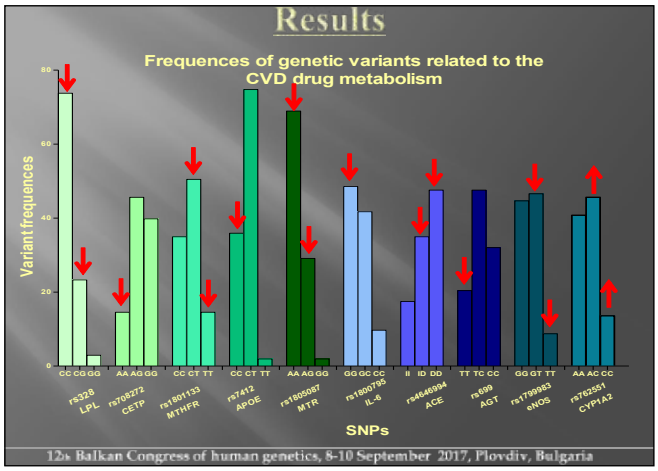

Results
Frequences of genetic variants related to the CVD drug metabolism
Variant frequences
SNPs
12th Balkan Congress of human genetics, 8-10 September 2017, Plovdiv, Bulgaria


Results
Frequences of genetic variants related to the Methotrexate toxicity
Variant frequences
rs1801133 MTHFR
rs1801131 MTHFR
rs1805087 MTR
rs1801394 MTRR
rs1695 GSTP1
rs4880 MnSOD/SOD2
SNPs
12th Balkan Congress of human genetics, 8-10 September 2017, Plovdiv, Bulgaria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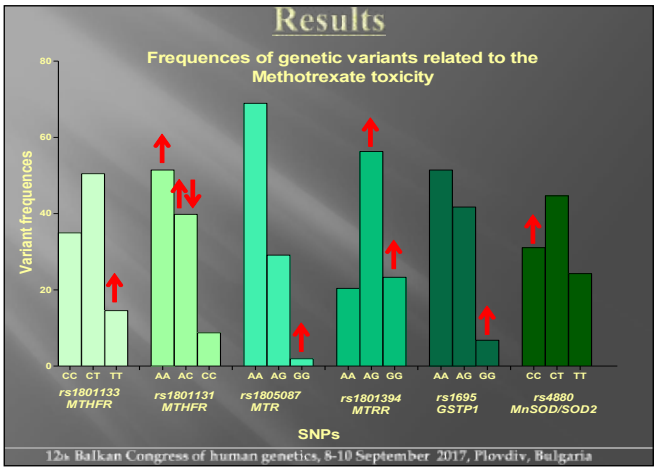
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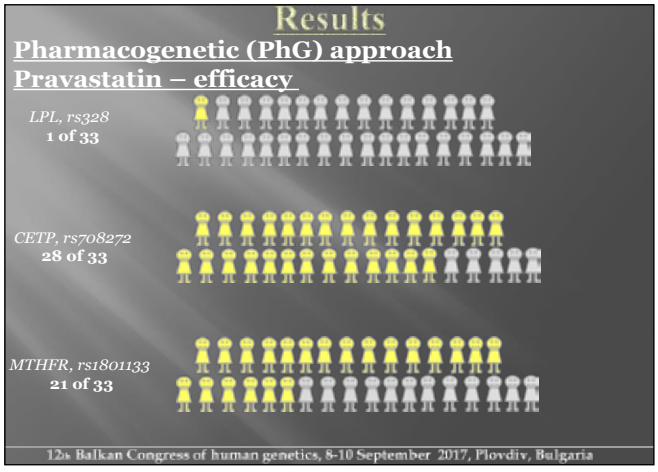
Results
Pharmacogenetic (PhG) approach
Pravastatin – efficacy
LPL, rs328
1 of 33
CETP, rs708272
28 of 33
MTHFR, rs1801133
21 of 33
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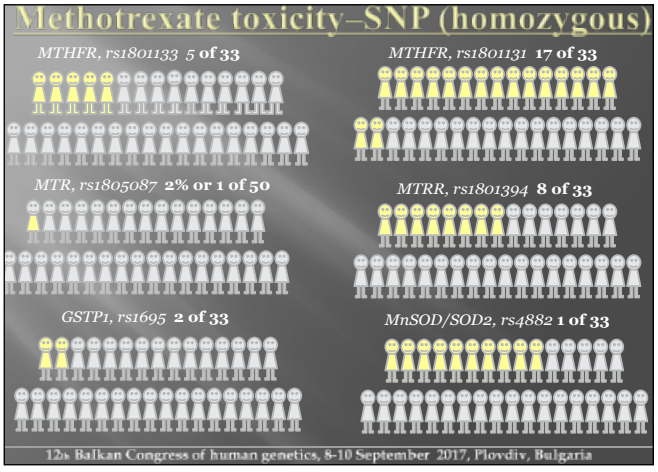
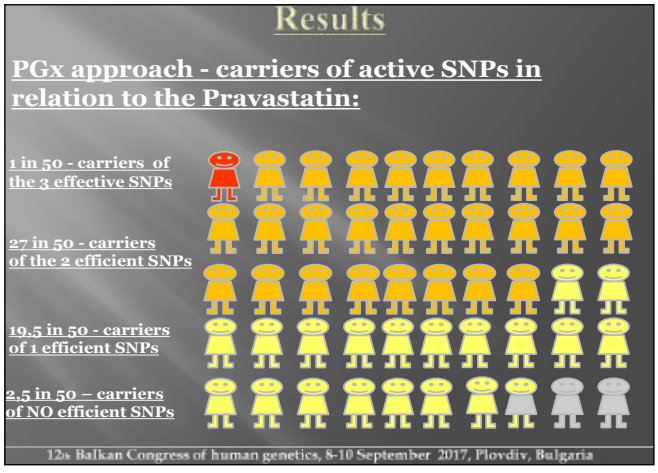

Methotrexate toxicity–SNP (homozygous)
MTHFR, rs1801133 5 of 33
MTHFR, rs1801131 17 of 33
MTR, rs1805087 2% or 1 of 50
MTRR, rs1801394 8 of 33
GSTP1, rs1695 2 of 33
MnSOD/SOD2, rs4882 1 of 33
12th Balkan Congress of human genetics, 8-10 September 2017, Plovdiv, Bulgaria


Results
PGx approach - carriers of active SNPs in relation to the Pravastatin:
1 in 50 - carriers of the 3 effective SNPs
27 in 50 - carriers of the 2 efficient SNPs
19,5 in 50 - carriers of 1 efficient SNPs
2,5 in 50 – carriers of NO efficient SNPs
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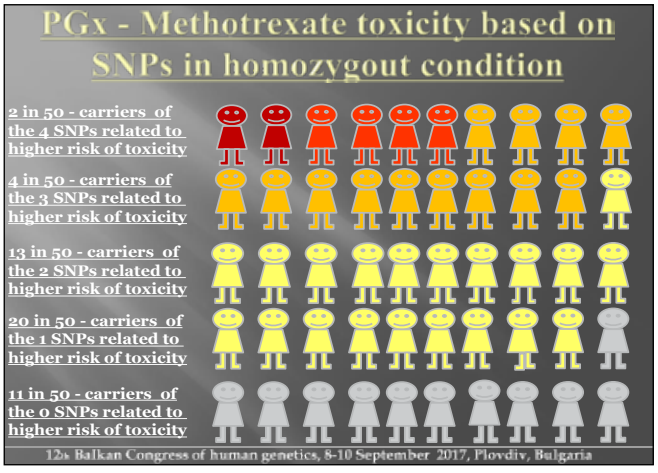
PGx - Methotrexate toxicity based on SNPs in homozygout condition
2 in 50 - carriers of the 4 SNPs related to higher risk of toxicity
4 in 50 - carriers of the 3 SNPs related to higher risk of toxicity
13 in 50 - carriers of the 2 SNPs related to higher risk of toxicity
20 in 50 - carriers of the 1 SNPs related to higher risk of toxicity
11 in 50 - carriers of the 0 SNPs related to higher risk of toxicity
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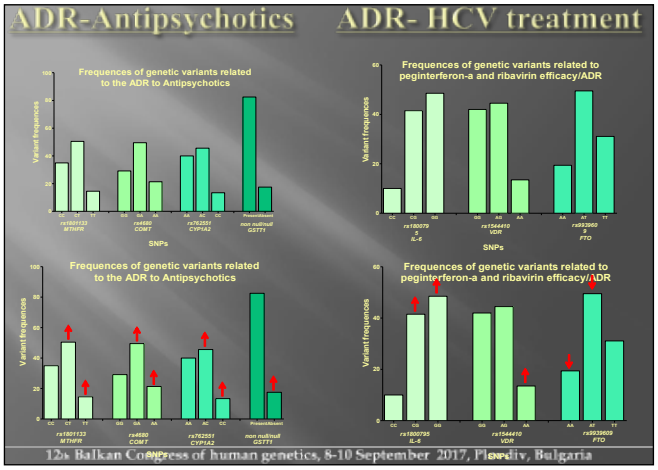

Conclusion

- We have determined the genotypic frequencies of 25 single polymorphic variants associated with the metabolism of a wide range of drugs.
- We have demonstrated that there is a genetic predisposition to interpatient variability in response to standard therapeutic doses.
- PGx approach can potentially be used to increase the clinical benefit and reduce the risk of adverse drug reactions (ADR) in people whose drug responses are not "average".

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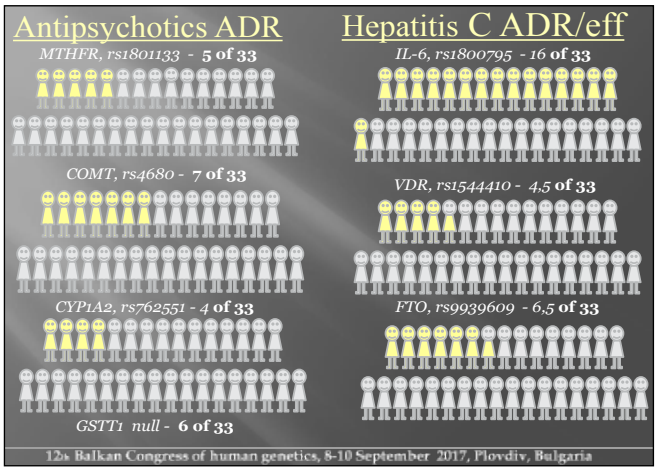

Request to the scientific community

I was really pleased to find at this Conference that a lot of scientific groups are working in the field of PG and PGx.

I would like to ask you to get together, to share our experience and to laid the foundations of PG database for Bulgarians.

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3 in 100 - carriers of the 3 SNPs related to higher risk of ADR, when treated with antipsychotics.

11 in 100 - carriers of the 2 SNPs related to higher risk of ADR

36 in 100 - carriers of the 1 SNPs related to higher risk of ADR

50 in 100 - carriers of the 0 SNPs related to higher risk of ADR

2 in 100 - carriers of the 3 SNPs related to higher risk of ADR and lower efficacy of HCV treatment.

12 in 100 - carriers of the 2 SNPs related to higher risk of ADR

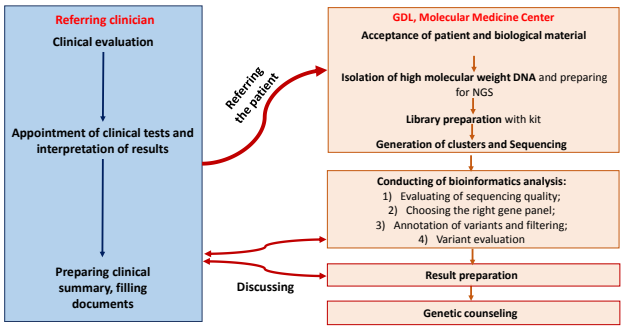
55 in 100 - carriers of the 1 SNPs related to higher risk of ADR

31 in 100 - carriers of the 0 SNPs related to higher risk of ADR

Thank You for Your attention!

NGS SEQUENCING IN SERVICE OF NEUROGENETICS IN BULGARIA

Darina Kachakova, Kalina Mihova, Ivan Popov, Ivanka Dimova, Emil Simeonov, Ivaylo Tournev, Ivo Kremensky, Vanyo Mitev, Radka Kaneva



Introduction

- There are more than 6000 known genetic disorders, many having some neurologic manifestations.
- Neurological conditions, disorders and syndromes are a huge group of more than 600 diseases.
- Many neurological phenotypes are characterized by extreme genetic heterogeneity and clinical variability
- Methods for genetic testing of neurological conditions
  - Sequencing: sequencing of single genes, tNGS, WES, WGS
  - Methylation analysis
  - Chromosome/genome analysis: aCGH, MLPA, karyotyping, FISH

PATIENT #	Referring CLINICIAN	DIAGNOSIS
13369R	Prof. Emil Simeonov	Epilepsy with complex partial seizures, temporal pseudopausal epilepsy
14104R	Prof. Emil Simeonov Prof. Ivo Kremensky	Mental retardation, encephalopathy, organic aciduria, congenital defect in glycosylation, atypical syndrome of Ret, West syndrome
14755R	Prof. Emil Simeonov	Neurodegenarative or metabolic encephalopathy, resistant epilepsy, facial dismorphism, progressive muscular hypotonia
12680R	Prof. Emil Simeonov Prof. Ivo Kremensky	Epilepsy with myoclonic-astatic attacks, Speech disorder, attention deficit disorder
14181R	Prof. Emil Simeonov	Familial myoclonic epilepsy
12686R	Prof. Ivo Kremensky	Genetic epileptic encephalopathy, Drave syndrome
13116R	Prof. Emil Simeonov	Drave syndrome, Lennox-Gastaut syndrome, encephalopathy
13966R	Prof. Emil Simeonov	Cataract-ataxia syndrome
14859R	Assoc. Prof. Ivanka Dimova	Noonan Costello syndrome
14733R	Prof. Ivo Kremensky	Polyneuropathy
35562	Prof. Emil Simeonov	Accelerated physical development in the background of severe neurological psychomotor development delay
7-3	Prof. Ivaylo Tournev	Myotonia

Introduction. tNGS

- Which genetic method for analysis of neurological diseases to use?
  - Which neurological diseases suitable for tNGS?

What did we find? Results from tNGS.

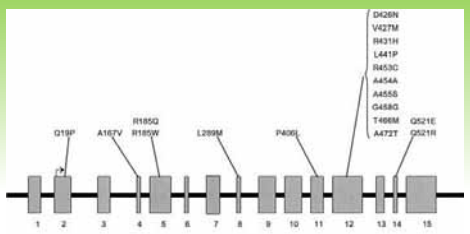
- Genetic diagnosis made in half of the analyzed patients.
- Genetic diagnosis changed in 4 of the patients
  - Results from tNGS for the patient 13369R referred to the lab with epilepsy estimated the diagnosis hyperprolinemia type I
  - For the patient 12680R with epilepsy genetic diagnosis was Allan-Herndon-Dudley syndrome
  - For the patient 14859R with Noonan – Costello syndrome genetic diagnosis was Cardiofacio cutaneous syndrome
  - We discovered a rare case of association of polyneuropathy with Klinefelter syndrome, confirmed with cytogenetic analysis

tNGS in patients with neurological conditions in Laboratory of genome diagnostics

What did we find? Results from tNGS.

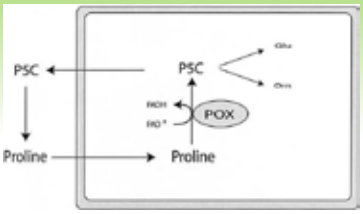
- Genetic diagnosis clarified the clinical diagnosis in two of the patients:
  - The diagnosis were clarified for the patient with cataract-ataxia syndrome
  - and for the patient with multiple differential diagnosis (mental retardation, encephalopathy, organic aciduria, congenital defect in glycosylation, atypical syndrome of Ret, West syndrome)

PATIENT #	DIAGNOSIS	RESULTS
13369R	Epilepsy with complex partial seizures, temporal pseudopausal epilepsy	Homozygous pathogenic variant **c.1292G>A (p.Arg431His)** in exon 11 of ***PRODH*** found leading to Hyperprolinemia type I
14104R	Mental retardation, encephalopathy, organic aciduria, congenital defect in glycosylation, atypical syndrome of Ret, West syndrome	Heterozygous pathogenic variant **c.2558G>A (p.Arg853Gln)** in exon 15 of ***SCN2A*** leading to West syndrome
14755R	Neurodegenarative or metabolic encephalopathy, resistant epilepsy, facial dismorphism, progressive muscular hypotonia	Found hemizygous pathogenic variant **c.1345delG (p.Gly449AlafsTer5)** in exon 5 of ***SLC16A2*** leading to Allan-Herndon-Dudley syndrome
12680R	Epilepsy with myoclonic-astatic attacks, Speech disorder, attention deficit disorder	Found **VUS** variants in *SERPINI1*, *SHANK3* and *CDKL5*
14181R	Familial myoclonic epilepsy	Pathogenic variants not found.
12686R	Genetic epileptic encephalopathy, Drave syndrome	Pathogenic variants not found.
13116R	Drave syndrome, Lennox-Gastaut syndrome, encephalopathy	Pathogenic variants not found.



From: Bender HU, Almashanu S, Steel G, Hu CA, Lin WW, Willis A, Pulver A, Valle D.
Functional consequences of PRODH missense mutations. Am J Hum Genet. 2005 Mar;76(3):409-20. Epub 2005 Jan 20.

PATIENT #	DIAGNOSIS	RESULTS
13966R	Cataract-ataxia syndrome	Found de novo heterozygous probably pathogenic variant **c.962G>A (p.Gly321Asp)** in exon 12 of ***KIF1A***
14859R	Noonan Costello syndrome	Found heterozygous pathogenic variant **c.1502A>G (p.Glu501Gly)** in exon 12 of ***BRAF*** leading to cardiofacio cutaneous syndrome
14733R	Polyneuropathy	Found Klinefelter syndrome. XXY
35562	Accelerated physical development in the background of severe neurological psychomotor development delay	Pathogenic variants not found.
7-3	Myotonia	Pathogenic variants not found.



Case 13369R

- Female 6 year old patient with epilepsy with complex partial seizures, temporal pseudopausal epilepsy, mental retardation
- Mother of the patient with two complex partial seizures at age 33. Having migraine since 18 years old.
- Gene panel: epilepsy and mental retardation: 718 genes

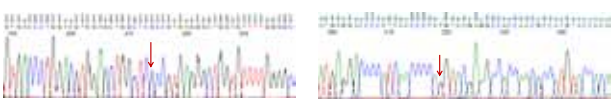
Metabolic test: proline levels in plasma

	Reference range uMol/L	Result from the test uMol/L
Patient	80 - 400	**935.71**
Mother		**758.8**
Father		248.77

Results

- Found homozygous variant: **c.1292G>A (p.Arg431His)** in exon 11 of the gene *PRODH* in the patient inherited from the father. In the mother this variant was not found

- In the mother we found heterozygous pathogenic variant c.1397C>T (p.Thr466Met) in *PRODH*.



Case 14755R

- Two years old male patient with neurodegenarative or metabolic encephalopathy, resistant epilepsy, facial dismorphism, progressive muscular hypotonia
- Gene panel for epilepsy, including encephalopathy, mental retardation, metabolic and mitohondrial diseases, defects in glycosilation - panel of 1128 genes

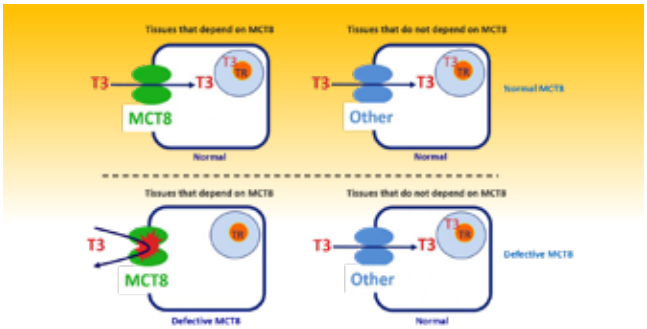
Results

- Heterozygous variants with unknown significance
  - Two variants in ***GLDC***: c.704C>G (p.Thr235Ser) in exon 5 inherited from the father and c.2894C>T (p.Ser965Phe) in exon 24 inherited from the mother ---> Glycine encephalopathy
  - De novo variant c.4850A>G (p.Asp1617Gly) in exon 9 of ***ANKRD11*** gene ---> KBG syndrome

- Hemyzygous pathogenic variant: c.1345delG (p.Gly449AlafsTer5) in exon 5 of *SLC16A2* gene inherited from the healthy mother ---> Allan-Herndon-Dudley (MCT8 -specific thyroid hormone cell transporter deficiency)

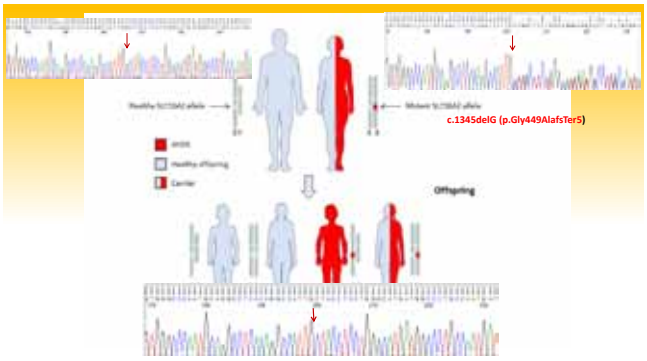
Conclusions

- With the implementation of tNGS in the diagnostic process, we have been able to shorten the "diagnostic odyssey" for many of the patients.
- Our results prove that tNGS is cost effective and efficient method, which allows a more precise diagnosis to be made for many complex neurological disorders, and could be considered earlier in the diagnostic practice.



Problems. Challenges in front of neurogenetics and NGS

- Genetic disorders often have a spectrum of symptoms and severity, which can make them hard to recognize clinically. Genetic heterogenity, phenotypic heterogenity due to varible expressivity, penetrance and influence of modifiers.
- Lack or wrong differential diagnosis.
- Many neurogenetic disorders are not yet described.
- Some mutations are somatic and do not occur in every cell, making them harder to detect with standard genetic testing.
- Some patients have more than one disorder.
- Testing can sometimes uncover "variants of uncertain significance," or genetic changes whose effects require further investigation.



Thank you for your attention!

SESSION 8-I

Moderators: Katya Kovacheva, Borislav Popov

Oral presentations:

- **Genetic heterogeneity of cardio vascular diseases associated with pathology of great vessels**
  S. Josifovska
- **Genetics and cardio-vascular diseases**
  M. Gospodinova
- **Recognition of syndromic forms of disorders of sexual differentiation**
  E. Sukarova Angelovska
- **Genetic background of steroid-resistant nephrotic syndrome in Bulgaria**
  V. Penchev
- **Molecular basis of developmental disorders: a view through the kidney filter**
  O. Beltcheva
- **Diagnostic and therapeutic approach in children with biliary atresia**
  M. Baycheva

GENETIC HETEROGENEITY OF CARDIOVASCULAR DISEASES ASSOCIATED WITH PATHOLOGY OF GREAT VESSELS

S. Josifovska, R. Vazharova, L. Balabanski, M. Malinov, A. Kaneva, S. Panov, D. Toncheva

Genetics of cardiovascular diseases

CVD cannot always be precisely diagnosed because of the large group of heterogeneous disorders that are involved.

Proper **genetic profiling** can significantly influence practitioner's therapy choice and general outcome.

With the advance of high-throughput technologies referred as **next generation sequencing (NGS)**, CVD have been associated with thousands of **new variants** in different genes introducing high prevalence of novel variants.

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Cardiovascular diseases (CVD)

Broad range of disorders of the **heart and blood vessels** including:

- **ischemic heart** disease
- **congenital heart** disease
- **rheumatic heart** disease
- **cerebrovascular** disease
- diseases of the **aorta and arteries** including hypertension and peripheral vascular diseases
- **cardiac arrhythmias**
- **cardiomyopathies** - diseases of the myocardium associated with cardiac dysfunction a) primary - due to genetic, non-genetic or acquired causes
  b)secondary - due to multi-organ or systemic disease*

*Mendis S, Puska P, Norrving B (2011) Global Atlas on Cardiovascular Disease Prevention and Control. World Health Organization, pp. 3–18. ISBN 978-92-4-156437-3

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Purpose of the study

The purpose was **genetic profiling** of patients with phenotype determined by cardio vascular diseases with pathology of great vessels.

We exploited the use of **NGS with a panel that includes 174 genes** connected to CVD in order to investigate the causes for clinical phenotype and its severity.

NGS simplifies detection of **more genes** connected to certain phenotype continuing where **Sanger** sequencing stoped in the past.

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Cardiovascular diseases (CVD)

CVD are the first cause of death worldwide with more than **17 million deaths annually** – World Health Organization's fact sheet reviewed in September 2016.

Over three quarters of this number are in **low- and middle-income** countries.

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Methodology

Clinical evaluation and diagnosis of patients was performed according to **standard procedures for the clinical assessment** of the hospital attended.

After informed consent from the participants, **blood samples** were obtained for **DNA extraction**.

Sequencing was performed on a **MiSeq System** by targeted next generation sequencing of **174 genes** included in **TruSight Cardio gene panel (Illumina)**.

Sequencing data was analysed by **Softgenetics NextGene Software** (ver 2.3.3).

Alignment was to the **human reference sequence (GRCh37/hg19)** and annotation and filtering of variants was done using the **VariantStudio Software**.

Variants in candidate genes were confirmed by **Sanger sequencing.**

Genetics of cardiovascular diseases

Clarification of the genetic causality is complicated by the genetic heterogeneity of the group.

There is a genetic contribution to most cardiovascular disease, but in actuality only a minority of patients with CVD have a traditional **monogenic disorder**.

Rate of **double or compound heterozygosity** is 3-5% *

*Ashley EA, Hershberger RE, et al (2012) Genetics and cardiovascular disease: a policy statement from the American Heart Association. Circulation 126(1):142-57

josifovskas@pmf.ukim.mk

Results

Double heterozygosity for novel ***ELN*:c.890-1G>A** and known ***SCN5A*: p.Gly9Val variant** was detected in a patient with **supravalvular aortic stenosis (SVAS)** and **pulmonary valve stenosis**.

SVAS is a systemic **elastin (ELN)** arteriopathy that affects the supravalvular aorta. *ELN* is a gene on chromosome **7q11.23** of 34 exons that encodes a protein involved in the **elasticity of various tissues and organs**.

Mutation in ***SCN5A*** (cytogenetic location: **3p22.2**) results in defective **SCN5A protein (Sodium Channel, voltage-gated, Type V, Alpha Subunit)**. The variant has been reported before and observed in patients with congenital long QT syndrome and patients with Romano-Ward syndrome .

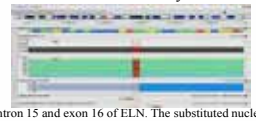

Fig. 1 Border between intron 15 and exon 16 of ELN. The substituted nucleotide is marked in red

Results

Heterozygous variants in **gene *ACTA2*** (cytogenetic location: 10q23.31) were detected in two patients:

- **p.Lys52Glu** in a patient with **aneurysm of abdominal aorta**
- **p.Arg258Cys** in a heterozygote patient with **aortic dissection type III**. The mutation is known and detected in autosomal dominant inheritance of familial form of **thoracic aortic aneurysms.**

The *ACTA2* gene provides instructions for making a protein called smooth muscle **alpha (α)-2 actin**, found in **smooth muscle cells** that line the layers of the walls of the **arteries**. It contributes to the ability of these **muscles to contract**, which allows the arteries to maintain their shape instead of stretching out as blood is pumped through them.

Results

Patient with **family history** and clinical diagnosis of **Marfan's syndrome:**

- heterozygous, probably pathogenic variant in gene ***FBN1*:p.Cys982Arg.**
- variant is novel and it has not been published so far.
- this substitution would disrupt protein's function according to predictors (MetaSVM Score: 1.098; MetaLR Pred: Damaging) so it is probably pathological variant.

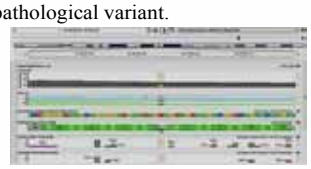

Fig. 2 Exon 25 of *FBN1*. The substituted nucleotide is marked,

Results

Marfan syndrome is a genetic disorder (1:5000) that affects the body's connective tissue.

Features associated with Marfan syndrome are **aortic dilation or aneurysm, aortic dissection, mitral valve prolapse-MVP,**
long arms and legs, tall and thin body type, scoliosis or kyphosis,
flexible joints, myopia, early glaucoma, sudden lung collapse, asthma etc

Three patients were presented with involvement of great vessels (bicuspid aortic valve, aortic dilation), tall stature and/or scoliosis resembling Marfan syndrome but did not meet systemic score >7 for the disease.

None of them had pathogenic or probably pathogenic variants in *FBN1* gene, 15q21.1, associated with the syndrome. The *FBN1* gene produces **fibrillin-1,** a protein that is transported out of cells into the extracellular matrix, where they attach to each other and to other proteins to form **microfibrils**. Microfibrils form **elastic fibers**, which enable the skin, ligaments, and blood vessels to stretch.

Conclusion

Our results are in accordance to previous findings of high heterogeneity of genetic background of cardio vascular diseases with pathology of great vessels.

This method proved to be useful in determining mutation status in correlation to the severity of clinical phenotype and can further clarify cases where clinical status could not be explained only by single gene mutation detected by standard methods.

Results confirm the importance of genetic association studies involving NGS in terms of establishing such analysis for clarifying clinical diagnosis with large implication on treatment and prognosis of patients and their families.

Results

First patient:

- mitral valve prolapse and aortic dilation
- heterozygote for two variants with extremely low frequency and unknown clinical significance in genes ***TTN* (titin), p.Thr8843Met and *ELN* (elastin), p. Gly518Ser.**
- software functional predictors put both variants in a group of **probably benign**.
- certain pathologic mutations in gene *TTN* (2q31.2), can lead to autosomal dominant forms of **dilated cardiomyopathy** and mutations in *ELN* (7q11.23) lead to a **supravalvular aortic stenosis**.

Acknowledgments:

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Prof. dr Draga Toncheva
Dr. Radoslava Vazarova
MSci. Lubomir Balabanski

Results

Second patient:

- scoliosis, bicuspid aortic valve and mitral valve prolapse
- no mutations in any of the genes in the panel.

Third patient:

- known heterozygous pathogenic variant in gene ***CBS* (Cystathionine-beta-synthase), p.Ile278Thr.**
- variants in *CBS* **(21q22.3)**, are phenotipcally expressed as **Homocistinuria** in compound heterozygotes and homozygotes
- Marfan syndrome and homocystinuria have some similar symptoms

Thank you on your attention

Corresponding author:

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GENETICS AND CARDIO-VASCULAR DISEASES

M. Gospodinova

Epidemiology

- HCM 1:500
- DCM 1:2500
- ARVC from 1:1000 to 1:5000
- RCM (non-amyloid) 1:100000?

Maron B.J et al. Circulation 2006; Elliott P et al. Eur Heart J 2008

Genetic variants in CVD

- Rare mutations responsible for diseases, such as cardiomyopathies, familial hypercholesterolemia, Marfan syndrome.
- Common polymorphisms that modulate the predisposition to complex diseases with a weak effect at individual level.

Inherited Cardiomyopathies

- HCM>60%
- DCM>50%
- ARVC > 70%
- Non-amyloid RCM ?

Michels M. et al. Neth Heart J. 2007; Mary Sweet BA et al. Expert Opin Orphan Drugs. 2015; Azaouagh A. et al. Clin. Res. Cardiol. 2011

Cardiomyopathies

- Cardiomyopathies are a heterogeneous group of heart muscle diseases associated with mechanical and/or electrical dysfunction that predispose patients to heart failure, rhythm and conduction disturbances and sudden cardiac death.
- Most of the cardiomyopathies are genetic diseases

Pattern of Inheritance

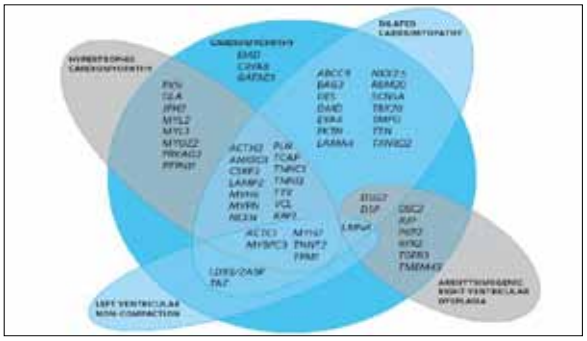
- Autosomal-dominant - 80%
- X- linked - 10%
- Autosomal-recessive - 7%
- Matrilineal - 3%

Charron P. et al. Eur Heart J. 2010

History

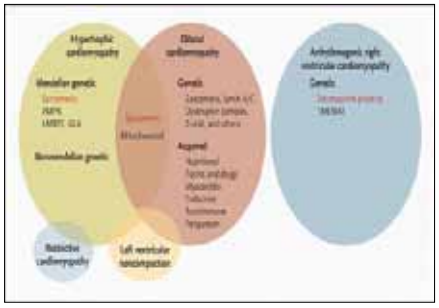
- The first genetic mutation, causing cardiac disease was identified in 1990 in French Canadian family with HCMP – point mutation in MYH7 gene.

Genotype Heterogeneity

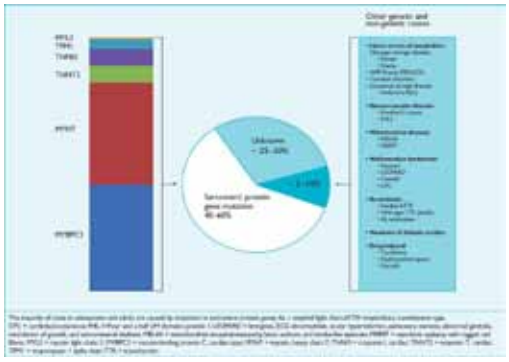


Phenotype Heterogeneity

- MYH7
  - HCM
  - RCM
  - DCM
- LMNA
  - DCM
  - ARVC
- Desmosome genes
  - ARC
  - DCM



Hypertrophic Cardiomyopathy



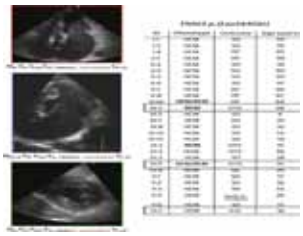
Authors/Task Force members et al. Eur Heart J 2014;eurheartj.ehu284

European Heart Journal

JACC Journals

From: **The MOGE(S) Classification of Cardiomyopathy for Clinicians**

J Am Coll Cardiol. 2014;64(3):304-318. doi:10.1016/j.jacc.2014.05.027



One gene – different phenotypes

Dilated Cardiomyopathy

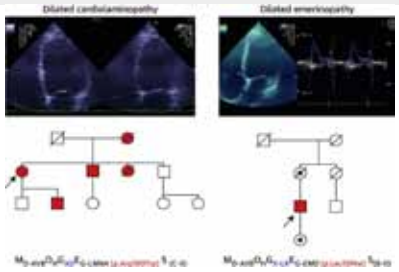
- More than 60 implicated genes.
- Truncating variants in TTN - most common (14.6%)
- More rare variants (MYH7, 5.3%; LMNA, 4.4%; TNNT2, 2.9%)

Genetics in Medicine (2017) 19, 192–203

JACC Journals

From: **The MOGE(S) Classification of Cardiomyopathy for Clinicians**

J Am Coll Cardiol. 2014;64(3):304-318. doi:10.1016/j.jacc.2014.05.027



Similar phenotype – different genes

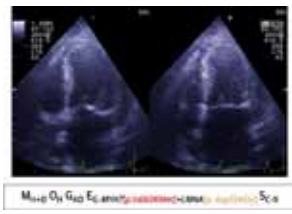
Dilated Cardiomyopathy

- Neuro-muscular disorders and DCM
  - Duchenne and Becker muscular dystrophy
  - Some Limb-Girdle MD mutations
  - Laminopathies - lamin A/C (LMNA) – Emery-Dreifus – increased risk of SCD

JACC Journals

From: **The MOGE(S) Classification of Cardiomyopathy for Clinicians**

J Am Coll Cardiol. 2014;64(3):304-318. doi:10.1016/j.jacc.2014.05.027

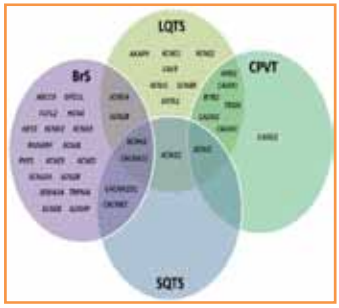


Double mutation carrier
(more severe phenotype)

Arrhythmogenic RV cardiomyopathy

- RV myocardium is replaced by fibrous and fatty tissue.
  - ventricular arrhythmias, SCD
- Mutations in 5 genes, coding desmosomal proteins
  - plakophilin 2, plakoglobin, desmoplakin, desmoglein 2, and desmocollin 2

Channelopathies



Pathologic changes in ion channels as a result of genetic mutations

Malignant arrhythmias and SCD in normal heart (structure and function).

Conclusion

Molecular genetics gives us:

- New understanding of pathogenesis and natural history of CVD.
- New possibilities for the diagnosis.
- Hope for new treatment.

RECOGNITION OF SYNDROMIC FORMS OF DISORDERS OF SEXUAL DIFFERENTIATION

E. Sukarova Angelovska, M. Kocova, G. Ilieva, V. Anastasovska, M. Krstevska-Konstantinova, G. Filev

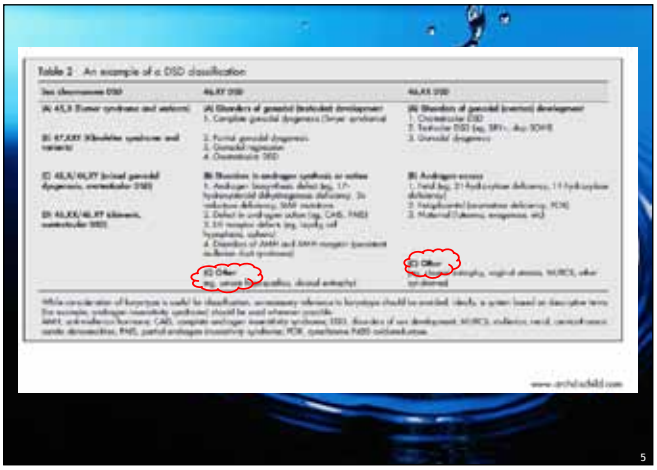


Table 2 An example of a DSD classification

Sex chromosome DSD	46,XY DSD	46,XX DSD
(A) 45,X (Turner syndrome and variants)	(A) Disorders of gonadal (testicular) development 1. Complete gonadal dysgenesis (Swyer syndrome) 2. Partial gonadal dysgenesis 3. Gonadal regression 4. Ovotesticular DSD	(A) Disorders of gonadal (ovarian) development 1. Ovotesticular DSD 2. Testicular DSD (eg, SRY+, dup SOX9) 3. Gonadal dysgenesis
(B) 47,XXY (Klinefelter syndrome and variants)		
(C) 45,X/46,XY (mixed gonadal dysgenesis, unilateral or bilateral DSD)	(B) Disorders in androgen synthesis or action 1. Androgen biosynthesis defect (eg, 17-hydroxysteroid dehydrogenase deficiency, 5α-reductase deficiency, StAR mutations) 2. Defect in androgen action (eg, CAIS, PAIS) 3. 5α-reductase deficiency (eg, copies of steroid 5α-reductase, alpha) 4. Disorders of AMH and AMH receptor (persistent Müllerian duct syndrome)	(B) Androgen excess 1. Fetal (eg, 21-hydroxylase deficiency, 11β-hydroxylase deficiency) 2. Fetoplacental (aromatase deficiency, POR) 3. Maternal (ingestion, exogenous, etc)
(D) 46,XX/46,XY chimeric, unilateral or bilateral DSD	(C) Other (eg, urogenital sinus anomalies, cloacal anomaly)	(C) Other (eg, vaginal atresia, müllerian anomalies, MURCS, other syndromes)

While consideration of karyotype is useful for classification, an necessary reference to karyotype should be avoided ideally, a system based on descriptive terms for anatomic, endocrine (testicular) synthesis) should be used wherever possible.
AMH, anti-müllerian hormone; CAIS, complete androgen insensitivity syndrome; DSD, disorders of sex development; MURCS, müllerian, renal, cervicothoracic somite abnormalities; PAIS, partial androgen insensitivity syndrome; POR, cytochrome P450 oxidoreductase

www.orphanet.com

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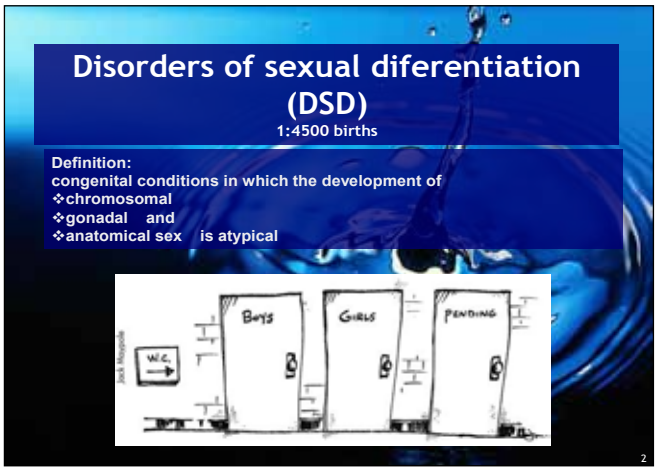

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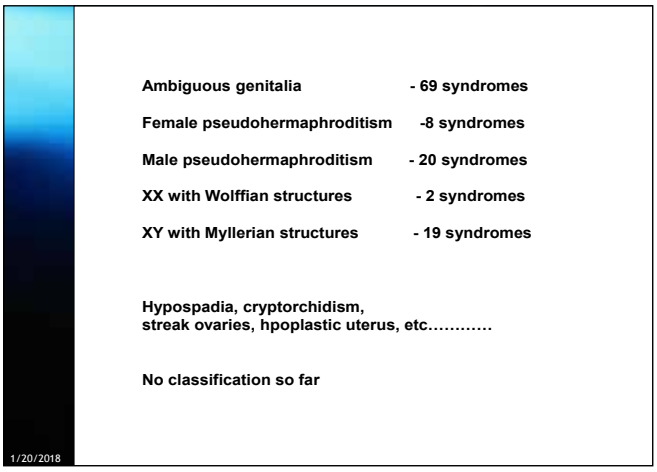

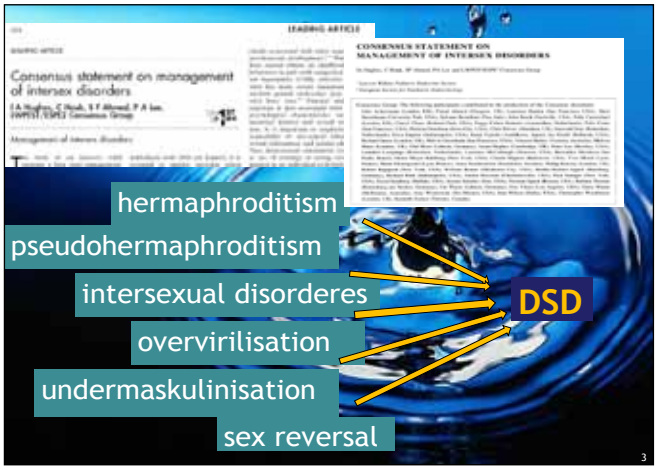

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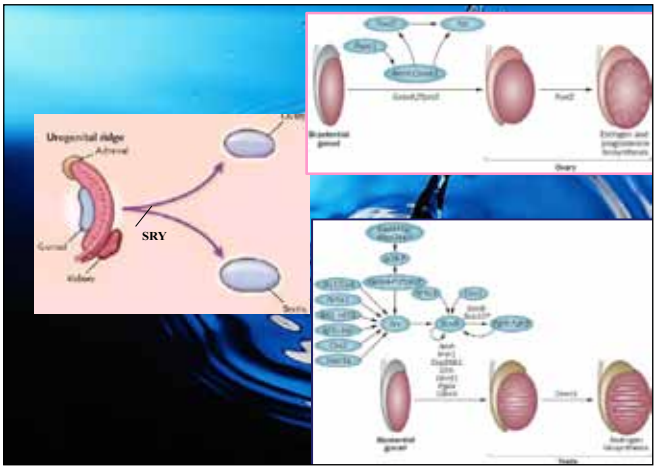

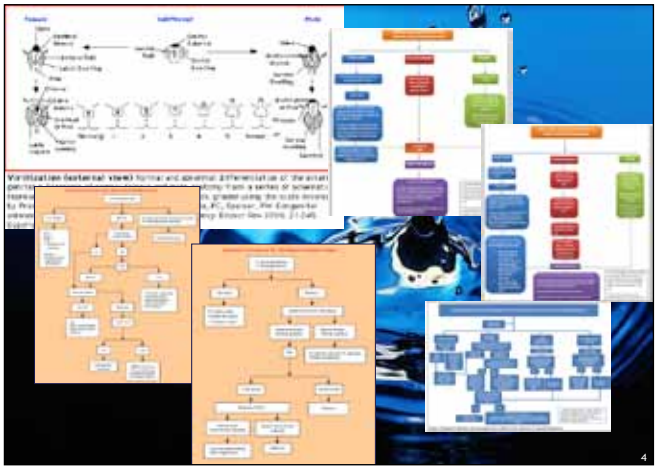

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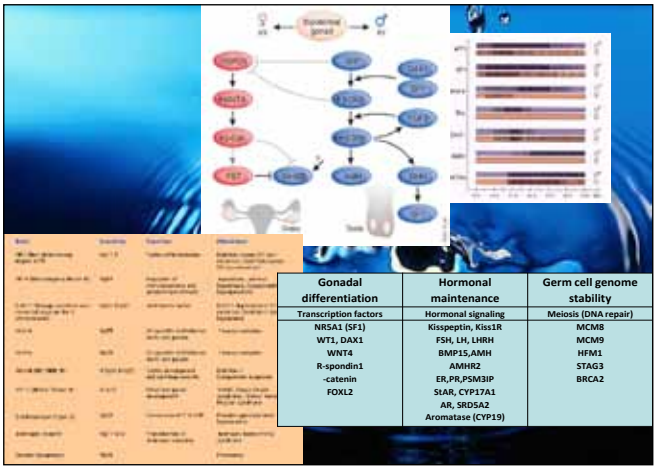


Materials and methods

14 patients with syndromic DSD

- **11 with XY karyotype, (4SRY positive)**
- **3 with XX karyotype, (2 SRY negative)**

Excluded - CAH, Turner sy, Klinefelter sy, isolated hypospadia, Swyer, AIS, Meyer-Rokitanski..

Associated anomalies

System	Patiens	More frequent
CNS	6	Hydrocephalus, corpus callosum agenesis, microcephalus, hypoplasio vermis
Developmental delay / mental retardation	9 (2 early neonatal deaths)	Mild/moderate/severe
Cardiovascular system	5	VSD, ASD, Tetralogia Fallot, atresio a.pulmonalis, hypoplastic right ventricle
Intestinal system	3	Anal atresia, Hirshprung, esophageal fistula
Urinary system	5	Kidney agenesia/hypoplasia, VUR, vesical extrophy
Musculo-skeletal system	10	Artrogryphosis, rib agenesia, vertebral anomalies, sacral agenesia, metaphyseal flaring, pes equinovarus, Sprengel anomaly

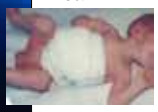
	karyotype	SRY	Barr bodies	Prader staging	
P1	46,XY	/	-	P2	Costello syndrome
P2	46,XY	/	-	P4	Distal artrogryphosis
P3	46,XY	/	/	P4	Multiple synphalangism sy
P4	46,XY	-	/	P3	Vater sy
P5	46,XY	-	-	P2	Vater sy
P6	46,XY	+	-	P3	Del Y
P7	46,XY	/	-	P3	MURCS
P8	⇒	+	-	female	46,XY,del 9p
P9	⇒	+	-	female	46,XY, der(10q), t(Xp;10q)mat
P10	⇒	/	-	P2	46,XY,der(9p), t(4q;9p)mat
P11	46,XY	+	-	P3	Smith-Lemli-Opitz sy

Associated anomalies


P2 artrhrogryphosis


P9 VUR

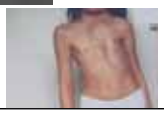

P11 artrhrogryphosis


P6 Hirshprung anomaly


P7 rib agenesis


P5 VUR, sacral agenesis


P8 metaphyseal wiedening


P13 scoliosis, Sprengel anomaly

	karyotype	SRY	Barr bodies	CAH screen	Prader staging	
P12	46,XX	-	+	-	P4 dup	??
P13	46,XX	/	+	-	P1	MURCS
P14	46,XX	/	+	/	P2	Extrophy vesicae

Facial dysmorphism


p1


p10


p6


p5

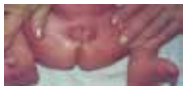

p7


p11


p8


p14


p9

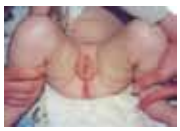

p10

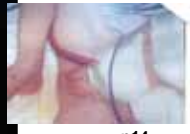

p1


p6


p4


p8


p5


p14

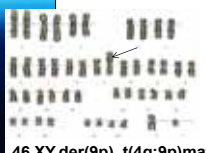

p9


p2

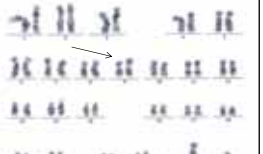
Possible mechanisms


p10


p8

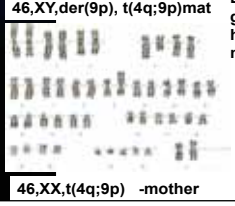

46,XY,der(9p), t(4q;9p)mat

DMRT1/DMRT2 gene haploinsuficiency

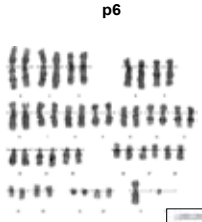

46,XY,del(9p), inv (9p;q)pat


Sex-determining gene(s) on distal 9p: clinical and molecular studies in six cases.

A Gene Involved in XY Sex Reversal Is Located on Chromosome 9, Distal to Marker D9S1779


46,XX,t(4q;9p) -mother

Possible mechanisms
p6



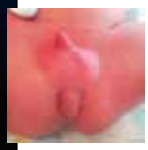
46,XY,del Y

SRY positive
Hypoplasio testis
Profound MR

Possible mechanisms ??
p12

46,XX
CAH screen negative
SRY negative
Barr body -positive

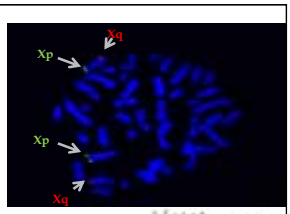
Anal atresia
Hirshprung
Sacral agenesia



Diphallus with ectopic bowel segment: a case report.

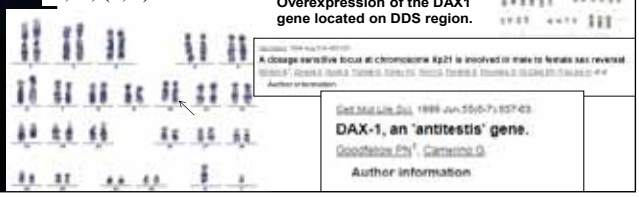
Possible mechanisms
p9

46,XY, t(X;10)mat SRY+



Mother
46,XX, t(X;10)

Overexpression of the DAX1 gene located on DDS region.

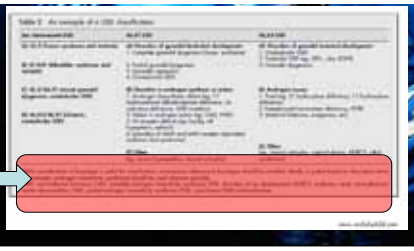


DAX-1, an 'antitestis' gene.

Author information

CONCLUSION

- **There are more than 130 syndromes associated with sexual ambiguity**
- **Some of them have recognized chromosomal or molecular defect, but most of syndromic DSD's still don't have established mutation or pathway and are commonly recognized clinically by the continuum of associated anomalies.**
- **Syndromes with DSD should be included into the DSD classification as a separate subgroup**



GENETIC BACKGROUND OF STEROID-RESISTANT NEPHROTIC SYNDROME IN BULGARIA

Valentin Penchev

Genetic background of SRNS

Gene	Protein	Mode of inheritance	Syndrome or extrarenal manifestations
Slit diaphragm associated			
CD2AP	CD2-associated protein	Autosomal recessive/ autosomal dominant	
NPHS1	Nephrin	Autosomal recessive	
NPHS2	Podocin	Autosomal recessive	
PLCE1	Phospholipase C, ε1	Autosomal recessive	
TRPC6	Transient receptor potential cation channel, subfamily C, member 6	Autosomal dominant	
Actin-cytoskeleton			
ACTN4	α-Actinin 4	Autosomal dominant	
ANLN	Anillin	Autosomal dominant	
ARHGAP24	Rho GTPase activating protein 24	Autosomal dominant	
ARHGDIA	RhoGDP dissociation inhibitor α	Autosomal recessive	
INF2	Inverted formin 2	Autosomal dominant	Charcot–Marie–Tooth
MYO1E	Nonmuscle myosin 1e	Autosomal recessive	
Mitochondrial proteins			
ADCK4	aarF domain containing kinase 4	Autosomal recessive	
COQ2	Coenzyme Q2 4-hydroxybenzoate polyprenyl transferase	Autosomal recessive	Seizures, encephalopathy
COQ6	Coenzyme Q6 monooxygenase	Autosomal recessive	Sensorineural deafness
MTTL1	tRNA-LEU	Unknown	Mental retardation, diabetes mellitus, MELAS syndrome
PDSS2	Prenyl diphosphate synthase subunit 2	Autosomal recessive	Encephalomyopathy, Leigh syndrome

M. N. Rheault, R. A. Gbadegesin, J Pediatr Genet 2016;5:15–24.

Introduction
Pediatric kidney and urinary tract disorders

- **Kidney disease in children, although in most cases highly treatable and with no long term consequences, may lead to chronic or end-stage renal disease with their significant morbidity and mortality. Among the most common paediatric kidney and urinary tract disorders are:**
  - **Nephrotic syndrome (NS)**
    - **incidence - between 1:50 000 and 1:500 children;**
    - **10-20% do not respond to steroid treatment and classified as SRNS;**
    - **burden – 20-40% of SRNS patients will develop ESRD;**
    - **at least 39 genes for SRNS have been identified so far.**
  - **Congenital anomalies of the kidney and urinary tract (CAKUT)**

Genetic background of SRNS

Adhesion and glomerular basement membrane proteins			
COL4A3	α3 type IV collagen	Autosomal recessive	Sensorineural deafness
COL4A4	α4 type IV collagen	Autosomal recessive	Sensorineural deafness
COL4A5	α5 type IV collagen	X-linked	Sensorineural deafness
ITGA3	Integrin α3	Autosomal recessive	Interstitial lung disease, epidermolysis bullosa
ITGB4	Integrin β4	Autosomal recessive	Epidermolysis bullosa
LAMB2	Laminin β2	Autosomal recessive	Pierson syndrome
Nuclear transcription factors			
LMX1B	LIM homeobox transcription factor 1β	Autosomal dominant	Nail–patella syndrome
NXF5	Nuclear RNA export factor 5	X-linked	Cardiac conduction disorder
SMARCL1	SMARCA-like protein	Autosomal recessive	Schimke immunoosseous dysplasia
WT1	Wilms tumor 1	Autosomal dominant	Denys-Drash, Frasier syndrome
Others			
CFH	Complement factor H	Autosomal recessive	Atypical hemolytic uremic syndrome
CUBN	Cubilin	Autosomal recessive	Megaloblastic anemia
DGKE	Diacylglycerol kinase ε	Autosomal recessive	Atypical hemolytic uremic syndrome
MEFV	Pyrin	Autosomal recessive	Mediterranean fever
NEIL1	Nei endonuclease VIII-like 1	Autosomal recessive	
PMM2	Phosphomannomutase 2	Autosomal recessive	Congenital defects of glycosylation
PTPRO	GLEPP1	Autosomal recessive	
SCARB2	Lysosomal integral membrane protein type 2	Autosomal recessive	Action myoclonus, renal failure syndrome
WDR73	WD repeat domain 73	Autosomal recessive	Galloway-Mowat syndrome
ZMPSTE24	Zinc metalloproteinase STE24	Autosomal recessive	Mandibuloacral dysplasia

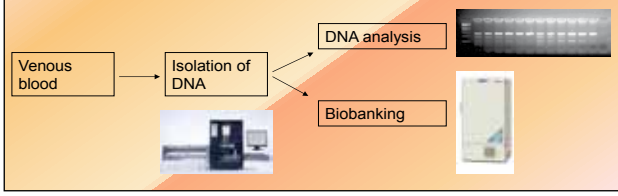
M. N. Rheault, R. A. Gbadegesin, J Pediatr Genet 2016;5:15–24.

Introduction
The importance of genetic testing in families with SRNS

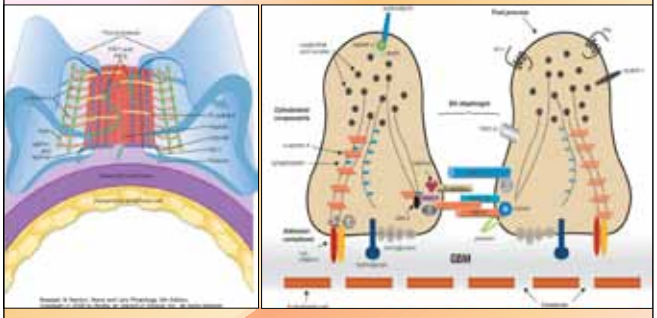
- ❑ **SRNS is a clinically and genetically heterogeneous group of diseases characterized by massive proteinuria, albuminuria and oedema;**
- ❑ **As a genetic background more than 39 single gene causes are known;**
- ❑ **Most commonly affected are *NPHS1*, *NPHS2*, *ACTN4*, *CD2AP* (responsible for the structure of glomerular filter) and *WT1* (important transcription factor involved in embryogenesis)**
- ❑ **Genetic testing in SRNS patients facilitates the choice of treatment;**
- ❑ **Genetic testing in SRNS patients help to perform proper genetic counselling for the affected families;**
- ❑ **Identifying the causative variants allows better understanding of the molecular causes of the disease which will facilitate better diagnosis algorithms;**
- ❑ **Early diagnosis allows better treatment options.**

Materials and methods

- ❑ **For the goal of the present study 28 patients with SRNS from 24 families were recruited;**
- ❑ **After signing informed consent venous blood was taken as initial clinical material;**
- ❑ **Isolated genomic DNA is stored in the Biobank of the Molecular Medicine Center;**
- ❑ **DNA analysis was focused on *NPHS2* and *WT1*, the two genes most commonly affected in non-Finnish patients.**



Introduction
Structure of the slit diaphragm



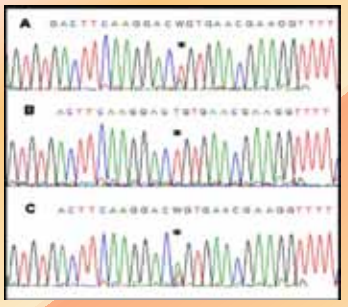
Results and discussion

The genetic cause of the disease was determined in five families

Gene	Mutation	Zygosity
WT1	p. Ser395Tyr	heterozygous
WT1	p. Cys428Ser	heterozygous
NPHS2	p.Gly140Aspfs * 40	homozygous/ compound heterozygous
NPHS2	p.Leu169Pro	homozygous/ compound heterozygous

Results and discussion

A unique case of maternal WT1 mutation p.Cys428Ser mosaicism was documented.



A – Mother
B – Father
C - Child

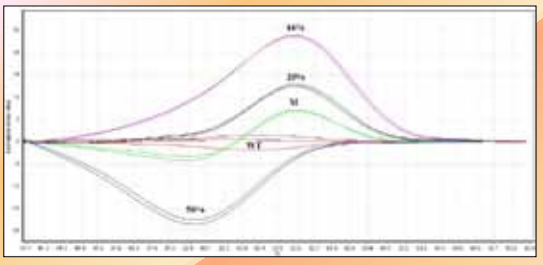
Funding

The study was funded under grants:

- National Science Fund
  DUNK01-2/2009 and DMU03/73-2011
- Council of Medical Silence, Medical University of Sofia
  MU 29/2007 and MU14/2008

Results and discussion

For mosaicism determination qPCR HRM was performed.



MMC team

- MSc students: E. Boiadjieva
  P. Botev
  P. Georgieva
- Senior scientists: O. Beltcheva, PhD
  assoc. prof. R. Kaneva, PhD
  acad. prof. V. Mitev, MD, PhD, DSc

Conclusions

- **NPHS2 and WT1 mutations do not have major contribution for SRNS in Bulgarian patients;**
- **Genetic testing in SRNS facilitates both the choice of treatment (i.e. good response to cyclosporine in p.Leu169Pro carriers; decision making in case of transplantation) and genetic counselling (i.e. possibilities for prenatal testing);**
- **In addition, identifying the causative variants allows us to better understand the molecular causes of the disease;**
- **Two novel heterozygous WT1 mutations (p. S395Y and p. Cys428Ser) were detected and a unique case of maternal WT1 mutation mosaicism was documented;**
- **In order to identify the genetic cause of the disease in larger proportion of the patients we need to expand the screening panel.**

Thank you for the attention!

Collaborators

- SBAL Paediatric Diseases:
  assoc. prof. A. Boueva, MD, PhD
  assoc. prof. P. Miteva, MD, PhD
  assist. prof. D. Roussinov, MD, PhD
  Sv. Marinova, MD

MOLECULAR BASIS OF DEVELOPMENTAL DISORDERS: A VIEW THROUGH THE KIDNEY FILTER

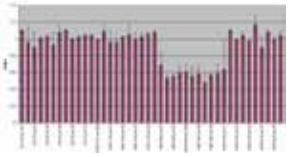
Olga Beltcheva

The case of HNF1β

A boy with bilateral hyperechogenity detected prenatally.

No family history of renal disease or diabetes.

De novo mutation, a heterozygous deletion of the entire HNF1β gene, was detected by means of MLPA.



Paediatric kidney and urinary tract disorders

Kidney disease in children, although in most cases highly treatable and with no long term consequences, may lead to chronic or end-stage renal disease with their significant morbidity and mortality. Among the most common paediatric kidney and urinary tract disorders are:

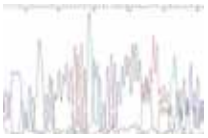
1. Nephrotic syndrome (NS)
2. Congenital anomalies of the kidney and urinary tract (CAKUT)
   - incidence - 3.3 per 1 000 births in Europe;
   - 20-30% of all anomalies identified during the prenatal period;
   - burden – 30-50% of CAKUT patients will develop ESRD;
   - 30% associated with non-renal malformations;
   - more than 35 genes have been identified so far.

The case of PAX2

A girl with renal dysplasia, diagnosed accidentally during ultrasonography for abdominal pains at the age of 6.

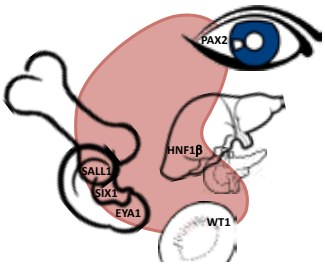
A familial case - mother diagnosed with ESRD and bilateral hypoplasia.

A heterozygous frameshift mutation (c.75_76dupGG, p.Val26GlyfsTer4) was found in both mother and child in PAX2.



CAKUT – our set of patients

Phenotype	Number of patients
Families / Females / Males Unilateral / Bilateral	65 / 25 / 44 15 / 37
Renal cysts/dysplasia/hyperechogenicity	32
Renal hypoplasia	5
Renal agenesis	7
Vesicoureteral Reflux (VUR)	3
Megaureter/Monoureter	11
Hydronephrosis	20
Atypical Hemolytic Uremic Syndrome (aHUS)	1
Nephronophthisis (NPH)	2
Extra-renal: • Heart anomalies • Skeletal anomalies • Mental retardation • Sensory deficits • Metabolic syndrome	 4 4 5 6 1



Candidate gene screening for "simple" cases

Initially, we chose to screen for mutations the most commonly affected genes known to contribute to the CAKUT pathology:

1. HNF1β (TCF2) – renal cysts and diabetes syndrome
2. PAX2 – renal-coloboma syndrome
3. SALL1 - Townes-Brocks branchio-oto-renal-like syndrome
4. EYA1 – Branchio-oto-renal syndrome 1
5. SIX1 – Branchio-otic syndrome with sporadic kidney involvement

All five genes code for transcription factors with key role for embryonic development.

Sanger sequencing and MLPA was used for mutation detection.

Some practical aspects

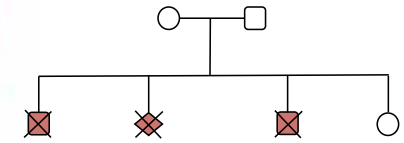
The difficulties

1. Incomplete penetrance
2. Variable phenotype
3. Di (poly?)genic inheritance

The solutions

1. High-troughput techniques that give information for greater number of loci
2. Multiple data base analysis and extensive review of literature (published cases, animal experiments)

Nephronophthisis with digenic inheritance



death at 2 m.
renal hypodysplasia,
renal and liver failure

renal dysplasia

death at 4 m.
renal hypodysplasia,
renal and liver failure

NPHP3 p.Arg577*
p.Ala1203Valfs*16

NPHP3 p.Arg577* (F)
p.Ala1203Valfs*16 (M)
NPHP4 p.Asp3Tyr (F)

Complex approach for complex cases

Carrying out genetic testing in patients with complex phenotype gives us a unique chance to look at the grater picture and uncover common developmental pathways involved in a number of pathologies.

Using high-throughput techniques, such as aCGH and NGS, we can identify novel genes, some of which may be key players in the embryonic development.

The knowledge of the gene and the pathway affected in each individual will allow better treatment, improved prognosis and accurate genetic counselling.

Some practical aspects

The difficulties

1. Incomplete penetrance
2. Variable phenotype
3. Di (poly?)genic inheritance

The solutions

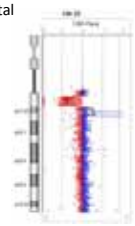
1. High-troughput techniques that give information for grater number of loci
2. Multiple data base analysis and extensive review of literature (published cases, animal experiments)
3. Collaboration with medical professionals
4. Collaboration with the families

A complex case Di George

A girl with unilateral renal agenesis, heart abnormalities, mental retardation, umbilical hernia , cloudy eye spot and macroglossia.

Sporadic case in a family with no history of developmental disorders.

A heterozygous deletion in *22q11.21* was detected with aCGH, later confirmed with MLPA.



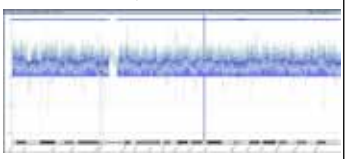
Trisomy 8

A boy with hydronephrosis of the right kidney and cystic left kidney with loss of parenchymal tissue. Epidermoid cyst on the forehead. High degree myopia (7 diopters). Mild learning disabilities.

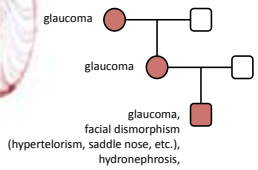
Sporadic case in a family with no history of developmental disorders.

Trisomy of chr 8 was detected with aCGH, with suspected mosaicism.

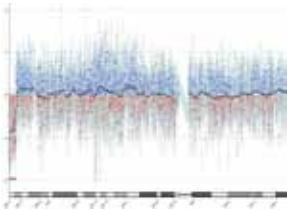
Confirmed with cytogenetics.



6p25.3-25.2 deletion



Affected genes: DUSP22, IRF4, EXOC2, HUS1B, FOXQ1, FOXF2, FOXC1, GMDS, MYLK4

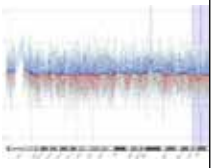


LPP partial deletion

A boy with nephrocalcinosis of the right kidney, hydronephrosis and dysplasia of the left kidney. Congenital adrenal hyperplasia (genetic diagnosis of 21 hydroxylase deficiency).

Sporadic case in a family with no history of developmental disorders. Heterozygous partial deletion of LPP (lipoma preferred partner) detected with aCGH.

Haploinssufficiency of LPP has been reported in a single patient with renal hypoplasia, heart, aesophageal and rib abnormalities (VACTERL).



Collaborators

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The making of the kidney

DIAGNOSTIC AND THERAPEUTIC APPROACH IN CHILDREN WITH BILIARY ATRESIA

M. Baycheva, P. Yaneva, P. Hadjiiski, R. Shentova, D. Kofinova, Ch. Zhelev

Introduction

- Biliary atresia (BA) is a destructive inflammatory obliterative cholangiopathy of neonates that affects in variable extent both intra- and extrahepatic bile ducts
- No analogous pathological process exists in older children or adults
- Obstruction of the biliary tree results in severe cholestasis leading to cirrhosis which is progressive and if untreated, leads to death by the age of 2 years
- BA is the leading cause for liver transplantation in children worldwide

Characteristics

- ~20% of all cases are associated with other congenital anatomical abnormalities
- The most common is biliary atresia splenic malformation syndrome (BASMS), reported in about 10% of European and US series
- Includes polysplenia (90%), situs inversus (50%), and unusual vascular anomalies such as absence of an inferior vena cava and a preduodenal portal vein
- Possible association with maternal diabetes (exposure of the developing embryo to a hyper- or hypoglycaemic environment?)
- BA has been described with several other genetic abnormal findings, such as trisomy 18 and 21

Hartley JL et al. Lancet 2009
Davenport M et al. J Pediatr 2006

Characteristics

- BA is an isolated finding in the remaining 80–90% of neonates
- The theory is that in these newborn babies the pathological obliterative process begins later (perhaps in the perinatal period) than it does in those of syndromic origin (beings in the embryonic phase)
- Some cases of isolated biliary atresia are associated with cystic changes – cystic biliary atresia, and half of those are detectable on antenatal ultrasound scan (from 20 weeks of gestation), showing that even in isolated non-syndromic cases the biliary tree has abnormal changes well before birth

Hartley JL et al. Lancet 2009

Epidemiology

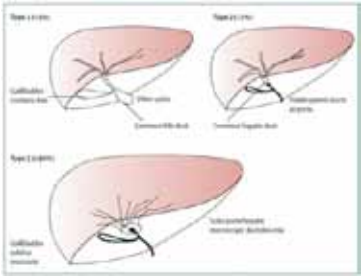
- BA is a rare disease
- Prevalence in Europe 1:18,000 live births
- In the world, the reported incidence varies from 5-32:100,000 live births, and is highest in Asia and the Pacific region
- Females are affected slightly more often than males

Etiology

- The etiology of BA is unknown
- The pathogenesis is still unclear, despite the efforts of many researchers

Classification

- syndromic/non-syndromic
- fetal/perinatal
- surgical classification



Hartley JL et al. Lancet 2009

Pathogenesis

- There are different hypotheses including genetic predisposition, infections or toxic insults during pregnancy, immune dysregulation and autoimmune-mediated bile duct destruction, abnormalities in bile duct development because of genetic mutations
- The cause is probably multifactorial, leading to a common anatomical substrate – **obliterative extrahepatic cholangiopathy**

Feldman A, Mack C. JPGN 2015

Pathogenesis

- The current theory for the pathogenesis of BA proposes that during the perinatal period, a still unknown exogenous factor meets the innate immune system of a genetically predisposed individual and induces an uncontrollable and potentially self-limiting immune response, which becomes manifest in liver fibrosis and atresia of the extrahepatic bile ducts

Feldman A, Mack C. JPGN 2015

Infections and immune dysregulation

- Candidate viruses that may trigger the bile duct injury:
  - Cytomegalovirus (CMV)
  - Rotavirus
  - Reovirus
- A higher prevalence of CMV antibodies in the mothers of infants with BA, higher serum CMV-Ig M levels, and greater amounts of Ig deposits on the canalicular membrane of the hepatocytes in infants with BA have been reported
- Strong evidence for a perinatal CMV infection associated with BA was described

Feldman A, Mack C. JPGN 2015
Xu Y et al. Clin Pediatr (Phila) 2012

Gene mutation association

- China group analyzed single-nucleotide polymorphisms (SNPs) and susceptibility genes in BA through genome-wide association studies, and revealed a strong association of BA with the SNP rs17095355 on chromosome 10q24
- Two genes in the region of this SNP include X-prolyl aminopeptidase P1 (XPNPEP1) and adducin 3 (ADD3)
  - XPNPEP1 is expressed in biliary epithelia and is involved in the metabolism of inflammatory mediators
  - ADD3 is expressed in hepatocytes and biliary epithelia and is involved in the assembly of spectrin-actin membrane protein networks at sites of cell-to-cell contact. Defective ADD3 could result in excessive deposition of actin and myosin, contributing to biliary fibrosis
- A study from the US tested the association of SNPs on chromosome 10q24 and BA and found the strongest signal to be at rs7099604 within the ADD3 gene

Garcia-Barcelo MM et al. Hum Mol Genet 2010
Tsai EA at al. Hum Genet 2014

Infections and immune dysregulation

- An initial viral insult to the biliary tree leads to expression of previously sequestered ''self'' antigens from the damaged bile duct epithelial cells and elicit autoreactive TH1-cell-mediated inflammation and B-cell production of autoantibodies directed at duct epithelia
- The predominant cellular immune response in BA encompasses activated CD4+ and CD8+ T cells within portal tracts that produce TH1 cytokines (interleukin-2 and IFN-g) and macrophages secreting tumor necrosis factor (TNF)-a
- These lymphocytes have been found invading between bile duct epithelia, leading to degeneration of intrahepatic bile ducts

Feldman A, Mack C. JPGN 2015

Gene mutation association

- The identification of putative BA susceptibility loci not only opens new fields of investigation into the mechanisms underlying BA but may also provide new clues for the development of preventive and curative strategies

Garcia-Barcelo MM et al. Hum Mol Genet 2010

Diagnostic approach

- BA presents in the first weeks of life
- Early diagnosis of biliary atresia is very important because of the possibility of treating the disease with Kasai portoenterostomy
- There is still not an unified diagnostic approach worldwide but it is quite clear that **early diagnosis improves prognosis**, and also the outcome of liver transplantation, which is the treatment choice when portoenterostomy is not successful or not available

Infections and immune dysregulation

- Bile duct injury in BA may be initiated by a virus infection followed by a secondary autoimmune response targeting bile duct epithelia
- The damaged bile duct cells may express self-proteins that are recognized as foreign, and elicit autoreactive T-cell- mediated inflammation and B-cell production of autoantibodies

Feldman A, Mack C. JPGN 2015

Diagnosis

• Clinical features:	• Labs (at presentation):
➢ jaundice	➢ Bilirubin >100 µmol/L
➢ pale stool	➢ ALP >600 IU/L
➢ dark urine	➢ γGGT >100 IU/L
➢ coagulopathy	➢ AST, ALT 80-200 U/L
➢ failure to thrive	➢ Albumin, prothrombin time – normal at presentation
➢ hepatosplenomegaly and ascites	

Diagnosis

- Early detection of elevated conjugated bilirubin
- Raised conjugated (direct) bilirubin > 20% of total in a two-week-old infant **must** be evaluated
- All term babies >14 days, preterm >21 days
- Stool colour charts to help parents to identify abnormal stool

Diagnosis

- Cholangiography – Endoscopic retrograde or magnetic resonance cholangiopancreatography (ERCP/MRCP)
- Percutaneous transhepatic cholangiography (PTC)
- Intraoperative cholangiography during laparotomy/laparoscopy

Diagnosis

- Ultrasound scan (USS)
- Liver biopsy
- MRCP/PTC
- Radio isotope scan
- Laparotomy/laparoscopy

Diagnosis

- Radio isotope scan, previously available in Bulgaria
- New modalities (HIDA/TEBIDA)
- Low specificity

Diagnosis

- Ultrasound scan (USS)
- USS shows an enlarged liver, absence of biliary dilation, and typically an absent or contracted gallbladder after a 4 h fast
- 20% of cases might have a normal gallbladder
- Identification of hyperechogenic liver hilum (triangular cord sign) is a specific finding in almost all cases but depends on the expertise of operator thus the reported sensitivities vary from 49% to 73%

Our approach and expertise

- University Children's Hospital in Sofia, Department of Gastroenterology/Hepatology is the national paediatric liver center
- Annually 2-4 new referrals with biliary atresia among multiple referrals of infants with conjugated hyperbilirubinaemia and neonatal hepatitis

Diagnosis

- Liver histology (obtained by percutaneous biopsy) is the usual diagnostic method of choice in some European countries and in the last few years in Bulgaria
- It shows extrahepatic biliary obstruction by varying degrees of portal tract fibrosis, oedema, ductular proliferation, and cholestasis with the appearance of bile plugs; evidence of giant cell transformation might be present, making differentiation from other causes of neonatal hepatitis difficult
- If taken early in life (<6 weeks) might be not informative

Our approach and expertise

- We now analyze the results of the management of children with biliary atresia in Bulgaria trying to establish a diagnostic and therapeutic algorithm for early diagnosis, introduction of appropriate treatment and preparation for surgery
- Clinical data for a period of twenty years include 38 children with biliary atresia who underwent liver transplantation with or without Kasai portoenterostomy

Our approach and expertise

- All patients presented with typical symptoms – jaundice, pale stool, dark urine, and specific laboratory results – conjugated hyperbilirubinaemia and cholestasis
- Variable time of referral from the general paediatricians (4-16 weeks)
- All patients were admitted to the hospital with at least 14 days of close observation (by doctor/experienced nurse) of stool colour

Treatment

- Surgical – Kasai portoenterostomy done in small number of cases (1996-2006)
- No surgical teams are performing portoenterostomy at present in Bulgaria
- World experts say it should be done only in experienced hands
- 50% of patients will still require liver transplant afterwards – same in our center

Our approach and expertise

- During the follow up we found coagulopathy and failure to thrive in some of the children, which are important prognostic factors
- Hepatosplenomegaly and ascites are late signs (after the age of three months) and are related to cirrhosis

Treatment

- Adjuvant therapy – after Kasai portoenterostomy and prior to transplant
- Nutrition (MCT, calories, optimal growth)
- Fat soluble vitamins
- Steroids
- Antibiotics
- Ursodeoxycholic acid
- Complications – portal hypertension, hepato-pulmonary syndrome, infections
- Post transplant complications

Our approach and expertise

- In the first 10 years of the period radio isotope scans were done
- Not possible at present, also low specificity
- PTC in single cases
- Liver biopsy

Treatment

- Pre-transplant assessment

➢ confirm the indication for Tx
➢ determine the severity of the disease
➢ consider appropriate treatments prior to Tx
➢ exclude contraindications
➢ identify active infections and assess the immunological status of the child
➢ rule out cardiac malformations that might need to be corrected before operation

Treatment

- Surgical – portoenterostomy (first described by the Japanese surgeon Morio Kasai in the 1950s) and liver transplantation remain the cornerstones of treatment of children with biliary atresia
- Adjuvant therapy – integrated multidisciplinary approach

Treatment

- Pre-transplant assessment

➢ therapeutic plan: immunizations, nutritional support to optimize growth, dental care, prevention or treatment of drug-induced side effects (osteopenia secondary to prolonged steroid intake)
➢ inform parents, and the patient if possible, on the Tx procedure and on the post-transplant period, prepare them to accept the issues and possible complications
➢ evaluate social status and logistic issues

Conclusion

- Establishing diagnostic and therapeutic algorithm in patients with biliary atresia is a challenge despite all achievements in hepatology
- Early diagnosis is essential and the efforts of paediatricians to refer those infants to a specialized unit must be part of a national strategy for all rare gastrointestinal and liver diseases
- Identifying actual problems in our country and establishing protocols for the GPs and general paediatricians would be crucial in improving prognosis

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Thank you for your attention!

SESSION 8-II

Moderators: Lyudmila Angelova, Trifon Chervenkov

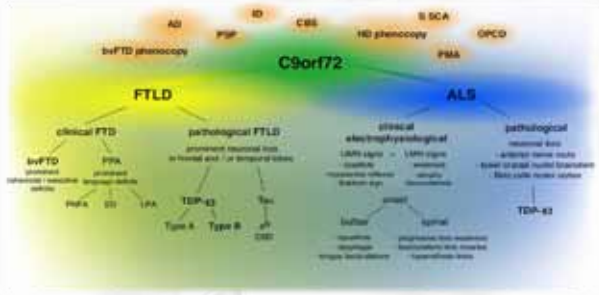
Oral presentations:

- **Study of ATXN2 repeat length in C9ORF72 expansion**
  A. Marjanovic
- **Spectrum of mutations in the CFTR gene of Albanian cystic fibrosis patients**
  G. Zoraqi
- **Genetically verified tuberous sclerosis complex in a cohort of fifteen Bulgarian families**
  M. Glushkova
- **Molecular profiling of papillary thyroid cancer by RNA expression and NGS sequencing platforms**
  D. Nikolova
- **Genetic profiling of advanced laryngeal carcinoma by NGS**
  S. Giragosyan

STUDY OF *ATXN2* REPEAT LENGTH IN *C9ORF72* EXPANSION CARRIERS

Marjanović Ana, Dobričić Valerija, Marjanović Ivan, Branković Marija, Janković Milena, Mandić Gorana, Stefanova Elka, Stević Zorica, Novaković Ivana, Kostić Vladimir

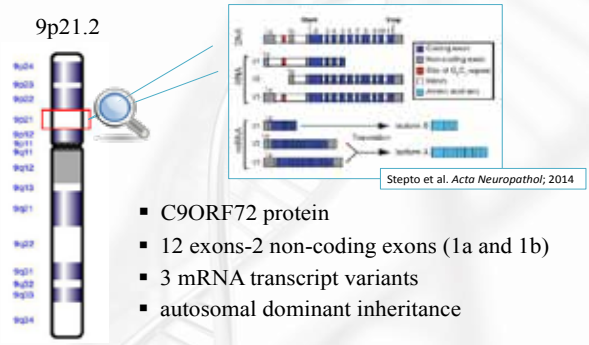
C9ORF72 phenotype spectrum



Van Mossevelde et al., Current Opinion in Genetics & Development ; 2017

C9ORF72

9p21.2

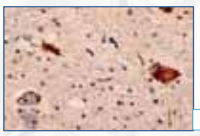


Stepto et al. *Acta Neuropathol*; 2014

- C9ORF72 protein
- 12 exons-2 non-coding exons (1a and 1b)
- 3 mRNA transcript variants
- autosomal dominant inheritance

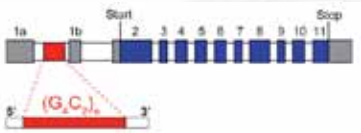
ATXN2

- CAG repeats in exon 1
- Spinocerebellar ataxia 2 (SCA2): CAG ≥33
- Intermediate *ATXN2* repeats (27-32 CAG repeats)
- *ATXN2* enhances TDP43 toxicity
- TDP43 inclusions in ALS



DeJesus-Hernandez et al. *Neuron*; 2011

C9ORF72



Stepto et al. *Acta Neuropathol*; 2014

- repeats = 2 - >4000
- normal range = 2 - 30

?

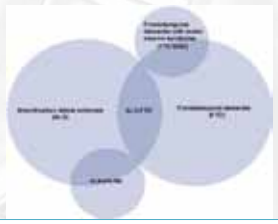
Material and Methods

- 280 ALS, 264 FTD, 159 AD, and 135 HD like patients (clinical criteria)
  - Blood sampling
  - DNA extraction

- Sizing of *C9ORF72* repeats:
  - Regular (fluorescent) PCR
  - Repeat-primed PCR

C9ORF72

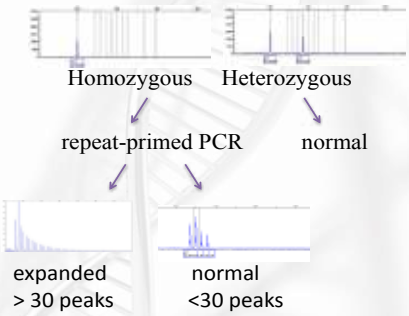
- Amyotrophic lateral sclerosis - ALS
- Frontotemporal dementia - FTD



adapted from Gjerde & Tysnes, *Tidsskr Nor Legeforen*; 2014

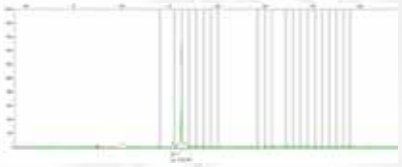
Material and Methods

- Regular PCR



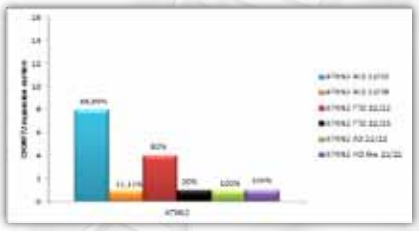
Material and Methods

- Sizing of *ATXN2* CAG repeats:
  - Regular (fluorescent) PCR



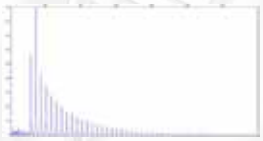
Results

- Intermediate *ATXN2* repeat length $(CAG)_{28}$
  - one (11.11%) ALS case carrying *C9ORF72* expansion
  - none in FTD, AD, nor HD like group.



Results

- **C9ORF72 (>30 peaks) : 3,21% (9/280) ALS, 1,89% (5/264) FTD, 0,74% (1/135) HD like patients**
- 0,63% (1/159) AD with border line repeat number

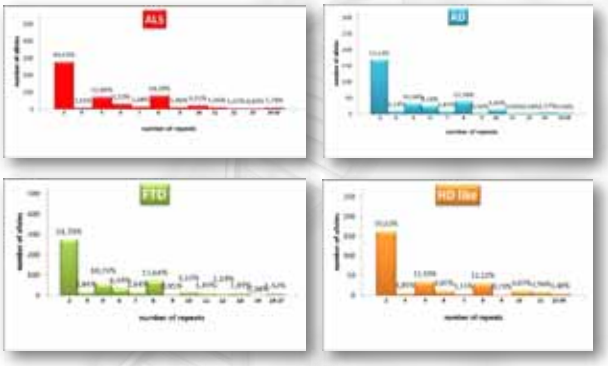


- Normal alleles: 2 – 27 repeats

Conclusion

- Intermediary CAG repeats in *ATXN2* were found in one ALS case.
- These repeats were not associated with FTD, AD nor HD like among *C9ORF72* expansion carriers.
- Further assessment is needed to evaluate the significance of *ATXN2* repeats among ALS patients.

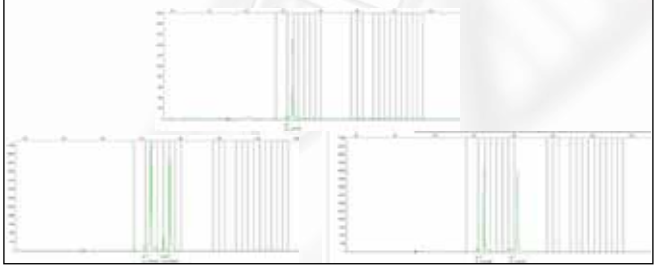
Results



Thank you for your attention!

Results

- *ATXN2* CAG repeat length in 16 C9ORF72 expansion carriers: 22-28 repeat units
- Most frequent allele was with 22 repeats



SPECTRUM OF MUTATIONS IN THE CFTR GENE OF ALBANIAN CYSTIC FIBROSIS PATIENTS

G. Zoraqi, E. Vevecka, L. Shundi, V. Falbo, M.R. D'Apice,G. Novelli

History of CF studies in Albania

- Limited data exist on the distribution of CF mutations in Albanian population. The most recent data published date back in 2002.
- In the review paper on worldwide distribution of CF mutations, Albania was shown having a pattern of 4 CF mutations of relative frequencies: p.Phe508del (72.4%), p.Gly85Glu (0.7%), p.Cys276X (0.7%), p.Arg1070Gln (0.7%) [Estevill X et al. 1997]. Novelli *et al.* 1992, reported p.Phe508del mutation with frequency of 75% (69/92 CF chromosomes).

Frequency of CFTR newborns in Europe

History of CF studies in Albania

- Nunes V et al. 1991, screened unknown Albanian CF alleles for 14 CFTR mutations (including **p.Gly542X and p.Ser549Arg (c.1647T>G)**, and no new alleles were identified (0/11).
- Mercier *et al.* 1994, found p.Arg1070Gln mutation in one CF Albanian patient.
- The other two mutations, p.Gly85Glu and p.Cys276X, presented in Bobadilla *et al.* 2002 may have been received as personal communications.

Frequency of CF newborns in Albania

- In the last 10 years (2006-2016) were registered ~ 35.000 newborns/year.
- Population 3.000.000 (Census 2011).
- From data of Cystic Fibrosis Center, Pediatric Hospital of Tirana , ~ 12 newborns were affected by CF per year.
- About 6 fetuses/year affected by CF were identified by prenatal diagnosis in our Center and by private labs.
- We have ~ 18 fetuses/year affected by CF in 35.000 newborns, means a frequency ~ 1/2000.

Our studies

- We found a complete different spectrum of CFTR mutations in Albania with respect to the last data presented by Bobadilla *et al.* 2002.

Frequency of CFTR newborns in Europe

Our studies

- In our preliminary data [Zoraqi G et al. 2006], we analyzed 58 CF patients by PCR-OLA protocol and found a pattern of 5 mutations:
- F508del (p.Phe508del)
- 621+1 G>T (c.489+1G>T)
- G85E (p.Gly85Glu)
- G542X (p.Gly542X)
- 721+1 G>T (c.579+1G>T)

Present study
Albanian CF patients

- 152 unrelated Albanian CF patients, originating from different regions of Albania, were referred to the CF Center in Tirana (70 males and 82 females, aged between 4 days and 21 years).
- The CF diagnosis was based on classical clinical signs of CF:
- **typical chronic pulmonary** (bronchiectasis, bronchitis and/or pneumonia, sinusitis, pulmonary fibrosis)
- **gastrointestinal tract disease** (ileus meconium, pancreatic insufficiency, hepatic fibrosis).
- About 90% of the patients had elevated sweat chloride tests (>60 mmol/l).

Diagnostic procedures

PCR-OLA protocol including 33 CFTR gene mutations (CF v3 Genotyping Assay)
7 mutations **83%**

↓

INNO-LiPACFTR36, INNO-LiPA CFTR Italian Regional
5 mutations **84%**

↓

Sequencing
12 mutations **85%**

Methods used in CF mutations identification

- We use PCR-OLA kit (33 mutations) as main protocol in our Center.
- Reverse Blot Strip Assay (ViennaLab+ NLM + InnoLipa)
- MLPA (MRC-Holland) deletions
- PCR & Sequencing

p.Phe508del (delF508)

- p.Phe508del is the most common mutation (70.06%).
- Similar frequency was found in Serbia and Montenegro (72%)[Radivojevic D et al. 2004], and lower frequencies were found in other Balkan countries, Croatia (58%) [Knezevic J et al. 2007], FYROM (62%) [Koceva S. Et al. 2001], Greece (53%)[Kanavakis E et al. 2003] and Bulgaria (62%) [Kremenski I et al. 2000].
- The distribution of CF mutations in various regions of Albania indicate that in 80% of the Albanian territory, the frequency of p.Phe508del mutation in CF patients is about 90%.

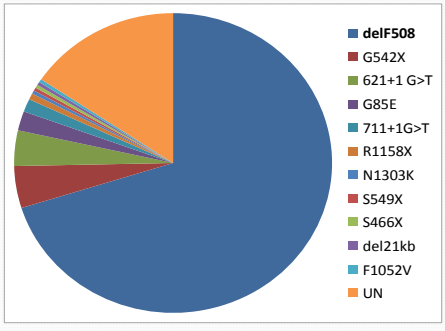
Mutations identified in 152 Albanian patients affected by CF

delF508	70,06 %	R1158X	0,71 %
621+1 G>T	4,27 %	N1303K	0,36 %
G542X	3,61 %	S466X	0,36 %
G85E	1,97 %	R1070Q	0,36 %
711+1G>T	1,31 %	CFdel2,3(21kb)	0,36 %
		F1051V	0,36 %
		E822X	0,36 %

c.489+1G>T (621+1 G>T)

- c.489+1G>T is the second most frequent mutation (4.3%).
- **Not randomly** distributed with frequencies in the North-west regions of Albania, about 30%.
- c.489+1G>T was found also in Greece (5.7%), predominantly in Northern Greece (12.1%), in Serbia (2.86%), in Croatia (1.04%), in FYROM (1.3%), and in Romania (0.8%)(WHO Report 32, 2002).
- The finding of c.489+1G>T mutation at higher frequencies (30%) in a part of Albania supports the hypothesis of a local geografic origin in Southern Europe.

Albanian CF Mutations



p.Gly542X (G542X)

- p.Gly542X is the third most frequent Albanian CF mutation (3.6%).
- We found this mutation **not randomly** distributed in Albania, higher frequencies in the North-west region at about 20% and almost not present in other regions.
- This mutation was found in all European populations [Estevill X et al. 1997], at frequency from 1-5%.

p.Gly85Glu (G85E)

- p.Gly85Glu is the fourth mutation (2%) found in Albanian patients and shows the highiest frequency with respect to the other European populations.
- This mutation is distributed in various regions of Albania and could be considered as local mutation of Albanian population.
- Moreover, p.Gly85Glu was found in other Balkan countries, in lower frequencies, as in Croatia (1.04%), in Greece (1%), in Bulgaria (0.9%) and in Turkey (1.3%) [Estevill X et al. 1997].

Unidentified alleles

- The combined use of techniques failed to detect about 16% of CF chromosomes.
- To find all mutations the entire CFTR gene should be analyzed by sequencing.
- Our next aim is the identification of entire Albanian CF spectrum of mutations.

c.579+1G>T (711+1G>T)

- c.579+1G>T is the fifth mutation (1.3%) present in Albania, a CF allele not found in other Balkan populations [Estevill X et al. 1997].
- This mutation was found in France (>1%) [Castellani et al. 2008], in Spain (1.6%) [M. J. Alonso et al. 2006] and in Southern Italy (1.3%) [Oscar Lao et al. 2003].

CF Prenatal Diagnosis in Albania

- Prenatal diagnosis was performed on 72 families, all having one CF child or CF family history.(2006-2016)
- Some of these families applied for prenatal diagnosis several times.
- Actually we check for delF508 mutation in prenatal diagnosis for Molecular Aneuploidies 21, 18, 13, XY.

Complex CF alleles in Albania

Were found two CF complex alleles, using entire gene sequencing method.

- **p.Arg1070Gln-p.Ser466X** allele was found in one CF patient as compound heterozygote p.Arg1070Gln /p.Ser466X.
- Was found previously in Balkan region, in Greece, in Serbia, Montenegro, and in Bulgaria [Krasnov K.V et al. 2008], confirming the Balkan regional pattern of this complex allele.
- **p.Glu822X- p.Phe1052Val** allele was found in one patient genotyped initially as p.Phe508del heterozygote, and the second mutation p.Glu822X was detected only through sequencing analysis.

Thank you!

Rare genotypes

- Rare genotypes were found in Albanian CF patients: p.Phe508del/p.Gly542X (7), p.Phe508del/ c.489+1G>T (8), p.Phe508del/p.Gly85Glu (5), p.Gly542X/p.Gly542X (1), c.489+1G>T / c.489+1G>T (1), p.Gly542X /c.489+1G>T (3), p.Gly85Glu/p.Arg1158X (1).
- We are interested to collect all clinical data on these patients and to find relationship between genotype and phenotype, or genotype – phenotype interactions.

CFTR genotypes characterized in 152 Albanian CF patients

Genotypes	Nr. of patients
p.Phe508del/p.Phe508del	91
p.Phe508del/p.Gly542X	7
p.Phe508del/ c.489+1G>T	8
p.Phe508del/p.Gly85Glu	5
p.Phe508del/ c.579+1G>T	1
p.Phe508del/p.Arg1158X	1
p.Phe508del/Asn1303Lys	1
p.Phe508del/p.Glu822X-Phe1052Val	1
p.Gly542X/p.Gly542X	1
c.489+1G>T / c.489+1G>T	1
p.Gly542X /c.489+1G>T	3
p.Gly85Glu/p.Arg1158X	1
p.Ser466X/p.Arg1070Gln-p.Ser466X	1
p.Phe508del/U*	15
c.579+1G>T/U*	3
p.Gly542X/U*	1
p.Ser549Arg/U*	1
c.54-5490_273+10250del21kb/ U*	1
U*/U*	14
TOTAL	152

CFTR Gene Mutations Identified in 304 Unrelated Albanian CF Chromosomes

Mutations	Nr. of chromosomes	Frequency (%)	Method
p.Phe508del	213	70.06	PCR-OLA
c.489+1G>T	13	4.27	PCR-OLA
p.Gly542X	11	3.61	PCR-OLA
p.Gly85Glu	6	1.97	PCR-OLA
c.579+1G>T	4	1.31	PCR-OLA
p.Arg1158X	2	0.65	RDB
Asn1303Lys	1	0.33	PCR-OLA
p.Ser549Arg	1	0.33	PCR-OLA
c.54-5490_273+10250del21kb/U	1	0.33	RDB
p.Ser466X	1	0.33	Sequencing
p.Arg1070Gln - p.Ser466X	1	0.33	Sequencing
p.Glu822X - p.Phe1052Val	1	0.33	Sequencing
U*	49	16.1	PCR-OLA, RDB
Total	304	100	

Methods

- In some cases direct sequencing of particular exons was used for the identification of the second mutation in compound heterozygotes and to confirm some rare mutations found by RDB method. In 5 patients whole CFTR gene sequencing was used. Using PCR-OLA kit the CF mutations were analyzed by ABIPRISM310 DNA Genetic Analyzer (Applied Biosystems).
- Some DNA samples presenting rare mutations were reanalyzed by sequencing in the laboratory of the National Centre for Rare Diseases, Istituto Superiore di Sanità (Rome, Italy).

Diagnostic procedures

- All patients were firstly analyzed by PCR-OLA protocol (Abbott, Applied Biosystems) including 33 CFTR gene mutations (Cystic Fibrosis v3 Genotyping Assay).
- In a second step, CF patients not genotyped or partially genotyped by PCR-OLA protocol were analyzed by line probe assay method (INNO-LiPACFTR19 and INNO-LiPACFTR17+Tn Update, INNO-LiPA CFTR Italian Regional, Fujirebio Europe).
- MLPA protocol (MRC-Holland, Amsterdam, the Netherlands) was used to screen CFTR gene deletions in 10 patients from 30 without a CF genotype.

Spectrum of CFTR mutations in Albania

- We found a complete different spectrum of CFTR mutations in Albanian population with respect to the last data presented by Bobadilla *et al.* 2002
- In our preliminary data in 2006, we analyzed 116 CF chromosomes (58 CF patients) by PCR-OLA protocol and found a pattern of 5 mutations: p.Phe508del, c.489+1G>T, p.Gly85Glu, p.Gly542X and c.579+1G>T [Zoraqi G et al. 2006]
- We found the same core pattern of mutations, but with higher frequency of p.Phe508del (77.6%), c.489+1G>T (4.3%), p.Gly85Glu (3.5%) and c.579+1G>T (2.6%) and lower frequency of p.Gly542X (2.6%) respect to the present study.

Methods

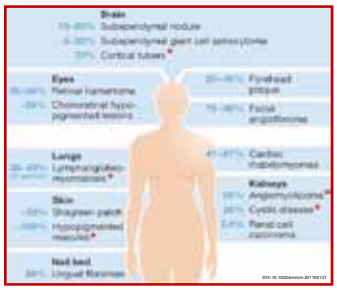
- Ten from thirty unidentified CF patients were analyzed in Policlinico "Tor Vergata" (Rome, Italy) using various protocols, RDB, MLPA and partial sequencing. Rare mutations, such as p.Arg1070Gln (R1070Q), p.Arg1158X (R1158X) and c.54-5490_273+10250del21kb (CFTRdelex. 2, 3 (21kb) were identified in this lab.
- Five from thirty unidentified CF patients were analyzed through sequencing and as result one complex CF allele (p.Phe1052Val (E822X) -F1052V) and a rare mutation (Ser466X, S466X) were identified.

GENETICALLY VERIFIED TUBEROUS SCLEROSIS COMPLEX (TSC) IN A COHORT OF FIFTEEN BULGARIAN FAMILIES

Glushkova M., Bojinova V., Koleva M., Dimova P., Bojidarova M., Litvinenko I., Todorov T., Mitev V., Todorova A.

TUBEROUS SCLEROSIS COMPLEX

- Autosomal dominant disorder
- Typical clinical symptoms
- Frequency 1/6000 to 1/10,000 live births
- Diagnosis based on well-established clinical criteria or positive results from genetic testing



RESULTS

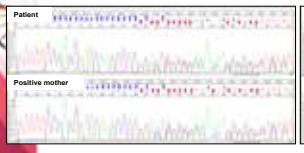
№	Clinical Symptoms	TSC2 location	TSC2 mutations	TSC1 location	TSC1 mutation	Family history
1	1 HM; 1 "café au lait macule"; focal epilepsy	exon 34	c.4473delA, p.Val1492Cysfs*84 ¥			positive mother, 3 HMs
2	2 HMs; FAs; periventricular calcifications; early GTCE (1st month); West syndrome (from 7 month)	intron25	c.2838-122G>A (splice-site)			De novo
3	HMs; cardiac rhabdomyoma; subependymal nodules with calcifications; West syndrome	exon 37	c. 4830G>A, p.Trp1610* ¥			De novo
4	HMs; cortical dysplasias; subependymal nodules; ID; West syndrome; TRE	-	-	exon 15	c.1966G>T, p.Gly656* #	na
5	> 5 HMs; FAs; ungual fibroma; SPs; cortical dysplasias; SEGA - operated; subependymal nodules; focal epilepsy with secondary generalization; ID with autism	exon 17	c.1769T>C, p.Leu590Pro			na
6	HMs; FAs; bilateral retinal hamartomas; multiple subependymal nodules; subcortical tubers; focal epilepsy with secondary generalization	exon 34	c.4051G>T, p.Glu1351* #			positive mother with renal AML
7	HMs; facial angiofibromas; multiple cortical tubers; renal angiomyolipomas; SEGA; focal epilepsy with secondary generalization		Deletion of exon 1-16 #			De novo
8	HMs; FAs; cardiac rhabdomyoma; symptomatic epilepsy	-	-	exon 22	c.2698_2699delCA, p.Gln900Glu*2 #	positive mother with HMs; FAs, SPs, subependimal calcifications
9	HMs; 3 cardiac rhabdomyoma; West syndrome; ID	exon 38	c.4949A>G, p.Tyr1650Cys			positive mother (mosaic mutation) with HMs, SPs, renal AML, epilepsy, ID
10	HMs; FAs; SPs; SEGA; subcortical tubers; ID with autism; West syndrome; GTCE	exon 42	c.5228G>A, p.Arg1743Gln			No family; positive brother with the same symptoms without SEGA
11	5 HMs; FAs; subependymal noduls; cortical dysplasias; GTCE	-	-	exon 15	c. 1525C>T, p.Arg509*	na
12	HMs; FAs; subependymal nodules with calcifications; focal epilepsy	exon 26	c.2954_2957dupATGT, p.Val987Cysfs*19 #			positive father with HMs, FAs; brother with multiple HMs; FAs; focal epilepsy
13	FAs; ungual fibromas; multiple renal cysts (bilateral); subependymal and periventricular nodules	TSC2 / MLPA	-	TSC1 / MLPA	-	This proband has a male with symptoms of TSC and healthy daughter
14	HMs; periventricular calcificate; West syndrome; intellectual disability	-	-	exon 15	c. 1453G>T, p.Glu485*	na - mother with HMs; FAs; calcificate; grandfather with HMs
15	*Romanian case* HMs; FAs; 1SP; 1"café au lait macule"; periventricular calcificate (CT scan)			exon 6	c.433_434delCA, p.Gln145Valfs7*	This proband has a child with TSC: HMs; FAs; 1SP; multiple tubers; calcified periventricular nodules; AMLs; focal epilepsy

RESULTS from Sanger sequencing

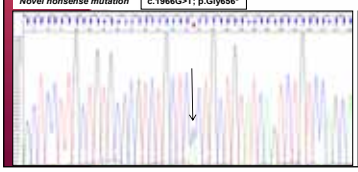
TSC1 gene variants

Novel frameshift mutation

c.2698_2699delCA; p.Gln900Glu*2



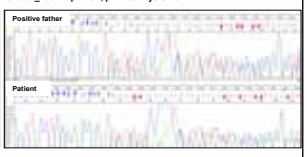
Novel nonsense mutation c.1966G>T; p.Gly656*



TSC2 gene variants

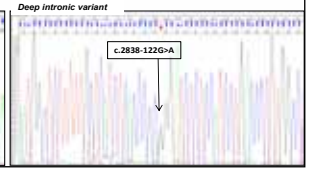
Novel frameshift mutation

c.2954_2957dupATGT, p.Val987Cysfs*19



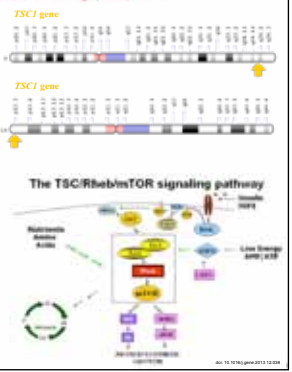
Deep intronic variant

c.2838-122G>A

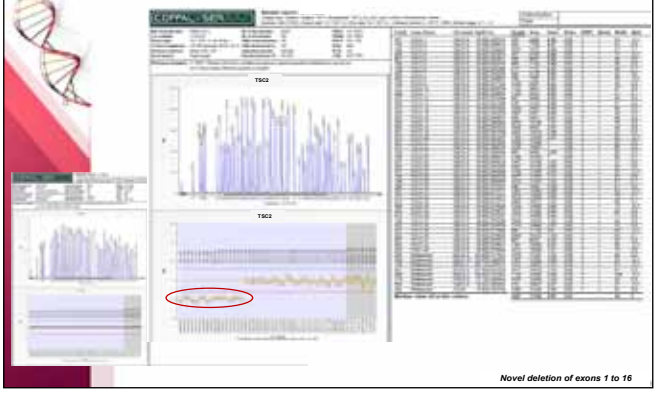


TUBEROUS SCLEROSIS COMPLEX

- TSC result from mutations in *TSC1* gene encoding Hamartin or *TSC2* gene encoding Tuberin
- TSC1-TSC2 complex inhibite the activity of mTOR and cascade of other downstream kinases
- TSC mutation leads to hyperactivation of these signaling pathways



RESULTS from MLPA analysis



Novel deletion of exons 1 to 16

MATERIALS and METHODS

- 14 Bulgarian patients and one Romanian patient
- DNA isolation
- PCR amplification
- The TSC mutation screening by Sanger sequencing

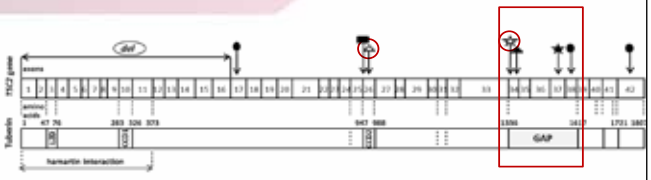
1st step: Sanger sequencing of exons and exons/introns borders of the *TSC2* gene

2nd step: Sanger sequencing of exons and exons/introns borders of the *TSC1* gene

3rd step: MLPA analysis for large deletions/duplications along the genes *TSC2* and *TSC1*

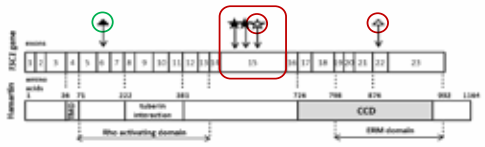
- Comparison of the detected genetic variants with the published databases

Distribution of the detected mutations in the *TSC2* gene



- Distribution of the disease-causing mutations along the *TSC2* gene and their localization along the protein domains of the tuberin
- **The mutations are spread through the whole gene with rough concentration of four of them within the GAP domain**

Distribution of the detected mutations in the *TSC1* gene



- Distribution of the disease-causing mutations along the *TSC1* gene and their localization along the protein domains of the hamartin
- **Three nonsense mutations are localized in exon 15, one of the largest gene exons**
- **We detected larger number of TSC1 mutations then the reported in the literature**

- **I would like to thank the physicians for the clinical description of the TSC cases.**
- **I am very grateful to the patients and their families for their cooperation.**

THANK YOU FOR YOUR ATTENTION!

The study was partially supported by Medical University Sofia, № Д-131/2017

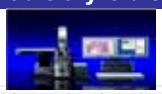
CONCLUSIONS

- The TSC diagnosis was confirmed in all but one of the cases;
- In 40% of our cases the family history was positive;
- In three cases de novo mutation was detected;
- In 40% of the cases the parents were not available for genetic testing;
- 5 novel mutations in both genes were found;
- It is difficult to do genotype-phenotype correlation because of the heterogeneity of the disease;
- The phenomenon of genetic heterogeneity might further play a role in clinical variability among families with the same diagnosis and within a single family which makes the genetic testing and genetic counseling in TSC affected families very important task;
- Our results add novel findings in the genetic heterogeneity and pathogenesis of TSC.

MOLECULAR PROFILING OF PAPILLARY THYROID CANCER (PTC) BY RNA EXPRESSION AND NGS SEQUENCING PLATFORMS

Nikolova D., Zembutsu H., Vidinov K., Sechanov T., Ivanova R., Balabanski L., Vazharova R., Hammoudeh Z., Weidner S., Nakamura Y., Toncheva D.

Genome-wide gene expression profiles of sporadic thyroid cancer



- ✓ It is the **first** study investigating the gene expression pattern of thyroid cancer (**PTC**) samples in **Bulgaria**.
- ✓ **LMM**
- ✓ 54676 transcripts and > 35500 known human genes were analyzed using oligonucleotide microarray analysis
- ✓Functional analysis was subsequently performed

Papillary thyroid cancer (PTC)

- ➢ **Thyroid carcinoma** – 1% of cancer patients
- ➢ Increasing frequency
- ➢ Sex dependent (women:men=3:1)
- ➢ Sporadic and familial cancer

➢ The aim of our study was:

To reveal the genetic background of sporadic **TC** and to analyze genetic predisposing variants to ***FPTC***.

CRITERIA FOR SELECTION

38,500 genes (54,676 transcripts)

↓ genes which were considered as "P" in more than 50% of the cases (11 cases)

20,216 transcripts

genes which were **up-regulated** and present in more than 50% of these cases

genes which were **down-regulated** and present in more than 50% of these cases

1,718 transcripts | **2757 transcripts**

up-regulated genes more than 5x vs.normal

genes which were down-regulated more than 5x vs.Normal

248 transcripts | 142 transcripts

BACKGROUND

- Familial thyroid cancer (**FTC**) is **rare**, accounting for <10% of thyroid cancer cases.

FTC

Familial Medullary TC (**FMTC**)

• ~ 3% of thyroid cancer cases germline point mutations in the *RET*
• MEN2A, MEN2B
• AD

Familial non-Medullary TC (**FNMTC**)

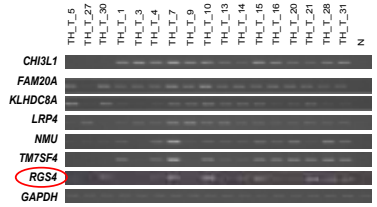
fPTC fFTC

•~6 % of sporadic NMTC are familial
• Little info about the genetic predisposition to familial PTC and FTC

EXPERIMENTAL STRATEGY - 1

- SELECTION OF CANDIDATE GENES -

1. **Have high expression in tumors;**
2. **Have low or no expression in normal (healthy) tissues;**
3. **Their products are secreted proteins, have a significant function in signal transduction processes or are enzymes.**

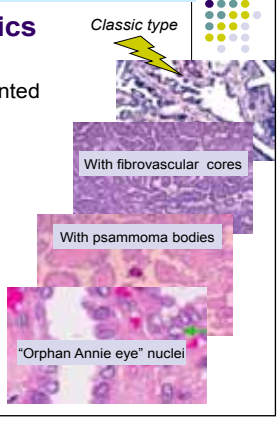


FPTC

Characteristics

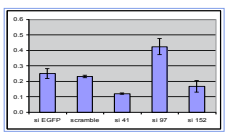
FPTC in FNMTC has a well-documented predisposition to:

- multicentric disease
- bilateral disease
- local invasion
- extrathyroidal extension
- lymph node metastases
- recurrence
- specific histology.

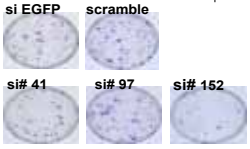


Inhibitory effect of siRNA constructs against RGS4 on the growth of FTC-133 thyroid cancer cell line

MTT ASSAY



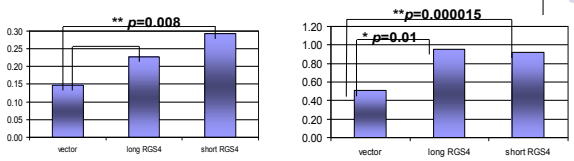
COLONY FORMATION ASSAY



- ✓ Positive suppression of *RGS4* gene expression;
- ✓ Notable retardation of the analyzed cell lines;
- ✓ MTT tests show a significant difference between transfected and non-transfected cells.
- ✓ This effect was again proved by a decrease in *RGS4* RNA by semi-quantitative RT-PCR analysis.

Growth assay for monitoring of RGS4 effect

48 h after transfection **72 h after transfection**



✓ The growth test that the RGS4 expression stimulates cells to increase in number.

Our data gives a hope, that RGS4 gene is a promising molecular gene for target therapy.

The molecular therapy is based on a genetic approach through siRNAs.

The study must be extended also in animals and followed by clinical trials in humans.

Common variants

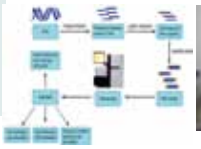
Presence:
* In two out of four affected families; SIFT value – "deleterious"

Variants	Families	rs	Clinical Significance	POLYPHEN-2 Score
FANCA_p.Ser1088Phe	**F2, F3**	**rs17233497**	**untested**	**possibly_damaging (0.933)**
FANCB_p.Gly335Glu	**F3,F4**	**rs41309679**	**untested**	**probably_damaging (0.999)**
HOXB13_p.Gly84Glu	**F3,F4**	**rs138213197**	**Uncertain; pathogenic**	**probably_damaging (0.999)**
MET_p.Thr1010Ile	**F2, F3**	**rs56391007**	**uncertain**	**probably_damaging (1)**
MUTYH_p.Val22Met		rs3219484	benign	benign (0.185)
SLX4(FANCP)_p.Pro245Leu		rs199929086	benign	benign (0.011)
FANCM_p.Thr1600Ile	**F1,F3**	**rs61746943**	**benign**	**possibly_damaging (0.701)**

FPTC - NGS analysis

- Heterogeneous
- BRAF
- Genetic studies have been contradictory
- *RET, TRK, MET, APC, PTEN*, and *TSHR* (Khan A., Thyroid,2010)
- Familial disease

- TruSight Cancer Panel
- Covers exonic and non-coding DNA in exon-flanking regions at 50 bp.
- Variant Studio Software used for analysis, **Polyphen-2 and SIFT**



Illumina sequencing process

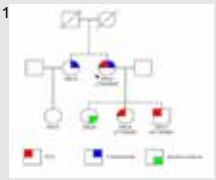
MiSeq, Illumina

Specific variants *in single families*

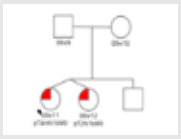
Variants	rs	Nucleotide change	POLYPHEN-2	Clinical significance	Families
RB1_p.Lys729Thr	rs150600740	A/G	probably_damaging(0.999)	-	**F1**
RET_p.Arg982Cys	rs17158558	T/C	possibly_damaging(0.861)	-	**F2**
WRN_p.Ala701Glu		C/A	probably_damaging(1)	-	**F2**
ERCC4 (FANCQ) _p.Arg415Gln	rs1800067	G/A	possibly_damaging(0.801)	untested	**F2**
MC1R_p.Arg151Cys	rs1805007	C/T	probably_damaging(0.993)	-	**F3**
PMS2_p.Asn775Ser	rs17420802	G/A	probably_damaging(0.996)	benign	**F3**
TSC2_p.Gly440Ser	rs45484298	G/A	possibly_damaging(0.863)	uncertain	**F3**
WRN_p.Thr1262Arg	rs78488552	C/G	probably_damaging(0.942)	uncertain	**F3**
FANCF_p.Pro320Leu	rs45451294	G/A	probably_damaging(0.994)	untested	**F3**
PRF1_p.Ala91Val	rs35947132	G/A	probably_damaging(0.973)	-	**F4**
RAD51D_p.Glu253Gly	rs28363284	T/C	probably_damaging(0.986)	benign	**F4**
ERCC4 ((FANCQ) _p.Ile706Thr	rs1800069	T/C	probably_damaging(0.998)	untested	**F4**

Study design

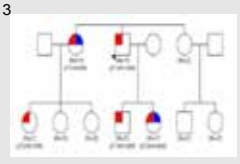
Family 1



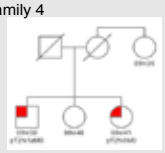
Family 2



Family 3



Family 4



RESULTS

1. Missense mutations in the coding sequences of Fanconi anemia (FA) genes

4/17 SNPs are missense mutations in FA group genes (*FANCA*_p.Ser1088Phe, *FANCM*_p.Thr1600Ile,*FANCB*_p.Gly335Glu, *FANCF*_p.Pro320Leu, FANCQ_p.Arg415Gln, FANCQ_p.Ile706Thr).
Mutations in DNA repairing genes play critical role in families with a predisposition to FPTC and, at the same time, their emergence provokes and speeds up the onset of FPTC in carrier family members.

2. Polymorphisms in other genes related to DNA repair

*RAD51D (FANCR)*_p.Glu253Gly; PMS2_p.Asn775Ser

3. Variants in protooncogenes and tumor suppressor genes

4/17 SNPs are situated in such genes. *RB1*_p.Lys729Thr (F1); *RET*_p.Arg982Cys (F2); *TSC2*_p.Gly440Ser (F3); *MET*_p.Thr1010Ile (F2,F3)

4. Polymorphisms in other genes

*WRN*_p.Ala701Glu; *MC1R*_p.Arg151Cys; *WRN*_p.Thr1262Arg; *PRF1*_p.Ala91Val – specific for a single family; *HOXB13*_p.Gly84Glu (F3,F4)

Results

Cancer Sequence Panel (Illumina ®)
94 cancer related genes, 284 common SNPs

23 individuals analyzed, 13 patients and 10 unaffected relatives

41 SNPs in 28 genes identified

Criteria set:
- General frequency less than 10%
- Potential damaging effect on the protein function

17 SNP variants

Common for two out of four investigated families (5 SNPs)

Specific for each family (12 SNPs)

Conclusions

- We report **17 missense variants** with low frequency and high prediction score to damage the protein function.
- Twelve of the variants (12) have been specific for a certain family; while 5 are common for 2/4 families.
- We underline the presence of possibly causative variants belonging to **FA (Fanconi anemia)** complementation group causing cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair.
- Two variants have been reported also in **oncogenes** (RB1, rs150600740 and RET, rs17158558) and one in TSC2 **tumor-suppressor gene** (rs45484298).
- Our validated NGS panel constitutes an **optimum method** for the identification of possibly causative variants in our families with PTC.

Future projects

Molecular Tests in Cytologically Indeterminate Lesions of Thyroid

- **One third of cases after FNA remain diagnostically "indeterminate" group**

- **IHC** is available but lacks reproducibility and
- **Gene mutation analysis** is su… …ancies but low specificity
- **Gene expression** … for detecting benign lesions
- **Next-ge…** …is promising but needs more validations **(Thyr…**
- **MicroR…** …ing has great potential for both risk stratification and predicting prognosis … is limited by significant variations in sensitivity and specificity

Taken together, molecular tests are a promising option for classifying cytologically indeterminate thyroid nodular lesions

Thank you for your attention!

GENETIC PROFILING OF ADVANCED LARYNGEAL CARCINOMA BY NGS

Silva Giragosyan, Todor Popov, Veronica Petkova, Gergana Stancheva, Darina Kachakova, Kalina Mihova, Vanyo Mitev, Diana Popova, Radka Kaneva

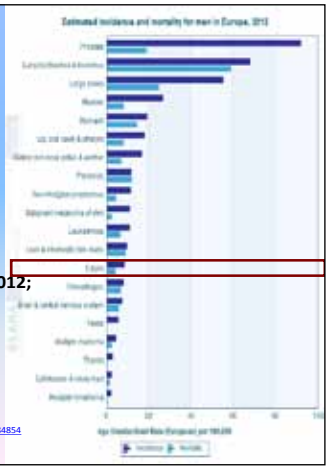
Laryngeal cancer

- ✓ 5 – years survival (2006 – 2012) - 60.7% (National Cancer Institute, NCI, USA); in advanced stage – 44%[1];
- ✓ High Mortality:
  - Late diagnosis – 2/3 in advanced stage;
  - Lymph node metastasis-18-32%;
  - Local recidives [2];
- ✓ Etiological Factors:
  - Abuse with tobacco smoking and tobacco products;
  - Abuse with alcohol beverages;
  - Workplace exposures;
  - HPV infection;
  - Gastroesophageal reflux;
  - Family history [3,4].

1. Megwalu UC et al. 2014, JAMA Otolaryngol Head and Neck Surg
2. Lorenzo J et al 2017, Eur Arch Otorhinolaryngology
3. World Cancer Report 2008, IACR
4. World Cancer Report 2012, IACR

Laryngeal cancer

- ✓ The second most common Head and Neck Cancer (25-30%)[1];
- ✓ 90-95% are Squamous cell carcinoma;
- ✓ Most affected are men, with a prevalence in 6th and 7th decade
- ✓ Men women ratio: 7:1 (GLOBCAN 2012; IACR); 9:1 (EUCAN 2012, IACR);
- ✓ Take 13th place of incidence and mortality in men (EUCAN 2012,IACR)

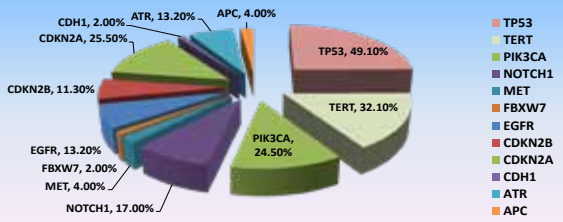


1. Meng Lian et al 2013, PLOS one, http://dx.doi.org/10.1371/journal.pone.0084854

✓Unlike other cancer types, there are no predictive biomarkers, and no new targeted therapies have been approved for HNSCC other than Cetuximab – approved by the FDA in March 2006.
✓Major part of the research studies are focused on HNSCC, and a few of them investigated LSCC as a separate from HNSCC disease.

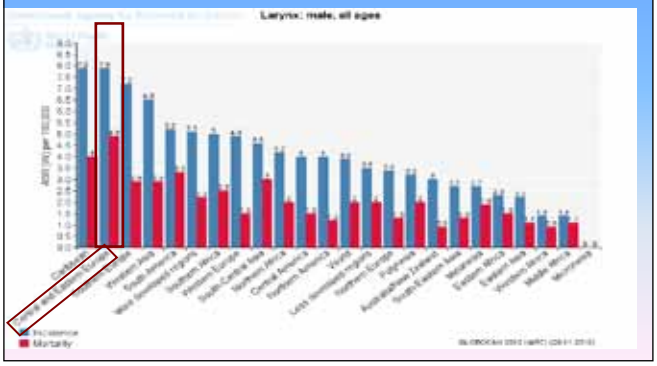
Table 1. Most frequent mutated genes in HNSCC

(Morris LGT et al 2016 *JAMA Oncol. doi:10.1001/jamaoncol.2016.1790)*



Laryngeal cancer

- ✓ In 2012, worldwide, are diagnosed 156.877 (1.1%) new cases of LSCC and are registered 83.376 (1%) LSCC deaths (GLOBCAN 2012; IACR);



The Aim

Tumor heterogeneity in larynx cancer is a known feature of these tumors, but the "driver" somatic mutations and processes in which they are involved are not extensively studied. There are significant gaps in knowledge of molecular mechanisms of LSCC, and the mutational status of LSCC is still poorly investigated.

The aim of this research is by using Next Generation Sequencing (NGS) to detect the mutation spectrum in genes that play a major role in the pathogenesis of advanced laryngeal cancer.

Laryngeal cancer

- ✓ The incidence in 2013 of LSCC in Bulgaria is 8,9 per 100 000, and the mortality is 5,6 per 100 000 („Cancer Incidence in Bulgaria, 2013" Volume XXIV, 2015);

Materials and Methods:

- ✓In the current study are involved fresh-frozen tumour tissue samples from 15 patients in advanced LSCC.
- ✓Patients were recruited at the Ear, Nose and Throat Department, University Hospital "Queen Joanna - ISUL" Sofia during the period 2010-2013.
- ✓Median age 57.8 ± 6.68 (age range 41-73).
- ✓None of the patients in this study had received chemotherapy or radiotherapy before surgery.

Table with Distribution of the clinicopathological characteristics of the patients

Features	N(%)
Gender	
Male	15 (100)
Female	0
Age	
> 55	9 (60)
≤ 55	6 (40)
T stage	
T1/2	0
T3	3 (20)
T4	12 (80)
N stage	
Negative	0
Positive	15 (100)
G stage	
G1	2 (33)
G2	10 (67)
G3	3 (20)
Localization	
Glottic	4 (27)
Supraglottic	11 (73)
Tabacco smoking	
Yes	15 (100)
No	0
Alcohol	
Yes	13 (87)
No	2 (13)

Materials and Methods:

TruSeq Amplicon - Cancer Panel

✓Illumina TruSeq Amplicon Cancer Panel (TSACP) -Routine NGS test to detect hotspot mutations in 48 genes.

✓>35 kb target sequence.

✓The sequencing was performed on the MiSeq.

✓ The data was analyzed by VarSeq Software (v.1.4.6):

Filters:
- Read depth: >100x;
- VAF: 5%;
- Variants with effect (without intron and synonymous variants);
- Data base: COSMIC; dbSNP; SIFT; Polyphen2 HVAR; Mutation Tester; FATHMM; ClinVar etc.

Cancer Gene Panel Includes:

ABL1	ERBB4	JAK2	PIK3CA
AKT1	FBXW7	JAK3	PTEN
ALK	FGFR1	KDR	PTPN11
APC	FGFR2	KIT	RB1
ATM	FGFR3	KRAS	RET
BRAF	FLT3	MET	SMAD4
CDH1	GNA11	MLH1	SMARCB1
CDKN2A	GNAQ	MPL	SMO
CSF1R	GNAS	NOTCH1	SRC
CTNNB1	HNF1A	NPM1	STK11
EGFR	HRAS	NRAS	TP53
ERBB2	IDH1	PDGFRA	VHL

illumina

Summary and discussion of the results

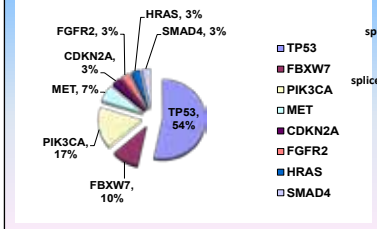
- ✓ We found altogether 29 pathogenic and one benign drug-response variant.
- ✓ The most frequently mutated gene was TP53 with 15 pathogenic variants in 15 patients and 1 drug-response variant to cisplatin, fluorouracil, paclitaxel and others, present in 13 patients (c.215C>G) (worse outcome).
- ✓ Four new variants in TP53 gene with pathogenic prediction (c.536A>G; c.546C>A; c.193C>T; c.641A>G) were found.
- ✓ Pathogenic mutations of TP53 particularly, are significantly associated with local recurrence, radioresistance, and short survival in HNSCC[1].

1. Skinner H et al. 2010, DOI: 10.1158/1078-0432.CCR-11-2260

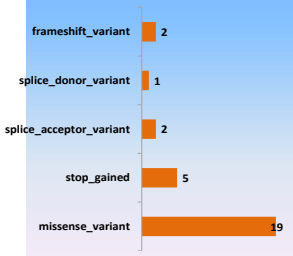
Distribution of detected pathogenic variants by genes and types

We found altogether 29 pathogenic and one benign, drug-response variant. Pathogenic variants were found in all of the advanced LSCC patients.

Distribution of the pathogenic variants by genes



Distribution of the pathogenic variants by type



Summary and discussion of the results

- ✓ The second commonly mutated gene was PIK3CA, with five pathogenic variants in five patients. Two of them were new (c.3118A>G; c.3031delC).
- ✓ Head and neck cancer patients with PIK3CA activating mutations are sensitive to an m-TOR/PI3K inhibitors. They may serve as predictive biomarker for treatment selection[1].
- ✓ Three pathogenic variants in FBXW7 gene were found, two of which new (c.1136A>G; c.1428C>G).
- ✓ MET gene was presented with two pathogenic variants, one was found in three patients and one was new (c.3359G>A). MET gene activation is correlated with lymph node metastasis and it is suggest the potential therapy in combination of MET inhibitors[2]
- ✓ One mutation was found in each FGFR2 (new variant: c.880G>T), HRAS (c.37G>C) and SMAD4 (new variant: c.394C>G).

1. Lui WM et al, 2013 DOI: 10.1158/2159-8290.CD-13-0103
2. Nisa L et al. 2014 http://dx.doi.org/10.1016/j.pharmthera.2014.04.005

Table with pathogenic variants in advanced LSCC patients

GENE	POSITION IN cDNA (HGVS)	AMINOACID SUBSTITUTION	VARIANT TYPES	INCIDENCE	PATHOGENICITY
TP53	c.215C>G	p.Pro72Arg	missense_variant	86.7% (13/15)	Benign, drug-response allele
TP53	c.536A>G	p.His179Arg	missense_variant	6.7% (1/15)	Pathogenic prediction
TP53	c.844C>T	p.Arg282Trp	missense_variant	6.7% (1/15)	Pathogenic
TP53	c.637C>T	p.Arg213Ter	stop_gained	6.7% (1/15)	Pathogenic
TP53	c.638G>T	p.Arg213Leu	missense_variant	6.7% (1/15)	Pathogenic
TP53	c.994-1G>A		splice_acceptor_variant	6.7% (1/15)	Pathogenic
TP53	c.546C>A	p.Cys182Ter	stop_gained	6.7% (1/15)	Pathogenic prediction
TP53	c.535C>T	p.His179Tyr	missense_variant	6.7% (1/15)	Pathogenic
TP53	c.672+1G>A		splice_donor_variant	6.7% (1/15)	Pathogenic
TP53	c.193C>T	p.Gln65Ter	stop_gained	6.7% (1/15)	Pathogenic prediction
TP53	c.641A>G	p.His214Arg	missense_variant	6.7% (1/15)	Pathogenic prediction
TP53	c.473G>A	p.Arg158His	missense_variant	6.7% (1/15)	Pathogenic
TP53	c.586C>T	p.Arg196Ter	stop_gained	6.7% (1/15)	Pathogenic
TP53	c.743G>A	p.Arg248Gln	missense_variant	6.7% (1/15)	Pathogenic
TP53	c.403dupT		frameshift_variant	6.7% (1/15)	Pathogenic
TP53	c.713_714del	p.Cys238Ter	stop_gained	6.7%	Pathogenic

Conclusion of the results

- In conclusion, a pilot molecular profiling of LSCC with NGS revealed TP53 and PIK3CA as commonly mutated genes, consistent with previous studies.
- The data adds to the spectrum of mutations in key driver genes.
- Somatic mutations found in LSCC tumours could provide better opportunities for predicting response to existing and finding targets for new therapies.

PIK3CA	c.1633G>C	p.Glu545Gln	missense_variant	6.7% (1/15)	Pathogenic
PIK3CA	c.3118A>G	p.Met1040Val	missense_variant	6.7% (1/15)	Pathogenic prediction
PIK3CA	c.3031delC		frameshift_variant	6.7% (1/15)	Pathogenic prediction
PIK3CA	c.1624G>A	p.Glu542Lys	missense_variant	6.7% (1/15)	Pathogenic
PIK3CA	c.1633G>A	p.Glu545Lys	missense_variant	6.7% (1/15)	Pathogenic
FBXW7	c.1136A>G	p.His379Arg	missense_variant	6.7% (1/15)	Pathogenic prediction
FBXW7	c.1428C>G	p.Ser476Arg	missense_variant	6.7% (1/15)	Pathogenic prediction
FBXW7	c.1513C>G	p.Arg505Gly	missense_variant	6.7% (1/15)	Likely Pathogenic
MET	c.3029C>T	p.Thr1010Ile	missense_variant	20% (3/15)	Likely Pathogenic
MET	c.3359G>A	p.Gly1120Asp	missense_variant	6.7% (1/15)	Pathogenic prediction
CDKN2A	c.151-2A>T		splice_acceptor_variant	13.3% (2/15)	Pathogenic
FGFR2	c.880G>T	p.Val294Leu	missense_variant	6.7% (1/15)	Pathogenic prediction
HRAS	c.37G>C	p.Gly13Arg	missense_variant	13.3% (2/15)	Pathogenic
SMAD4	c.394C>G		missense_variant	6.7% (1/15)	Pathogenic prediction

Thank to your attention!

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POSTER SESSION

Poster jury:

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Bruck type 1 syndrome due to a mutation c.831dupC in FKBP10 gene in Bulgarian Roma

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Introduction

Bruck type 1 syndrome (OMIM 607063) is an autosomal recessive disease, characterized by congenital contractures with pterygia, postnatal short stature, severe limb deformities, onset of fractures in infancy or early childhood and progressive scoliosis (McPherson and Clemens, 1997). It is caused by homozygous mutations in *FKBP10* gene on17q21 chromosome. Bruck syndrome-2 (609220) is caused by homozygous mutation in the PLOD2 gene (601865) on chromosome 3q24. Van der Slot et al. (2003) stated that they were unaware of any phenotypic differences between the 2 forms of Bruck syndrome.

Aim

To describe the clinical features of 10 affected, belonging to the Wallachian Roma group of basketmakers, residing in Kostenetz, Dolna Banya and Maritza, Sofia region (Figure 1).

Material and methods

We identified 10 affected individuals, 6 female and 4 male, belonging to one extended pedigree. All of the patients underwent physical, neurologic and orthopaedic examinations. In four affected metabolic screening from serum and urine, nerve conduction studies (NCS) and electromyography (EMG), ECG and echocardiography, ventillatory assessments, X-rays of thoracic spine, lung and heart, and neuroophthalmological assessment were performed. Blood or saliva were taken from all patients to isolate DNA and perform a molecular genetic analysis.

Results

All patients were born from normal pregnancies and births but with congenital contractures in the lower limbs. All of the affected had growth delay. Only three patients had achieved ambulation with assistive devices around the age of three years.

The disease evolution encompasses contractures in the hip, shoulder joints and thoracic deformities, related with frequent pulmonary infections. In three affected spontaneous rib fractures have been documented.

The clinical examination in all the patients revealed:

- Short stature
- Short neck with limited mobility
- Short body
- Pectus excavatus
- Severe right-convex thoracolumbar scoliosis with a formed gybus in the proximal thoracic spine (Figure 2)
- Severe flexion contractures in the hip, knee and elbow joints and milder contractures in the shoulder joints and wrists, where the movements were painful and heavily limited,
- Complete ankylosis in the hip joints
- Equinovarus deformities of the feet with pronounced plantar hygromy

Figure 2. Severe skeletal deformities in two patients Bruck type 1 syndrome .

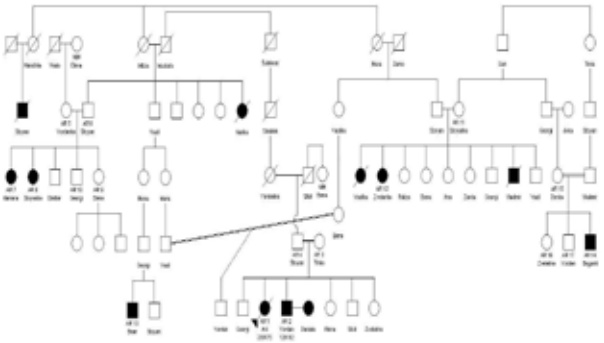

Figure 1. Pedigree of the affected with Bruck type 1 syndrome.

NCS, EMG, neuro-ophthalmological evaluation and metabolic screening were normal in the evaluated patients.

Ventillatory assessment revealed respiratory insufficiency with severely reduced vital capacity up to 25% of normal range.

Echocardiography was consistent with right ventricular cardiac insufficiency with mitral and tricuspid regurgitation.

In all patients a homozygous mutation c.831dupC was found in the *FKBP10* gene.

Conclusion

In Roma cases with congenital contractures, short stature and severe limb deformities c.831dupC in the *FKBP10* gene should be ruled out. The cardiac and respiratory follow up of these patients is crucial, as somatic complications are the most common cases for death.

Novel form of complicated hereditary spastic paraplegia (SPG78), due to mutations in the ATP13A2/PARK9 gene

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Introduction

Hereditary spastic paraplegia (HSP) is one of the most genetically heterogeneous neurodegenerative condition as currently more than 55 causative genes have been identified [1]. This disorder is characterized by progressive spasticity and weakness of the lower limbs and can be classified according to the mode of inheritance as autosomal-dominant (AD), autosomal-recessive (AR) or X-linked and according to the clinical features as pure or complex [2] . ATP13A2 (OMIM 610513) is a lysosomal P5-type transport ATPase, for which the transported substrate remains unidentified. Molecular defects in *ATP13A2*-gene have been associated with Kufor-Rakeb syndrome and neuronal ceroid lipofuscinosis [3].

Aim

To describe the clinical features of a Bulgarian family with complex HSP with three affected siblings, carrying a novel mutation in *ATP13A2/PARK9* gene and to present the underlying pathogenic mechanisms.

Material and methods

All three affected received a systematic neurological, neuropsychological, electrophysiological assessments and brain Magnetic resonance imaging (MRI) to evaluate affection of multiple neurological systems. In the Bulgarian family we performed whole exome sequencing and homozygosity mapping. For further clarification of the underlying pathogenic mechanisms biochemical and immunocytochemical experiments in COS-1 and HeLa cells and patient-derived fibroblasts were completed.

Results

The disease presentation in our patients was dominated by an adult-onset lower-limb predominant spastic paraparesis, ataxia, cognitive impairment with more pronounced executive dysfunction. Nerve conduction studies revealed involvement of the peripheral motor and sensory nerves (table 1). Neuroimaging investigations revealed pronounced vermian and hemispheric cerebellar atrophy (figure 1).

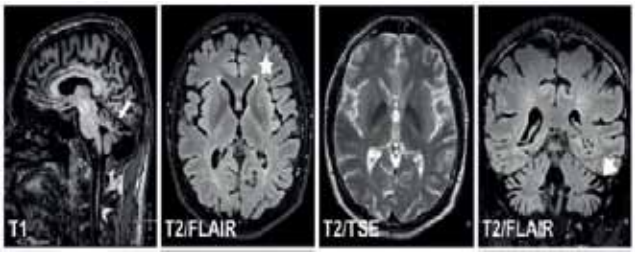

Figure 1. Brain MRI of patients HSP84.II.3 with vermian and hemispheric cerebellar atrophy (white arrowheads) and 'ear of the lynx' sign (T2 hyperintensity of the anterior fornix of the corpus Callosum (asterisk)

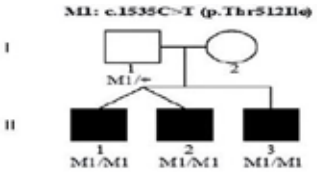

Figure 2. Pedigree of the affected Bulgarian patients

The affected were carrying homozygous p.Thr512Ile (c.1535C4T) mutation in ATP13A2 (figure 2). Our biochemical and immunocytochemical experiments demonstrated that the HSP-associated mutations cause loss of ATP13A2 function due to transcript or protein instability and abnormal intracellular localization of the mutant proteins, ultimately impairing the lysosomal and mitochondrial function with additional evidence that disease-causing mutations can affect the catalytic autophosphorylation activity of ATP13A2 (figure 3).

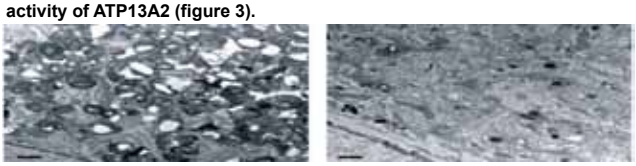

Figure 3. Lysosomes are excessively present in Thr512Ile-HSP fibroblasts (A) compared to controls (B). The majority of lysosomes contain aberrant storage material consisting of whirls and stacks of membranes.

Table 1. Clinical phenotype of the affected Bulgarian patients

Patient	HSP84-1	HSP84-3	HSP84-4
mutation (cDNA)	1535C>T;1535C>T	1535C>T;1535C>T	1535C>T;1535C>T
mutation (protein)	Thr512Ile;Thr512Ile	Thr512Ile;Thr512Ile	Thr512Ile;Thr512Ile
sex	m	m	m
age at onset (y)	30	33	30
age at examination (y)	50	40	50
disease severity			
Landmarks of disability (max 4)	4	3	4
SARA (max 100)	20 (50y)	8 (40y)	21 (50y)
Period until dependency on walking aid after clinical onset	13	8	13
Period until loss of ambulation after the clinical onset	18	n.a.	18
presenting symptom	gait disorder, dysarthria	gait disorder, dysarthria	gait disorder, dysarthria
cognitive deficits	slight verbal memory deficit	none	slight verbal memory deficit
behavioral and psychiatric symptoms	none	none	none
pyramidal and peripheral motor system			
UL/LL spasticity	-/+	-/+	-/+
UL/LL weakness	-/+	-/+	-/+
increased tendon reflexes UL/LL	+/+	+/+	+/+
muscle atrophy	-	-	-
Babinski sign	+	+	+
extrapyramidal motor system			
brady-/hypokinesia	-	-	-
dystonia	-	-	-
tremor	-	-	-
spinocerebellar system			
oculomotor disturbance	+	+	+
dysarthria	+	+	+
limb/gait ataxia	+/+	+/+	+/+
sensory system			
surface sensation deficit	+	+	+
vibration/joint position deficit	+/-	+/-	+/-
bladder	voiding	normal	normal
rectum	normal	normal	normal
Imaging	cerebellar > cortical atrophy	mild cortical atrophy, periventricular WM changes	cerebellar > cortical atrophy
Nerve conduction studies	axonal motor and sensory polyneuropathy	L5-S1 radiculopathy	axonal motor and sensory polyneuropathy
VEP		prolonged	
AEP	prolonged	prolonged	prolonged

Conclusion

In conclusion, loss of ATP13A2 function causes a combination of lysosomal and mitochondrial dysfunction that affects multiple neuronal populations. This translates to a spectrum of neurological disorders ranging from complicated HSP to KRS.

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CYTOTOXIC T-LYMPHOCYTE ANTIGEN-4 GENE POLYMORPHISM AND THE RISK FOR CHRONIC LYMPHOCYTIC LEUKEMIA IN MACEDONIAN POPULATION

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ABSTRACT

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a glycoprotein molecule that is transiently expressed on the surface of activated T-cells and delivers an inhibitory signal to the T cell. An A to G polymorphism at position 49 of the CTLA-4 first exon, which results in substitution of threonine with alanine, has been associated with several autoimmune disorders like Graves' disease, Hashimoto thyroiditis and Type 1 Diabetes mellitus. Some studies have reported association of this CTLA-4 polymorphism with the higher risk and susceptibility for Non Hodgkin Lymphoma (NHL) and Chronic Lymphocytic Leukemia (CLL). The aim of our study was to investigate a possible association of this CTLA-4 polymorphism with the risk for CLL in our population.
We have examined this CTLA-4 polymorphism in 130 CLL patients and 100 Control subjects. Genotyping was performed by using PCR-RFLP methods.
Our results did not demonstrate significantly different CTLA-4 genotypes distribution in patients with CLL (G/G=16, A/G=53, A/A=61) compared with controls (G/G=10, A/G=39, A/A=51), p=0.777. A strong correlation was observed between the presence of the CTLA-4 G allele and the development of AIHA (CLL with AIHA, A/A=8, G/A=20, G/G=2, versus CLL without AIHA, A/A=53, G/A=33, G/G=14; p=0.004). Interestingly, this correlation was less significant when we compared DAT positive and DAT negative CLL patients, regardless of the presence of AIHA (p=0.021).

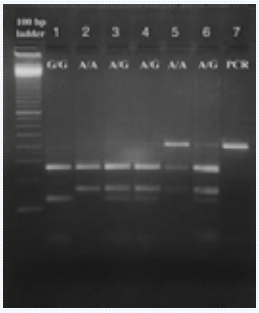
INTRODUCTION

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a glycoprotein molecule that is highly homologous to CD28 and reacts with higher affinity to B7.1 and B7.2 on antigen-presenting cells. It is transiently expressed on the surface of activated T-cells and delivers an inhibitory signal to the T cell. An A to G polymorphism at position 49 of the CTLA-4 first exon, which results in substitution of threonine with alanine, has been associated with several autoimmune disorders like Graves' disease, Hashimoto thyroiditis and Type 1 Diabetes mellitus. Some studies have reported association of this CTLA-4 polymorphism with the higher risk and susceptibility for Non Hodgkin Lymphoma (NHL) and Chronic Lymphocytic Leukemia (CLL). The aim of our study was to investigate a possible association of this CTLA-4 polymorphism with the risk for CLL in our population.

MATERIALS AND METHODS

We examined this CTLA-4 polymorphism in 130 CLL patients. In our series, 30 of these 130 CLL patients had a prior history of autoimmune hemolytic anemia (AIHA). Control subjects were 100 healthy individuals. Genotyping was performed by using PCR-RFLP methods.
Genomic DNA was isolated from peripheral blood leukocytes and the specific DNA fragment that contains polymorphic site was amplified using polymerase chain reaction (PCR).
Amplified DNA fragments were digested with 1U of restriction enzyme BbvI at 37°C for 16 hours followed by an electrophoresis on a 2% agarose gel.

Results from electrophoretic analysis of digested fragments for CTLA-4:



RESULTS

Table 1. CTLA-4 genotype distribution between CLL patients and controls.

CTLA-4 +49 A/G	A/A	A/G	G/G	p value
CLL patients (n=130)	61 (46.9%)	53 (40.7%)	16 (12.4%)	***0.777***
Controls (n=100)	51 (51%)	39 (39%)	10 (10%)	

Table 2. CTLA-4 genotype distribution between CLL patients with and without AIHA.

CTLA-4 +49 A/G	A/A	A/G	G/G	p value
CLL with AIHA (n=30)	8 (27%)	20 (66%)	2 (6.6%)	***0.004***
CLL without AIHA (n=100)	53 (53%)	33 (33%)	14 (14%)	

Table 3. CTLA-4 genotype distribution between DAT+ and DAT- CLL patients.

CTLA-4 +49 A/G	A/A	A/G	G/G	p value
DAT+ CLL patients (n=41)	12 (29%)	23 (56%)	6 (15%)	***0.021***
DAT- CLL patients (n=89)	49 (55%)	30 (34%)	10 (11%)	

CONCLUSIONS

In conclusion, our results did not demonstrate significant difference in the distribution of genotypes or allele frequencies for CTLA-4 polymorphisms between the CLL patients and controls and this polymorphism is not associated with susceptibility to CLL, contrary to the results from other studies. But, our data indicate that the G allele of CTLA-4 gene is more frequent in CLL patients with AIHA and/or anti-erythrocytes antibodies and may predispose to development of AIHA in patients with CLL.

CYTOTOXIC T-LYMPHOCYTE ANTIGEN-4 GENE POLYMORPHISM AND THE RISK FOR TYPE 2 DIABETES IN MACEDONIAN POPULATION

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ABSTRACT

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a CD28 homologue which plays an important role in negative regulation of T cell response. An A to G polymorphism at position 49 of the CTLA-4 first exon, has been associated with several autoimmune disorders like Graves' disease, Hashimoto thyroiditis and Type 1 Diabetes mellitus. Some studies have reported a role of this CTLA-4 polymorphism in the susceptibility and clinical form of type 2 diabetes also. The aim of our study was to investigate a possible association of this CTLA-4 polymorphism with the risk and susceptibility for type 2 diabetes in Macedonia. We have examined this CTLA-4 polymorphism in 95 consecutive patients with type 2 diabetes and 120 healthy controls. Genotyping was performed by using PCR-RFLP methods. Our results did not demonstrate significantly different distribution for CTLA-4 genotypes in patients with type 2 diabetes (G/G=9, A/G=42, A/A=44) comparing with healthy controls (G/G=12, A/G=46, A/A=62), p=0.68. There was no significant difference in the allele frequencies between patients with type 2 diabetes (G=60, A=130) and healthy controls (G= 70, A=170), p=0.588.
In conclusion, our results did not demonstrate significant difference in the distribution of genotypes or allele frequencies for CTLA-4 polymorphisms between the patients with type 2 diabetes and controls in Republic of Macedonia.

INTRODUCTION

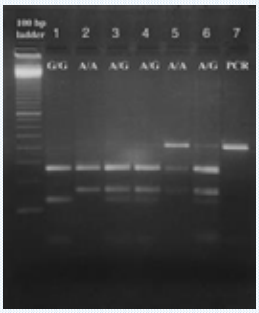
Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a glycoprotein molecule that is highly homologous to CD28 and reacts with higher affinity to B7.1 and B7.2 on antigen-presenting cells. It is transiently expressed on the surface of activated T-cells and delivers an inhibitory signal to the T cell. An A to G polymorphism at position 49 of the CTLA-4 first exon, which results in substitution of threonine with alanine, has been associated with several autoimmune disorders like Graves' disease, Hashimoto thyroiditis and Type 1 Diabetes mellitus. Some studies have reported a role of this CTLA-4 polymorphism in the susceptibility and clinical form of type 2 diabetes also. The aim of our study was to investigate a possible association of this CTLA-4 polymorphism with the risk and susceptibility for type 2 diabetes in Macedonia.

MATERIALS AND METHODS

We have examined this CTLA-4 polymorphism in 95 consecutive patients with type 2 diabetes and 120 healthy controls. Genotyping was performed by using PCR-RFLP methods. Genomic DNA was isolated from peripheral blood leukocytes and the specific DNA fragment that contains polymorphic site was amplified using polymerase chain reaction (PCR).
Amplified DNA fragments were digested with 1U of restriction enzyme BbvI at 37°C for 16 hours and analyzed with electrophoresis on a 2% agarose gel.

Patients characteristics	
Median age (years)	64.8±7.6
Sex (females/males)	52/43
Median duration of DM (years)	12.5±5.8
Number of patients on insulin (%)	43 (45%)
Median duration of insulin treatment (years)	5.6±5.1
Average HbA1c	8.5±2.0%.
Number of patients with diabetic retinopathy (%)	54 (57%)

Results from electrophoretic analysis of digested fragments for CTLA-4:



RESULTS

Table 1. CTLA-4 genotype distribution between DM type 2 patients and controls.

CTLA-4 +49 A/G	A/A	A/G	G/G	p value
DM type 2 patients (n=95)	44 (46.3%)	42 (44.2%)	9 (9.5%)	***0.68***
Controls (n=120)	62 (51.7%)	46 (38.3%)	12 (10%)	

Table 2. CTLA-4 allele frequencies between DM type 2 patients and controls.

CTLA-4 +49 A/G	A allele	G allele	p value
DM type 2 patients (n=95)	130 (68.4%)	60 (31.6%)	***0.588***
Controls (n=120)	170 (70.8%)	70 (29.2%)	

CONCLUSIONS

In conclusion, our results did not demonstrate significant difference in the distribution of genotypes or allele frequencies for CTLA-4 polymorphisms between the patients with type 2 diabetes and controls in Republic of Macedonia.

FCGR2A AND FCGR3A VARIANTS ARE NOT ASSOCIATED WITH RESPONSE TO RITUXIMAB IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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University Clinic for Hematology, Medical Faculty, Skopje, Republic of Macedonia.

ABSTRACT

Chronic lymphocytic leukemia(CLL) is the most common form of adult leukemia in Western world. Chemoimmunotherapy with rituximab, fludarabine and cyclophosphamide (R-FC) has prolonged progression free survival (PFS) and overall survival in CLL patients. FCGR2A is polymorphic and has two alleles: FCGR2A-H131 allele having a higher affinity for human IgG2, comparing to FCGR2A-R131. The gene for FCGR3A has also two polymorphic variant alleles: FCGR3A-158V variant with higher affinity for Fc gamma receptor than 158F variant. These FCGR polymorphisms may influence antibody-dependent cytotoxicity(ADCC), complement-dependent cytotoxicity(CDC) and direct proapoptotic effect. The aim of our study was to investigate a possible association of these two polymorphisms with response to R-FC therapy in CLL patients.

We have analyzed these polymorphisms in 90 CLL patients treated with R-FC. DNA was isolated from peripheral blood mononuclear cells and genotyping was performed by using PCR/RFLP methods. Distribution of genotypes was compared by using a chi-squared test or Fisher's exact test.

Distribution of genotypes in our patients was: 33% H/H, 49% H/R and 18% R/R for FCGR2A and 43% V/V, 40% V/F and 17% F/F for FCGR3A. Rate of CR and PR were similar irrespective of the FCGR variants and our results did not demonstrate significantly different genotype distribution for FCGR2A (p=0.8001) or FCGR3A (p=0.1019) in CLL patients with complete, partial or no response to R-FC treatment.

INTRODUCTION

Chronic lymphocytic leukemia(CLL) is the most common form of adult leukemia in Western world. Chemoimmunotherapy with rituximab, fludarabine and cyclophosphamide (R-FC) has prolonged progression free survival(PFS) and overall survival(OS) in CLL patients. FCGR2A is polymorphic and has two alleles: FCGR2A-H131 allele having a higher affinity for human IgG2, compared to FCGR2A-R131. The gene for FCGR3A has also two polymorphic variant alleles: FCGR3A-158V variant with higher affinity for Fc gamma receptor than 158F variant. These FCGR polymorphisms may influence antibody-dependent cytotoxicity(ADCC), complement-dependent cytotoxicity(CDC) and direct proapoptotic effect. The aim of our study was to investigate a possible association of these two polymorphisms with response to R-FC therapy in CLL patients.

MATERIALS AND METHODS

MATERIALS

We have analyzed these polymorphisms in 90 CLL patients treated with R-FC. Median age of our patients was 62.7(36-78) and 63% were male. Number of patients with stage III/IV disease was 65(72%). Median WBC count at the start of treatment was 68.5(34-173x10^9/L). Percentage of previously treated patients was 51/90(56.6%). Average numbers of R-FC cycles were 4.3 and median PFS was 35.1 months. Median time of observation after treatment was 3.6 years (range:6 months-8 years). Response was evaluated 2 months after therapy according to National Cancer Institute (NCI) criteria. Complete response(CR) was achieved in 24/90(26.7%), partial response(PR) in 56/90(62.2%) and no response in 10/90(11.1%).

METHODS

•Genomic DNA was isolated from peripheral blood leukocytes

•For genotyping of these polymorphism, a specific DNA fragment that contains polymorphic site was amplified using polymerase chain reaction (PCR)

•Amplified DNA fragments were digested with 10U of restriction enzyme BstU I at 37°C for 16 hours for FCGR2A +494A/G and 10U of Nla III at 37°C for 16 hours for FCGR3A +559T/G, followed by an electrophoresis on a 2% agarose gel.

RESULTS

FCGR2A-131H/R	CR	PR	NR
H/H	8 (26.6%)	18 (60%)	4 (13.4%)
H/R	10 (22.7%)	29 (65.9%)	5 (11.4%)
R/R	6 (37.5%)	9 (56.3%)	1(6.25%)

X^2 = 1.648
P = 0.8001

FCGR3A-158V/F	CR	PR	NR
V/V	8 (20.5%)	29 (74.4%)	2 (5.1%)
V/F	11 (30.6%)	21 (58.3%)	4 (11.1%)
F/F	5 (33.3%)	6 (40%)	4 (26.7%)

X^2 = 7.7
P = 0.1019

CONCLUSIONS

We did not find significantly different distribution of FCGR2A and FCGR3a genotypes between patients and controls (p=0.366 & p=0.949). Rate of CR and PR were similar irrespective of the FCGR variants and our results did not demonstrate significantly different genotype distribution for FCGR2A (p=0.8001) or FCGR3A (p=0.1019) in CLL patients with complete, partial or no response to R-FC treatment.

Autosomal-Recessive Congenital Cerebellar Ataxia Is Caused by Mutations in Metabotropic Glutamate Receptor 1

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Introduction

Autosomal-recessive cerebellar ataxias (ARCAs) are a clinically and genetically heterogeneous group of disorders whose clinical and genetic classifications are still evolving as the field progresses and novel phenotypes and genes are described (1,2). Congenital cerebellar ataxias are a relatively small ARCA subset characterized by infantile onset of motor incoordination, developmental delay, and variable additional manifestations (3–10). Although a number of genes have already been implicated in ARCAs, many rare forms and their molecular basis remain to be discovered.

Aim

To describe a novel form of congenital cerebellar ataxia identified in patients of Roma ethnicity in Bulgaria

Material and methods

All ten affected, belonging to five different families received a systematic neurological, neuropsychological, electrophysiological assessments and brain Magnetic resonance imaging (MRI). We sequenced the exomes of 11 individuals—six patients and five parents. Exome capture (Illumina TruSeq) and sequencing (Illumina HiSeq 2000) were performed by Axeq Technologies (Seoul, South Korea).

Results

Patients presented with global developmental delay, moderate to severe stance and gait ataxia, dysarthria, mild dysdiadochokinesia, dysmetria and tremors, intellectual deficit, and mild pyramidal signs. Brain imaging revealed progressive generalized cerebellar atrophy, and inferior vermian hypoplasia and/or a constitutionally small brain were observed in some patients (Table 1 and Figure 1).

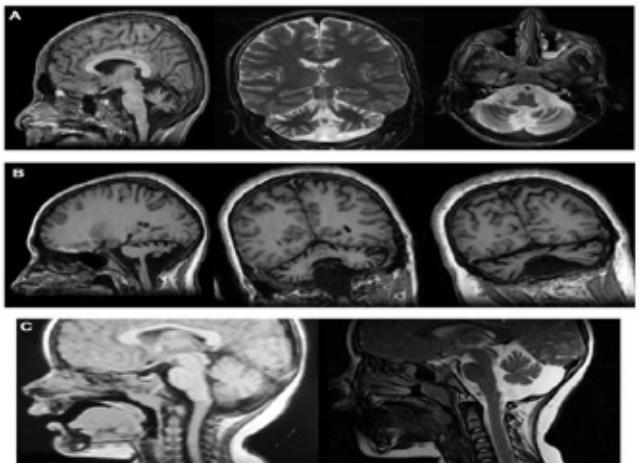

Figure 1. Brain Imaging of the Patients with Congenital Cerebellar Ataxia (A) Generalized cerebellar atrophy and inferior vermian hypoplasia in patient P II-2 (B) Moderate to severe cerebellar atrophy and inferior vermian hypoplasia in patient B IV-3 ((C) Evolution of MRI changes in patient V III-2. The left panel shows normal findings at age 1 year and 8 months, and the right panel shows moderate to marked cerebellar evident at 6 years of age.

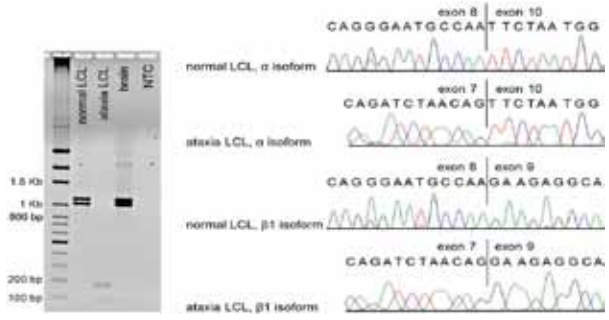

Figure 2. GRM1 Alternative Transcripts in Normal and Ataxia Cells

Table 1. Clinical Observations in the Ten Living Ataxia Patients

	Patient									
	LI 1	V III-2	V III-3	B IV-2	P II-2	B IV-3	P II-1	P II-3	M II-2	M II-1
Age (years) at examination	6	9	11	26	27	32	37	42	47	57
Sex	female	female	male	female	male	female	male	male	male	male
Pregnancy and delivery	no data	normal	intrauterial asphyxia	normal	lung defect at birth	normal	normal	normal	preterm	preterm
Age (years) at walking	4	4	5	childhood	never	1	never	never	3	no data
Age (years) at simple sentences	4	2	3	no data	never	no data	never	never	never	never
Height (cm)	120	126	137	147	138	143	151	150	154	152
Weight (kg)	22	40	42	42.5	23	42.5	50	60	47.1	45.1
Cerebellar Ataxia (SARA Scores)										
Gait (0–8)	4	5	5	5	8	4	7	8	6	7
Stance (0–6)	2	4	4	4	6	2	6	6	5	6
Sitting (0–4)	0	1	1	0	4	0	3	3	1	2
Speech disturbance (0–6)	2	2	2	2	no speech	2	no speech	no speech	no speech	no speech
Finger chase (R → L)/2 (0–4)	1	1	2	1	not tested*	1	1	1	2	1
Nose-finger test (R → L)/2 (0–4)	1	1	2	1	not tested*	1	1	2	1	0
Fast alternating hand movements (R → L)/2 (0–4)	1	2	2	1	not tested*	1	2	2	2	1
Heel–shin slide (R → L)/2 (0–4)	1	2	2	2	not tested*	1	2	3	2	2
Total SARA score (0–40)†	13/40	18/40	20/40	16/40	18/18	12/40	22/34	25/34	19/34	19/34
Oculomotor Signs										
Gaze-evoked horizontal nystagmus	no	no	no	yes	no	yes	yes	no	no	no
Hypometric saccades	no	yes	no	no	yes	no	yes	no	no	yes
Abduction deficit	yes	no	no	yes	yes	no	no	yes	no	no
Esotropia	no	yes	yes	no	yes	no	no	no	no	yes
Ptosis	no	no	no	yes	no	no	no	no	yes	yes
Additional										
Intellectual deficit	mild	mild	mild	moderate	profound	moderate	moderate	severe	severe	severe
Hypomyelinia	yes	yes	yes	yes	no	yes	yes	yes	yes	yes
Spasticity	no	no	no	no	no	no	no	yes	no	no
Polyneuropathy	no	no	no	no	mild	no	no	no data	no data	no data
Seizures	no	at 2 years	at 5 months	no	one abnormal EEG	no	no	no	no	no

The scoring of the eight SARA[17] items is shown in the first column; 0 indicates a lack of impairment, and higher scores indicate increasing severity. The following abbreviations are used: R, right; L, left; SARA, Scale for the Assessment and Rating of Ataxia; and EEG, electroencephalography.
*Profound intellectual deficit precluded these assessments in patient P II-2.
†The sum of figure is the maximal possible total SARA score, which can be lower than the theoretical maximum of 40 if some assessments weren ot possible (e.g., a dysarthria score is missing in patients who never learned to speak).

Conclusion

The study implicates mGluR1 in human hereditary ataxia. It also illustrates the potential of the Roma founder populations for mutation identification by exome sequencing.

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Clinical and genetic heterogeneity of myopathies in Bulgaria

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Introduction

With advances in the genetics of muscle disease, the term, myopathy, has expanded to include mutations in an increasing large list of genes. The current study aims to present the different myopathies, diagnosed up to now in Bulgaria with their clinical peculiarities and underlying gene defects.

Duchenne/Becker Muscular Dystrophy (DMD/BMD)

Duchenne/Becker Muscular Dystrophy (DMD/BMD) is an X-linked recessive disorder caused by a deficient or defective synthesis of dystrophin protein. DMD is the most common form of muscular dystrophy with an incidence of about 1 in 5000 live boys. Though primarily resulting in progressive muscle weakness, it affects various other organs as well (Figure 1). In 131 patients the diagnosis DMD/BMD was genetically confirmed: 117 with deletions in the dystrophin DMD gene, 3 with duplications and 11 with point mutations.

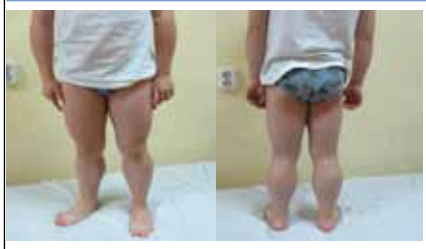


Figure 1. Pseudohypertrophy of calf muscles in a patient with DMD

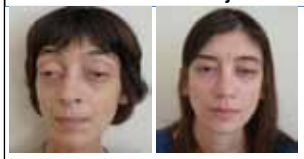
Facioscapulohumeral muscular dystrophy

Facioscapulohumeral muscular dystrophy comprises two genetically distinct types that converge on a common downstream pathway of the expression of the toxic protein DUX4. In Bulgaria we have 70 identified patients.

Figure 2. Scapular winging in a patient with FSHD.

Mitochondrial myopathies

Chronic progressive external ophthalmoplegia (CPEO) is a mitochondrial myopathy with slowly progressive, often symmetric blepharoptosis and limitation of ocular motility in all directions of gaze. We have 14 identified subjects with CPEO and CPEO+ syndromes.



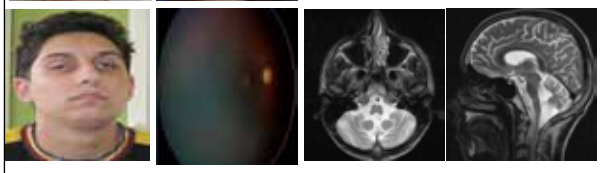


Figure 4. 4A, 4B, 4C, 4D Bilateral ptosis in patients with CPEO. 4E Optic atrophy. 4F, 4G. Cerebellar atrophy in a patient with CPEO+.

Limb girdle muscular dystrophy (LGMD)

Limb girdle muscular dystrophy (LGMD) is an umbrella term given to a group of rare, highly heterogeneous, autosomal neuromuscular disorders. To date over 50 genetic loci have been identified. In Bulgaria the most common forms of LGMD are inherited in autosomal recessive manner, encompassing LDMD 2A, 2C, 2G. LGMD 2C is caused by a founder C283Y mutation in the SGCG gene and is typical for Roma/Gypsy, while LGMD 2G was described in the religious minority of Bulgarian Muslims. Rare forms, such as LGMD 2B, 2J, 2L and 2Z have been found in single cases.

Distal myopathies

Distal myopathies. GNE myopathy is the most common distal myopathy, caused by p.I618T, an ancient founder mutation in the kinase domain of the GNE gene, identified in 58 Roma patients. The clinical features in the Bulgarian GNE group can be described with disease onset mostly in the third decade, but in individual cases, onset was as early as 10 years of age. The majority of patients had foot drop as the first symptom with early and severe involvement of the tibialis anterior muscle, and minimal or late impairment of m. quadriceps femoris (Figure 3).

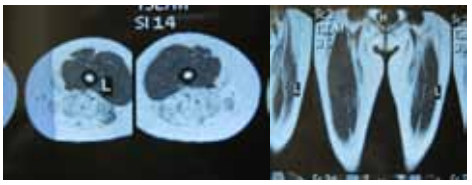


Figure 3. MRI of the thigh muscles in a patient with GNE myopathy

Metabolic myopathies

Pompe disease is a rare autosomal recessive neuromuscular disorder caused by acid α-glucosidase enzyme (GAA) deficiency and divided into two distinct variants, infantile- and late-onset. The late-onset variant is characterized by a spectrum of phenotypic variation that may range from asymptomatic, to reduced muscle strength and/or diaphragmatic paralysis. We have 6 patients, diagnosed with Pompe disease, all of them compound heterozygous.

Congenital muscular dystrophies (CMD)

Congenital muscular dystrophies (CMD) are a group of hereditary myopathies with predominantly autosomal recessive inheritance that are characterized by genetic and clinical heterogeneity. Their clinical course is broadly variable and encompasses congenital hypotonia, delayed motor development, progressive muscle weakness, joint contractures and dystrophic pattern on muscle biopsy. Mutations in the gene encoding the α2 chain of laminin1 lead to congenital muscular dystrophy type 1A (MDC1A), identified in 5 patients (Figure 5).

Figure 5. A patient with MDC1A with confluent white matter lesions on brain MRI.

Conclusion

In Bulgaria a diverse genetic muscle disorders have been diagnosed. Genetic diagnosis in these cases is important for future potential treatment and genetic counselling in the affected families.

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Niemann-Pick disease type B and type C in Bulgaria – genetic and clinical characteristics

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Introduction

Niemann-Pick (NP) disease is a multisystem disorder with a wide range of symptoms that vary in severity. NP disease is divided into four main types: type A, type B, type C, and type D These types are classified on the basis of genetic cause and the signs and symptoms of the condition.

- Mutations in the sphingomyelin phosphodiesterase 1 (*SMPD1*) gene, causing acid sphingomyelinase deficiency has been categorized as either neuronopathic NPD-A, with death in early childhood, or non-neuronopathic NPD-B. Afterwards it was discovered that intermediate forms to these two extremes occur.
- Niemann-Pick disease type C (NP-C) is a rare, autosomal recessive (AR) disease, caused by mutations either in the NPC1 or in the NPC2 gene, which lead to impaired intracellular lipid trafficking and subsequent accumulation of cholesterol and glycosphingolipids in various tissues.

Aim

To describe the clinical features and the causative mutations, related to both diseases (NPB and NPC) among Bulgarian population

Material and methods

All the affected- 25 patients with NPB and 16 with NPC underwent detailed neurological examination and neuro-ophthalmological, neuropsychological and psychiatric evaluations, as well as brain MRI, abdominal ultrasound and hearing tests.

Results

Niemann Pick type B

All affected NPB 25 subjects were Roma/Gypsy, homozygous for the same ancestral mutation, W391G in *SMPD1*.

They displayed a broad phenotypic intermediate spectrum, encompassing;

- Hepatosplenomegaly
- Recurrent respiratory infections
- Ataxia (Figure 1A)
- Extrapyramidal signs
- Peripheral neuropathy
- EEG abnormalities and epileptic seizures
- Cognitive impairment
- Psychotic episodes
- Retinal abnormalities (Figure 1B)

The clinical heterogeneity of W391G homozygotes points to additional factors, beyond SMPD1 and residual ASMase, which determine the localization, extent and severity of neural involvement. In practical terms, W391 is common in the Gypsy population and the diagnosis of NPD should be borne in mind despite the atypical course of the disease.

The overall carrier rate of W391G in *SMPD1* was 1.1%, with highest frequency of 4.6% among the Rudari.

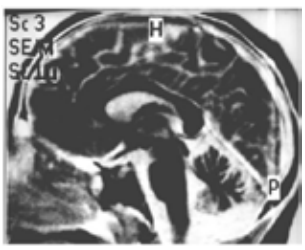

Figure 1. A Cerebellar atrophy; 1B Macular halo in a patient with NPB

Niemann Pick type C

Currently in Bulgaria 16 patients (Figure 2) with NP-C have been diagnosed, due to various mutations. The mean age at onset in our cohort is 11.36 (SD 8.47) years with a diagnostic delay varying between 1 and 23 years.

The main clinical features in the Bulgarian NPC patients were:

- Vertical gaze palsy
- Dysarthria
- Pyramidal involvement
- Cognitive impairment
- Ataxia
- Dystonia
- Epileptic seizures
- Gelastic cataplexy
- Organomegaly epileptic
- Brain MRI with hyperintense white matter lesions and/or cortical and/or cerebellar atrophy (Figure 3)

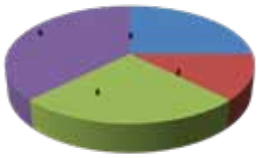

Figure 2. Distribution based on the age at onset of Bulgarian NPC patients.

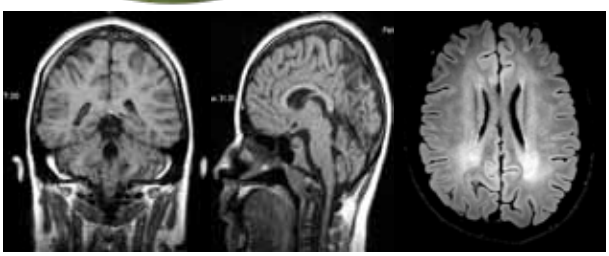

Figure 3. 3A and B. Cerebellar atrophy in a patient with NPC. 3C. Leukoencephalopathy in a NPC patient

Conclusion

Niemann-Pick disease is a group of multisystem lysosomal disorders. Diagnosis has often been a difficult task, due to the wide range in age of onset and clinical presentation of the disease, combined with the complexity of the cell biology laboratory testing, even in combination with genetic testing. Early diagnosis is crucial, since approved treatmenet and therapies under development can be offered.

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Estimation of relative telomere length in different MSCs

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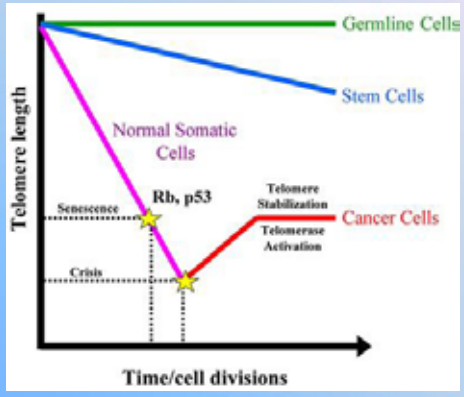
INTRODUCTION

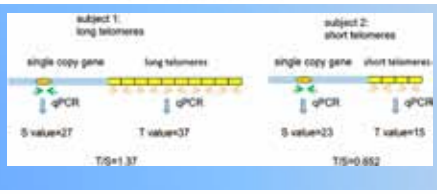
- Telomeres are the protective (TTAGGG)n repeats on the ends of eukaryotic chromosomes, essential for maintaining the genomic stability within adult somatic tissues. Due to end-replication problem, each cell division leads to telomere loss. Telomere shortening to a critical length triggers to cell senescence and apoptosis. Further erosion of telomeres over a critical length leads to chromosomal instability.

- The maintenance of telomere length in stem cells is an important factor for their self-renewal and differentiation. As a life span of stem cells is limited in "*in vitro*" condition, it is of crucial importance to estimate replicative potential of stem cells with preserved self-renewal and differentiation capacity. One of the most relevant marker in estimating of proliferative capacity of stem cells could be telomere length.



AIMS

The aim of this study was to estimate and compare proliferative capacity of various human mesenchimal stem cells (MSTs) by evaluating relative telomere length (RTL).



MATERIAL AND METHODS

- Telomere length evaluation was done using DNA isolated from MSCs of peripheral blood (PB-MSCs), umbilical cord (UC-MSCs), adipose tissue (AT-MSCs), dental pulp (SHEDs), periodontal ligament (PDL-MSCs), and from bone marrow stromal cell line (HS-5) as reference sample.
- Quantitative real-time PCR was performed for estimating of RTL by ordering ΔΔCt value (ΔΔC=Ct *telomere - Ct single copy gene;* ***ΔΔCt=ΔCt*** *sample - ΔCt reference,* $2^{-\Delta\Delta Ct}$)
- Cell proliferation was estimating by direct counting of live MSCs in 0.4% Trypan Blue solution after 3, 5, and 7 days of incubation, and by comparing MSCs to referent cells (HS-5 line).

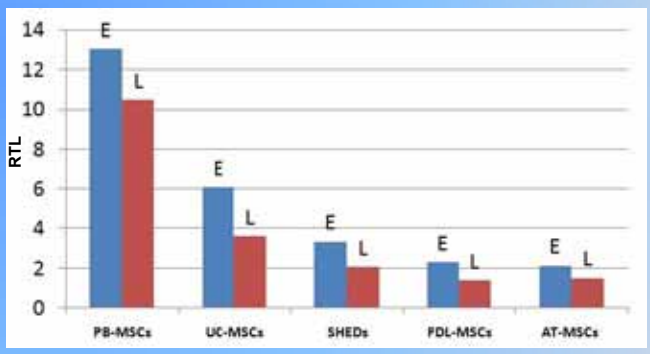


Figure 1. Relative telomere length (RTL) in different MSCs lines. E, early passages; L, later passages

RESULTS

- The median RTL values of all MSCs from early passage (E=4th) to later (L=10th) were as follows: PB-MSCs, E:13.03-L:10.46; UC-MSCs, E:6.06-L:3.60, PDL-MSCs, E:3.35-L:2.08, SHEDs E:2.3-L:1.4, and AT-MSCs, E:2.14-L:1.52. Figure 1.
- RTL for PB-MSCs was significantly higher in comparison to other MSCs, as in lower passage (p=0.04) as in higher passage (p=0.009).
- Intermediate value of RTL was observed in UC-MSCs, while the lowest RTL values obtained in PDL-MSCs, SHEDs, and AT-MSCs.
- Proliferative capacity of PB-MSCs was significantly higher compared to other MSCs, at any time point (3, 5, and 7 days). Figure 2.
- Proliferative capacity of PDL-MSCs, SHEDs, and AT-MSCs was the lowest, and could be related to RTL values.

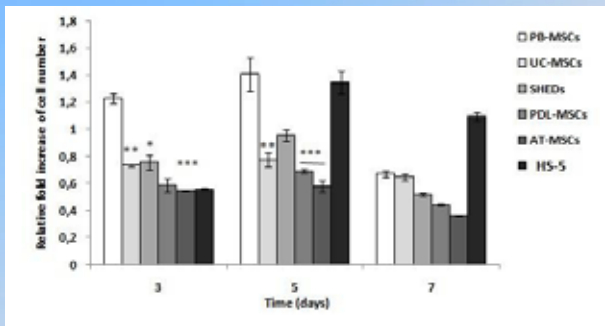


Figure 2. Proliferative capacity of different MSCs in various time point

CONCLUSION

- Shortening of MSCs telomere trough passages during cultivation was in association with their proliferative capacity.
- Telomere length could be useful marker in estimation of MSCs self-renewal before thier aplication in clinical protocols.

TUMOR NECROSIS FACTOR GENE POLYMORPHISMS AND THE RISK FOR CHRONIC LYMPHOCYTIC LEUKEMIA IN MACEDONIAN POPULATION

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ABSTRACT

Single nucleotide polymorphisms (SNP) in the genes for tumor necrosis factor beta (TNFB+252G/A) and tumor necrosis factor alpha (TNFA-308G/A) have been associated with many inflammatory and autoimmune diseases, but also they are associate with higher risk for lymphoproliferative disorders like Non Hodgkin Lymphoma (NHL) and Chronic Lymphocytic Leukemia (CLL).
The aim of our study was to investigate a possible association of these two gene polymorphisms with the risk for CLL.
We have analyzed 130 patients with chronic lymphocytic leukemia and 120 healthy controls. Thirty of these 130 CLL patients had also autoimmune hemolytic anemia (AIHA). Genotyping was performed by using PCR-RFLP methods.
Our results did not demonstrate significantly different distribution of the TNFβ+252 G/A genotypes in patients with CLL compared with controls, p=0.764.
We also didn't find significant difference in the genotype distribution of TNFα-308G/A between CLL patients and controls, p=0.612.
Our results demonstrated that the G allele of the TNFB+252G/A and A allele of the TNFA-308G/A were significantly more frequent among the CLL patients with AIHA compared to CLL patients without AIHA, p=0.003 and p=0.0002. These data indicate that the G allele of TNFB+252G/A and A allele of TNFA-308G/A, which are both associated with increased TNF secretion, are more frequent in CLL patients with AIHA suggesting that these two polymorphisms may predispose to the development of AIHA in CLL patients.

INTRODUCTION

Single nucleotide polymorphisms (SNP) in the genes for tumor necrosis factor beta (TNFB+252G/A) and tumor necrosis factor alpha (TNFA-308G/A) have been associated with increased TNF production and interleukin-6 secretion. TNF has potent proinflammatory effects, and is implicated in many inflammatory and autoimmune diseases, like inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, autoimmune thrombocytopenia and haemolytic anemia, but also they are associate with higher risk for lymphoproliferative disorders like Non Hodgkin Lymphoma (NHL) and Chronic Lymphocytic Leukemia (CLL).The aim of our study was to investigate a possible association of these gene polymorphisms (TNFB+252G/A and TNFA-308G/A) with the risk for CLL.

MATERIALS AND METHODS

MATERIALS

We analyzed 130 patients with CLL and 120 healthy controls. Thirty of these 130 CLL patients had also autoimmune hemolytic anemia (AIHA).

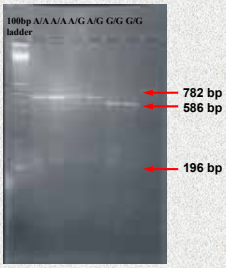
METHODS

•Genomic DNA was isolated from peripheral blood leukocytes and a specific DNA fragment that contains polymorphic site was amplified using polymerase chain reaction (PCR)
•Amplified DNA fragments were digested with 1.6 U of restriction enzyme Nco I at 37°C for 16 hours for (TNFβ +252 G/A) and (TNFα -308 G/A), followed by an electrophoresis on a 2% agarose gel.

RESULTS INTERPRETATION

Results from electrophoretic analysis of digested fragments for:

TNFβ (+252 G/A)



TNFα (-308 G/A)

RESULTS

Table 1. Genotype distribution of TNFβ and TNFα in patients with CLL and controls.

	TNFβ (+252 G/A) G/G	G/A	A/A	p values	TNFα (-308 G/A) G/G	G/A	A/A	p values
CLL patients (n=130)	18	43	69	0.764	97	29	4	0.318
Controls (n=120)	16	35	69		95	23	2	

Table 2. Genotype distribution of TNFβ and TNFα in CLL patients with and without AIHA.

	TNFβ (+252 G/A) G/G	G/A	A/A	p values	TNFα (-308 G/A) G/G	G/A	A/A	p values
CLL with AIHA (n=30)	8	12	10	0.018	82	17	1	0.0006
CLL without AIHA (n=100)	10	31	59		15	12	3	

CONCLUSIONS

Our results did not demonstrate significant difference in the distribution of genotypes or allele frequencies for both polymorphisms between the patients with CLL and controls contrary to the results from other studies in patients with NHL and CLL. But these data indicate that the G allele of TNFB+252G/A and A allele of TNFA-308G/A, which are both associated with increased TNF secretion, are more frequent in CLL patients with AIHA suggesting that these two polymorphisms may predispose to the development of AIHA in CLL patients.

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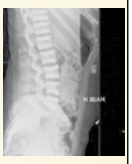
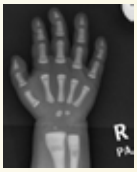
Клиничен случай на остеопетроза с негативен резултат за JAK-2 мутация





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Въведение: Остеопетрозата ("мраморна костна болест") представлява група редки наследствени заболявания на скелета, характеризиращи се с повишена рентгенографска костна плътност. Честотата на автозомно-рецесивната остеопетроза (АРО) е около 1 на 250 000 раждания, а на автозомно-доминантната остеопетроза (АДО) е 1 на 20 000 раждания. Остеопетротичните състояния значително се различават по изява и тежест на протичане, вариращи от неонатално начало с животозастрашаващи усложнения, като например класическата или "злокачествена" АРО, до случайното й откриване при рентгенография. Класическите АРО форми се характеризират с фрактури, нисък ръст, компресионни невропатии, хипокалциемия с придружаващи тетанични припадъци и животозастрашаваща панцитопения. Известно е, че JAK2 V617F мутацията се установява при 58% от пациентите с първична миелофиброза и е лош прогностичен маркер.

Материали и методи: Касае за момиче на 21 год. възраст с АРО с неонатално начало. Родено от втора патологично протекла бременност и нормално раждане. Към 40-дневна възраст е установена хепатоспленомегалия, долихоцефална конфигурация на главата, тежък анемичен синдром и промени в костите, по-късно и двустранна амавроза. Поставена е диагноза остеопетроза на 1-годишна възраст рентгенологично. Детето е изоставащо във физическото моторно развитие и често боледуващо. Посещава специализирано училище за слепи деца с много добра успеваемост. На 16-годишна възраст е поставена остеосинтеза след фрактура на лява бедрена кост. Проследявано е периодично от неврохирург и хематолог.
ДНК е изолирана от кръвна проба на пациентката с остеопетроза и нейната здрава сестра (25 год.). Генът *JAK2* е амплифициран със специфични праймери за V617F мутация. Изследването е извършено с конвенционален PCR, последван от гел електрофореза.

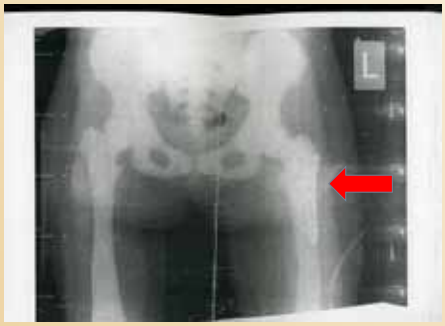
Резултати

Резултатите показват, че двете сестри не носят *JAK2* мутация. Това е един от случаите, който следва да се доизясни чрез допълнително изследване за генетични промени чрез таргетно екзомно секвениране.

1.

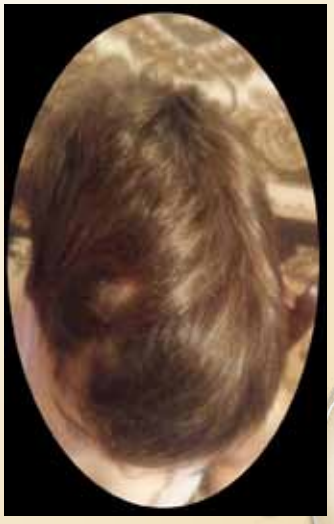
Фигура 1. Резултати от изследването на двете сестри, които показват че те не носят *JAK2* мутация; 1 – хетерозигот по мутацията; 2 и 3 – хомозиготи по нормалния алел (пациентката и нейната сестра)

2.



Фигура 2. Рентгенова снимка на поставена остеосинтеза, след фрактура на лява бедрена кост

3.



Фигура 3. Долихоцефална конфигурация на главата

Заключение

Липсата на JAK2 V617F мутацията при пациентите с първична миелофиброза е добър прогностичен белег. За изясняване на генетичната причина се предвижда търсене на други генетични маркери, асоциирани със заболяването.

Beta-1Adrenergic Receptor Gene Polymorphisms With Obstructive Sleep Apnea Syndrome In East of Turkey

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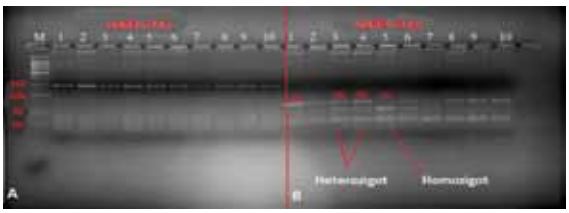
Introduction

Sleeping, which is an indispensable necessity for human health, serves to biologically and psychologically regenerate an organism. Sleeplessness can trigger various illnesses and weakens the labor force, contributing to overall economic losses (Punjabi, 2008). Obstructive sleep apnea syndrome (OSAS) is defined as diurnal sleepness that occur during sleep because of repeated obstructions in the upper airway (Kaparianos et al., 2006). Apnea is defined as the stoppage of airflow through the mouth and nose for a duration of 10 s or more. The clinical diagnosis of OSAS is related to several metabolic diseases such as, hypertension, diabetes, endocrine diseases, and obesity (Prentice, 2006). In recent years, progress has been made in diagnosing this disease using polysomnography (PSG) tests (Mokhlesi and Gozal, 2011).
In different countries, OSAS differs in terms of clinical findings, and the prevalence has been shown to increase in individuals 40-65 years of age (Ge et al., 2005; Zhang et al., 2005). The incidence rate of OSAS is 4% in men and 2% in women (Larkin et al., 2010). Approximately 0.9-1.9% of the Turkish population suffers from sleep apnea syndrome (Sengul et al., 2011). In the USA, 12 million people aged 30-65 years have OSAS, and approximately 25% have medium or severe apnea according to the Apnea Hypopnea Index (AHI) (Daghestani et al., 2012; Herr et al., 2013). In a study conducted in the others examined continuous snoring in 1453 individuals 30-60 years of age who appeared to be healthy. The researchers applied PSG to 602 subjects and found AHI values of >5 in 9% of women and 24% of men, AHI > 10 in 5% of women and 15% of men, and AHI > 20 in 4% of women and 9% of men (Young et al., 2003; Daghestani et al., 2012 The β-1 adrenergic gene receptors (ADRB1) are synthesized in the body in various cells that are targets of adrenaline and noradrenalin and are involved in regulating the cardiac, pulmonary, vascular, and central nerve systems (Fragoso et al., 2006).

Aim and Methods

The sympathetic nervous system and the adrenergic receptors play an important role in regulation of daily sleep and circularity system.. This study explored the associations between functional polymorphisms of the ,beta-1(β1)-adrenergic receptor genes and obstructive sleep apnea (OSA) in patients and healty controls.
Methods:We determined the distribution of the Ser49Gly and Arg389Glu polymorphisms of the beta-1 adrenergic receptor gene (ADRB1) in patients with obstructive sleep apnea syndrome as well as a control group in eastern Turkey. A total of 62 patients diagnosed with obstructive sleep apnea in a sleep laboratory and 78 control subjects were examined. Peripheral blood samples were taken from patients diagnosed with obstructive sleep apnea by polysomnography. DNA was extracted from blood samples and amplified using polymerase chain reaction. Amplification products were digested with restriction enzymes (EcoO109I and BcgI) to investigate gene polymorphisms. Restriction products were extracted from agarose gel electrophoresis and polymorphisms were analyzed using gel images.



ADRB1 Gene PCR amplifications and digesstion with RE enzymes

Result

The Ser49Gly polymorphism was observed in 11 of 62 (17.5%) patients and in 10 of 78 (13%) controls. The Arg389Gly polymorphism was observed in 18 of 62 (29 %) patients and in 11 of 78 (13.5%) controls

Table 1. Genotypic and Allelic Results of ADRB1 Gene Polymorpisms in the Patients and Controls

ADRB1			OSAS (n=62)							Control Group (n=78)				
Genotypes	GG	%	CC	%	GC	%	P	GG	%	CC	%	GC	%	P
Frequencies of Genotypes	33	0,53	7	0,11	22	0,36	0,41	64	0,80	8	0,10	6	0,10	0,515
Frequencies of Alleles	0,67		0,33					0,68		0,32				0.160

Discussion

In conclusion, there was no correlation among polymorphic frequencies between patient and control groups. Based on the results, these polymorphisms do not contribute to Ser49Gly the clinical diagnosis of this syndrome. However, the distribution of Arg389Gly polymorphisms may contribute to patients with a body mass index greater than 30 ($P < 0.05$).

Key words: ADRB1 gene polymorphisms; Obstructive sleep apnea syndrome Body mass index; Restriction fragment length polymorphism; Turkish population

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Clinical symptomatology and genetic investigation in Bulgarian patients with Leber's hereditary optic neuropathy

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Leber's hereditary optic neuropathy (LHON) is a rare maternally inherited disease caused by mitochondrial DNA point mutations in genes encoding the MT-ND1, MT-ND4, MT-ND4L and MT-ND6 subunits of complex I in the mitochondrial respiratory chain. The most common mutations MTND4*LHON11778A, p.R340H,MTND1*LHON3460A,p.A52T and MTND6*LHON14484C, p.M64V cause the disease in 90% of patients but there are also a number of less common mutations. LHON is characterized by bilateral acute or subacute visual failure and usually occurs in young males.

Purpose: An assessment of the clinical symptomatology and molecular genetic analysis in a cohort of Bulgarian patients with LHON

Subjects and methods: On the basis of the clinical evaluation and genetic investigation **12 patients (**9 males and 3 females), age between 7 and 41 years were diagnosed as having LHON. **A full neuroophthalmologic examination** including visual acuity testing, Goldmann kinetic or automated static threshold perimetry, slit lamp biomicroscopy, examination of the pupillary light reflex, direct ophthalmoscopy, optical coherence tomography, evaluation of ocular motility and genetic investigation was performed in all patients.

Results and discussion: The age at onset of visual failure ranged from 3 to 34 years (**average = 16,1 years).** Visual acuity ranged between 0,01 and 0,6 in the patients i.e. from legal blindness in 5 patients to some level of useful vision in 7 patients (Table 1). Bilateral central scotoma was the typical perimetric finding, revealed by visual field examination (Fig.1).

Visual acuity (metric)	Number of LHON patients (n=12)
0,4 – 0,6	1
0,1 – 0,3	6
0,05 – 0,09	1
< 0,05	4

Tabl. 1. Distribution of visual acuity in LHON patients

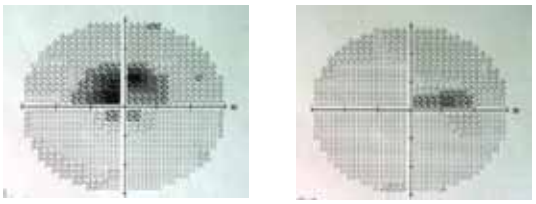

Fig. 1. Bilateral central scotoma in a LHON patient

In 3 patients, examined during the acute stage of optic neuropathy, ophthalmoscopy revealed an optic disc hyperemia, opacity of the peripapillary retinal nerve fiber layer, tortuous telangiectatic peripapillary retinal vessels (Fig.2) and in the remaining 9 patients examined after the acute stage bilateral optic atrophy was found. OCT revealed a marked thinning of the retinal nerve fiber layer especially of the temporal fibers (papillomacular bundle) (Fig. 3).

Fig. 2. Fundus photograph in acute stage of LHON

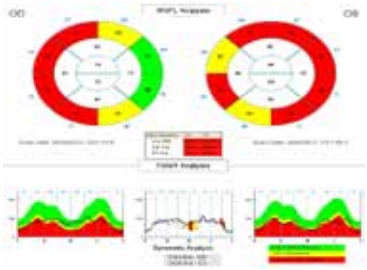

Fig. 3. OCT finding in a LHON patient

Seven patients from 2 unrelated families had family history of LHON and the remaining 5 patients were isolated cases in their families. Genetic testing revealed the following gene mutations causing LHON in our patients (Tabl. 2).

Gene mutation causing LHON	Gene	Number of LHON patients
G11778A, p. R340H	MT-ND4	3
G3460A, p. A52T	MT-ND1	2
G3635A, p. S110N	MT- ND1	4
G11778A, p. R340H T14484C, p. M64V	MT-ND4 MT-ND6	3

Tabl. 2. Mitochondrial genome mutations in Bulgarian LHON patients

Two out of three most common gene mutations causing LHON - MTND4*LHON11778A, p. R340H and MTND1*LHON3460A, p. A52T, were found in 5 of our patients, all sporadic cases. The G11778A mutation is associated with the most severe phenotype and worst visual outcome in LHON patients. Indeed in 2 out of 3 patients carrying MTND4*LHON11778A, p. R340H mutation visual acuity was the lowest in the whole group and ranged between 0,01 and 0,03.

Interestingly a digenic inheritance of MTND4*LHON11778A, p. R340H and MTND6*LHON14484C, p. M64V was detected in a family with 3 affected individuals – mother and her both sons. In the contrast to G11778A mutation associated with the poorest visual prognosis, the MTND6*LHON14484C, p. M64V mutation is reported to be associated with the most favorable visual outcome. In all 3 affected with digenic inheritance visual acuity ranged between 0,09 and 0,1, i.e. they have moderately reduced visual acuity with some level of useful vision. Most likely this a result of modifying effect of MTND6*LHON14484C, p. M64V mutation, preventing the appearance of severe phenotype, characteristic for the MTND4*LHON11778A, p. R340H mutation.

The rare mutation MTND1*LHON3635A, p. S110N was found in another family with four affected members – two sisters and both sons of the elder sister. This mutation is rare for the European population but it is described in Chinese families and in Russian families from Siberia.

Conclusion : A genetic investigation of the whole mitochondrial genome is necessary in all patients suspected of having LHON.

MULTIDISCIPLINARY APPROACH AND SPEECH, OCCUPATIONAL, AND PHYSICAL THERAPY IN A CASE OF JOUBERT SYNDROME

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Background and aims:
Joubert syndrome is autosomal recessive rare disease, characterized by finding of complex congenital malformation of the brainstem and agenesis or hypoplasia of the cerebellar vermis with key finding that comprises the *molar tooth sign* on magnetic resonance images. Speech, occupational, and physical therapy lead to improved prognosis.

Methods:
Case report.
Results:
We present the 20-months-old-child with facial dysmorphic features: prominent forehead, high rounded eyebrows, epicanthal folds, hypertelorism, depressed nasal bridge, anteverted nostrils, convergent strabismus, otapostatic ears, tongue protrusion, with nystagmus, developmental delay and hypotonia. MRI finding of central nervous system is characteristic with abnormal morphology of cerebellum and vermis agenesis. The management of individuals with Joubert syndrome is supportive and related to the different manifestations of the syndrome.

Conclusion:
Joubert syndrome is rare disease with estimated prevalence of 1:100.000. This heterogeneous syndrome is in association with genes on chromosomes: 6q23, 2q13, 12q21, 8q22, 16q12, 3p12.3-q12.3, 4p15, 9q34 and 11p12-q13. Developmental outcome in Joubert syndrome is variable. Multidisciplinary approach and speech, occupational, and physical therapy should be ordered to improve different manifestations of the syndrome.

DIAGNOSIS OF MYELOPROLIFERATIVE NEOPLASMS USING MOLECULAR METHODS FROM SINGLE CENTRE EXPERIENCE

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Introduction

Myeloproliferative neoplasms (MPN) are clonal blood disorders characterized by uncontrolled production of the myeloid line of cells. These groups of diseases consist of the following entities: Chronic myelogenous leukemia (CML), Polycytemia Vera (PV), Essential trombocytemia (ET), and Primary myelofibrosis (PMF). Although they derive from one cell, management of the MPNs is not uniform. The precise differentiation is an imperative in the management of these diseases.

Aim of the study

The aim of this study was evaluation of the hematological, biochemical and molecular diagnostic parameters which are important in the diagnosis of MPN.

Materials and methods

We have analized 143 patiens (63 men and 80 women) with median age of 61 (range 16-88) which were diagnosed at the University Clinic of Hematology- Skopje. The monitoring period of the patients was 13 months. The examined group consisted of: 44 patients with Polycytemia vera (PV), 71 patients with Essential trombocytemia (ET), 14 patients with Chronic myelogenous leukemia (CML), 12 patients with Primary myelofibrosis (PMF).

The values of full blood hemoglobin level and count of erythrocytes, leukocytes and thrombocytes, as well as the peripheral blood smear were examined among the hematological parameters, and values of LDH and blood iron were the evaluated biochemical parameters. The presence of BCR-ABL oncogene was investigated by RT-PCR methods (Figure 1). JAK2V617F mutation was analyzed by allele-specific PCR and confirmed with RFLP method (Figure 2).

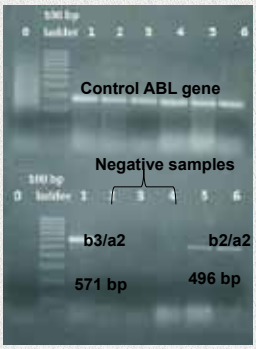

Figure 1. PCR products of two common transcripts of BCR-ABL oncogene: b2/a2 and b3/a2

Ladder-100bp
Control ABL gene
Samples 2,3,4 are negative for BCR-ABL oncogene
Samples 1,5,6 are positive for BCR-ABL oncogene

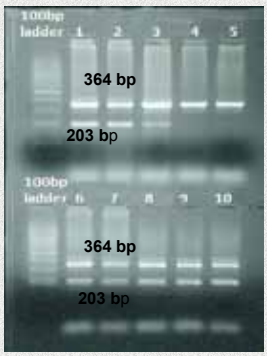

Figure 2. PCR products of JAK2V617F mutation

Ladder-100bp
Samples 1,2,3,6-10 are positive for JAK2V617F mutation
Samples 4,5 are negative for JAK2V617F mutation

Results

The results from the molecular testing showed presence of BCR-ABL oncogene in all patients with CML. The transcript b3/a2 with size of 571 bp was present in 64,3% of the patients, whereas b2/a2 with size of 496 bp, was present in 35,7% of the patients (Table 1) . JAK2V617F mutation was positive in 62,4% of patients with BCR-ABL negative myeloproliferative neoplasms. JAK2V617F mutation was detected in 86,4% of patients with PV and 59,2% of patients with ET (Table 2).

JAK2 V617F	Diagnosis						Total	
	PV		ET		PMF			
	N	%	N	%	N	%	N	%
Pozitiv samples	38	86.4	42	59.2	8	66.7	**88**	**62.4**
Negativ samples	6	13.6	29	40.8	4	33.4	**53**	**37.6**

BCL-ABL	N	%
Pozitive samples	14	**100**
- b3/a2	9/14	**64.3**
- b2/a2	5/14	**35.7**
Negative samples	0	**0**

Conclusion

In conclusion, molecular testing of BCR-ABL oncogene and JAK2V617F mutation are mandatory for the diagnosis and treatment of patients with MPN.

THE CASE OF THE TERMINAL DELETION OF THE LONG ARM OF THE CHROMOSOME 3

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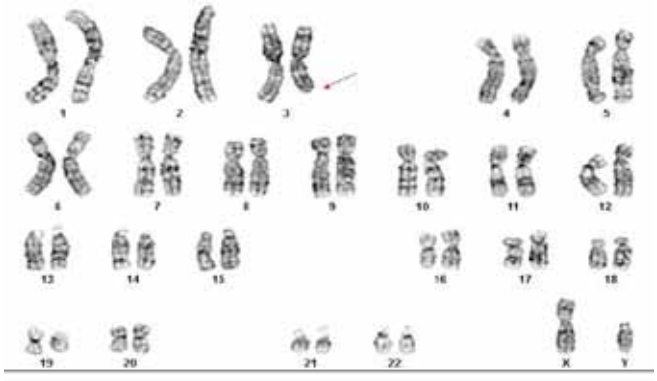
Last years we have seen an increase of the number of patients with mental, physical retardation and speech delay who get an primary medical and genetic consultation in Center of Medical Genetics of the National Children's Specialized Hospital "OKHMATDYT". Was identified: in 56% cases - aneuploidy, 21% - microdeletion syndromes confirmed by fluorescence hybridization in situ, 14% - unbalanced chromosomal rearrangements, and 9% - balanced chromosomal translocation.

Purpose: to improve the diagnosis of various microdeletion syndromes during the medical-genetic examination.

Methods: clinical, genealogical, standard chromosome analysis.

Results. The proband (boy, 1,5 y.o.) with complaints on chronic pneumonia resist to the treatment, retardation of psychomotor development and feeding problems was direct to the Center of Medical Genetic. Anamnesis: first pregnancy, premature birth (on the 36 week), born with weighing 1600 g, length of 43 cm, Apgar scores 6 points. At the time of the observation: weight - 5370g (-4 SDS), body length - 73cm (-2 SDS), head circumference - 38cm (-3 SDS). Phenotype: microcephaly, hook-shaped nose, bilateral exophthalmos, microstomia, auricle deformation, blond hair with many curls, single creases on both hands, defective finger`s line on both footsteps, bilateral cryptorchism. Abnormalities of the internal organs were not found.



Standard chromosome analysis: 46, XY, del(3)(q28),dn.
Parents`s karyotype is normal. Thus, the results of a laboratory examination is allowed to confirm in the patient unbalanced chromosomal abnormality - partial monosomy of the long arm of chromosome 3.

Fig.1. The patient's karyotype was identified as 46, XY, del(3)(q28).

Conclusion. All children with mental delay and growth retardation are recommended cytogenetic screening involving molecular diagnostic methods.

A 16-year old boy with LEOPARD syndrome

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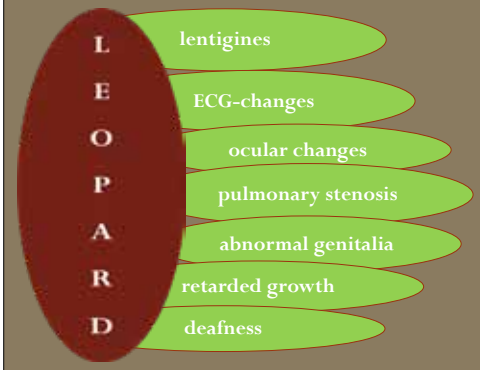
BACKGROUND. The very rare LEOPARD syndrome, also called Noonan syndrome with multiple lentigines (LS, about 200 cases in literature) is an autosomal-dominant multisystem disease, which was first described in 1969. PTPN11, RAF1 and BRAF are the genes known to be associated with LS, identifying molecular genetic testing of the 3 gene mutations in about 95% of affected individuals.

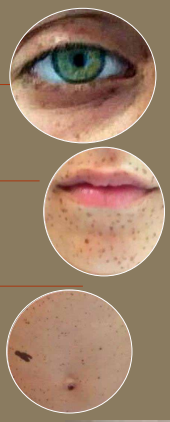


Diagnostic criteria (Voron et al., 1976)

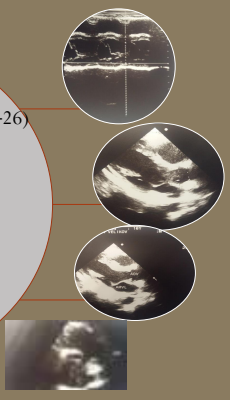
Multiple lentigines + 2 of other cardinal features	Absence of lentigines + 3 of the other cardinal features + First-degree relative with LS

CLINICAL CASE. A 16-year-old boy with short stature presented to us for the first time at 16 years of age. Our clinical diagnosis is LEOPARD syndrome. The boy is under follow-up by pediatric endocrinologist and pediatric cardiologist without treatment for the moment. DNA studies were undertaken.

multiple lentigines, including mucosae
ocular hypertelorism
blue eyes
anteverted ears
pectus excavatum
Height 15.8 cm below the lower boundery of mid-parental height
Pubertal stage Tanner II-III, normal penile development



IGF-1 - 261 ng/ml (t.r. 57-426)
Bone age - 3 years delay
ECG – superior axis, prolonged QT
EchoCG - apical aneurysm, Ao insufficiency 0-1, thickening of the wall heart chambers



References: Orphanet journal of rare diseases, 2008, Anna Sarkozy et al., LEOPARD syndrome – clinical features and gene mutations, E. Martinez-Quintana et al., 2012

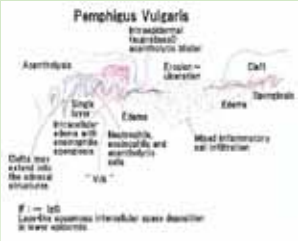
УНГ прояви на Пемфигус Вулгарис

Д-р Димитър Пазарджиклиев[1], Ангел Балинов[2], Благовест Петров[2], Гина Стойкова[2], Георги Николов[2], Севдалена Георгиева[2],бНевена Илиева[2]

1.Клиника по УНГ- УМБАЛ " Каспела"; 2.Студент IV курс МФ към МУ-Пловдив, специалност "медицина".

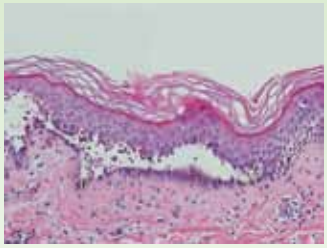
Въведение:

Пемфигус Вулгарис е автоимунно заболяване, което се характеризира с образуване на булозни обриви по кожата и лигавиците. Заболяването има идиопатична етиология, но е установено влиянието на няколко рискови фактори, сред които някои лекарства, неоплазми и разнообразни физически и химически дразнители. Засяга лица предимно на средна възраст и изисква продължително, понякога доживотно лечение с медикаменти потискащи имунната система. Реализира се посредством *акантолиза*, дължаща се на синтеза на IgG антитела срещу десмозомалния гликопротеин и десмоглеина (Dsg), които се съдържат в кератиноцитите.Това води до загуба на междуклетъчни връзки и разслояване на епидермиса.За една от причините за образуване на IgG антитела се счита дефект в HLA-системата (комплекс от гени, осигуряващ „ненападение" от страна на имунната система на собствени за организма структури).



◄ Схематично представяне на патогенезата на пемфигус вулгарис

Траен хистологичен препарат ►



Съществуват няколко клинични вида пемфигус:

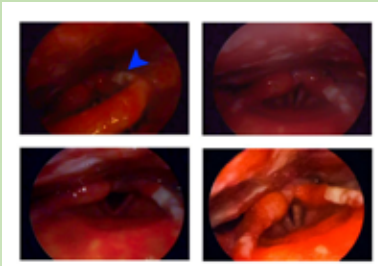
➢ **Пемфигус вулгарис** - най-разпространената форма на пемфигус.Увреждa най-често лица, чиято възраст е между четиридесет и шестдесет години.От своя страна този вид има два клинични типа,в зависимост от вида на доминиращите антитела: **Мукозната форма (ПВМ),** която се характеризира с предоминиране на anti-Dsg3 антителата. За разлика от това при **кожнолигавичната форма (МКПВ)** са налице както anti-Dsg3, така и anti-Dsg1 антитела.

➢ **Пемфигус фолиацеус** - представлява сравнително лека разновидност на състоянието.

➢ **Паранеопластичен пемфигус** - тази разновидност на **пемфигус** се смята за най-тежката. Тя се явява усложнение на раково заболяване, най-вече лимфом.

Клиничен случай:

Пациент на 44 годишна възраст с придружаващо заболяване-тиреоидит на Хашимото, постъпва в клиниката по повод дисфония и дисфагия, болки и чувство на чуждо тяло в гърлото с давност 1 месец. Лекуван амбулаторно от ОПЛ с локални антисептични средства, но без резултат.Проведена е мезофаринго- и риноскопия, които не показват патологични промени. Няма данни за везикули и булозни изменения.



Фотоматериал от фиброларингоскопичното изследване на пациента.

На индиректна ларингоскопия са установени налепи в двата recessus piriformis. Изписан е противогъбичен медикамент и пациентът е изпратен за консултация при кожен лекар, въпреки тогавашната липса на кожни изменения**. Едновременно с посещението на кожния лекар, пациентът получава булозни лезии в устната кухина.** Доказани са антитела, характерни за заболяването, посредством диагностична имунофлуоресценция. Изписан от кожна клиника с терапия за дома - метилпреднизолон по схема.

Обсъждане

Независимо от формата си,пемфигусът може да се изяви от страна и на ларинкса. Той се засяга сравнително често, но въпреки това убягва от ДД план на заболяването.

Заключение:

Препоръчва се **УНГ преглед да бъде рутинна част от диагностичните мероприятия**, тъй като изявите по съответните органи често съпровождат кожните форми. За диагностика е важно използването *на индиректна ларингоскопия* и *флексибилна ригидна ендоскопия*, в противен случай диагнозата се забавя с повече от месец и е причина за забавяне на адекватното лечение.

Източници:

1. The clinical phenotype of pemphigus is defined by the anti-desmoglein autoantibody profile
Masayuki Amagai, MD, PhD,a Kazuyuki Tsunoda, DDS,a,b Detlef Zillikens, MD,c Tetsuo Nagai, DDS, PhD,b and Takeji Nishikawa, MD, PhDa *Tokyo, Japan, and Wuerzburg, Germany*
2. Pemphigus vulgaris of the larynx and upper gastro-intestinal tract induced by radiotherapy
GIL BAR-SELA1, SHARON BAUM2, HENRI TRAU2 & ABRAHAM KUTEN1
1Division of Oncology, Rambam Health Care Campus and Rappaport Faculty of Medicine, Technion-Israel Institute of Technology Haifa, Israel and 2Department of Dermatology, Sheba Medical Center and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
3. http://www.dermpedia.org/baby-dermpedia-for-beginners/pemphigus-vulgaris

Пневморахис в следствие на некротизиращ фасциит на дълбоката шийна фасция.

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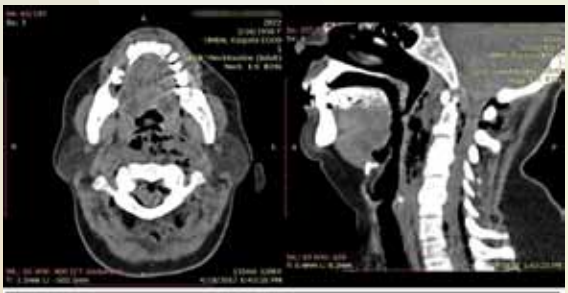
1.Клиника по УНГ- УМБАЛ " Каспела"; 2.Студент IV курс МФ към МУ-Пловдив, специалност "медицина".

ВЪВЕДЕНИЕ:

Пневморахисът представлява наличие на газ в спиналния канал. Обикновено е резултат от травма или има ятрогенна етиология. В литературата е описан един случай на пневморахис, дължащ се на сепсис, а настоящият случай е следствие на некротизиращ фасциит като усложнение на ретрофарингеален абсцес.



Фотоматериали от компютъртомографското изследване на пациентката.

Некротизиращият фасциит най-често засяга фасциите на коремната стена, перинеума и крайниците, а разположението му в цервикалния отдел представлява по-малко от 10% от случаите на съответното заболяване. Както всяка една инфекция, за възникване на заболяването е необходимо наличие на бактериален причинител. Най-честите изолирани бактериални щамове са стрептокок от група А (60 %), на второ място се нарежда Стафилококус ауреус, следвани от полимикробни инфекции, причинени от анаеробни микроорганизми.

В зависимост от причинителя, некротизиращият фасциит най-често се наблюдава при имунокомпроментирани пациенти, а засягането на здрави възрастни и деца е относително рядко явление. Често се развива при пациенти със съпътстващ захарен диабет, бъбречна недостатъчност, СПИН, чернодробна цироза, както и при болни с хематологични заболявания. За съжаление,ранната диагноза е възпрепятствана да бъде поставена поради разхвърляните оплаквания, както и от обратнопропорционалните на тежестта на заболяването некрози,клинични изяви и симптоми. Силната болка може да бъде изява на много причини, а подпухване, зачервяване и топла кожа се наблюдават при редица инфекции.

КЛИНИЧЕН СЛУЧАЙ:

Касае се за 61-годишна пациентка, с фебрилитет 39.8 ° C , остра болка в гърлото инеспособност за преглъщане на течности, храна или слюнка. Оплакванията прогресират в продължение на една седмица. Пациентката съобщава за провеждано домашно лечение с хомеопатични медикаменти. Също така пациентката е имала зловонен дъх и течаща слюнка, примесена с сиво-черен ексудат, без наличие на тризмус. Наблюдавано е зачервяване и подуване на фарингеалната стена с наличие на малка язва, от която изтича некротичен материал.

Клинични показатели:

Кръвно налягане- 120/70, сърдечна честота: 115 у/мин, WBC: 38x109 / l, glu: 26.4 mmol /l. (До момента не и е било провеждано лечение по повод диабета.)

На КТ на шия (виж снимката) се открива газова колекция в ретро- и латерофарингеалното пространство, както и в дълбоката шийна фасция, спиналния канал от C2 до Th2, субклавикуларно и по върховете на белия дроб.

Резултати: Ретрофарингеалното пространство е дренирано трансорално. Открита е некторична фасция, след което пространството е почистено с йод и разреден водороден пероксид. Латерофарингеалното пространство е дренирано външно, но там не са били открити некротични и гнойни колекции. Извършена е трахеотомия. По време на интензивното лечения е проведена терапия с Клиндамицин, Метронидазол, Меропенем, Амикацин.

Микробиологично се установява *Streptococcus pyogenes* от група А като бактериален причинител.

Хемодинамичните показатели в началото са били стабилни, пациентката - афебрилна, както и съответните симптоми на възпаление са били потиснати. След това обаче пациентката се влошава, последва хемодинамичен срив и кома 48 часа след операцията. Екзитус леталис настъпва 4 дни по-късно вследствие на полиорганна недостатъчност.

ДИСКУСИЯ:

1. Настоящият слуай засяга дълбоката фасция, диагнозата се потвърждадава от находката при резивизията на раната, и микробиологичното изследване.
2. Има наличие на предразполгащ фактор – новооткрит диабет.
3. Това, което забавя диагнозата, е, липсата на измения по кожата. (поради засягането на дълбоката фасция) Пациетката се предстявя в напреднал стадий на заболяването и екстензивни възпалителни изменения. На КТ достигащи купулите на БД, и ангажиращи спиналния канал от форамен магнум до Т2.
4. Двукратно се резивизира ретро- и латерофарингеалното пространство, но заболяването прогресира.
5. В литературата болшинство случаи засягат повърхностната фасция и кожата, независимо дали лезията е локализирана отпред на шията или отзад на тила. В конкретния случай измененията са в областта на дълбоката фасция и около гръбначния стълб, както и в спиналния канал. Това, от една страна, прави разпознаването на това тежко състояни по-трудно, а от друга- хирургичното дрениране невъзможно.
6. Макар и рядко НФ може да засегне дълбоката фасция на шията и да се имитира ретро и латерофарингеален бсцес. Агресивния характер на тази инфекция бързо ангажира спиналния канал, което се наблюдава рядко в останалите случаи на заболяването и което влошава още повече и без това лошата първоначална прогноза.

ИЗТОЧНИЦИ:

1.Pneumorachis caused by metastatic gas gangreneGeorge R. Thompson III□, George E. Crawford

Division of Infectious Diseases, Department of Internal Medicine, University of Texas Health Science Center at San Antonio,

San Antonio, TX 78229-3900, USA

Received 28 April 2008; accepted 16 August 2008

2. Cervical necrotising fasciitis with pharyngeal perforation: treatment and reconstruction

N.W. Yii; S.J. Quinn; L.C. Andersson; N.S. Niranjan; G.S. Kenyon

MOLECULAR ANALYSIS OF AZF (AZOOSPERMIA FACTOR) GENE MICRODELETIONS WITH QUADRUP REAL TIME POLYMERASE CHAIN REACTION METHOD IN INFERTILE TURKISH MALES

Gizem Koprululu , A. Ilter Guney

PURPOSE

Our study was planned to realize molecular diagnosis of Y chromosome microdeletions by Quadrupleks Real-Time Polymerase Chain Reaction method with using specific primers to the Sequence Tagged Site (STS) and by testing sensitivity of this method, to determine clinical significance of microdeletion detection in infertile Turkish male.

Our method is simple, cheap and quick and it has high confidence intervals than the other Y chromosome detection methods. In this study, we intend to adapt this method to our own routine.

METHODOLOGY

In this study, 6 infertile male were selected and examined in terms of Y chromosome AZF gene microdeletions. This study is a new generation method study, it is not population study. Our aim is to optimized this method to our laboratory conditions.For this reason, we worked with fewer patients. Peripheral blood samples were examined for Y chromosomes microdeletions by a Quadrupleks Real-Time Polymerase Chain Reaction amplification of sequence-tagged-sites (STS) of Y chromosome.

REAKSİYON A Primer adı	5'-3' sekans
SRY-F	GAATATTTCCCGCTCTCCGGA
SRY-R	CTCAAGAATGGAGCACCAGC
SRY-P	FAM-AAGCAGCTGGGATACCAGTGGAAAATGCT -BHQ1
sY86-F	GTGACACACAGACTATGCTT
sY86-R	AGGGTTGTCCCTCTGTGTGT
sY86-P	HEX-ATCAAGCTATGGCCAGGGCTGGTTCC-BHQ1
sY127-F	GGCTCACAAACGAAAAGAAA
sY127-R	CTGCAGGCAGTAATAAGGGA
sY127-P	ROX-ACTGGGAATCTACCAAAGCCCACTGTGTTCATG BHQ2
sY254-F	GGGTGTTACCAGAAGGCAAA
sY254-R	GCTGCTTTGGTAGATACGGTTC
sY254-P	CY5-TCGTGCCAAACACTGTTTTTGTTGGTGGAA-BHQ2

Table 1: Primer and Probe design for Reaction A

REAKSİYON B Primer adı	5'-3' Sekans
ZFX/Y-F	ACCRCTGTACTGACTGTGATTACAC
ZFX/Y-R	GCACYTCTTTGGTATCYGAGAAAGT
ZFX/Y-P	FAM-ACCAGCAAGGCAGAGAAGGCCATTGA-BHQ1
sY84-F	AGAAGGGTCTGAAAGCAGGT
sY84-R	GCCTACTACCTGGAGGGCTTC
sY84-P	HEX-AAGCTGGCTAACTCCTTTCAAAAGGTTTTGTCTT-BHQ1
sY134-F	GTCTGCCTCAACCATAAAACG
sY134-R	ACCACTGCCAAAACTTTCAA
sY134-P	ROXATAGATGGGGTTGATACTAAAGTTTAAAACATCTGGAACATT CTACT-BHQ2
sY255-F	GTTACAGGATTCGGCGTGAT
sY255-R	GTGGCTGCACATGACGAG
sY255-P	CY5-AGGTAGGTTTCAGTGTTTGGATTCCGCA-BHQ2

Table 2: Primer and Probe design for Reaction B

Primer and Probe Name	Lenght (bp)	Temprature (°C)
SRY-F	20	62
SRY-R	20	62
sY86-F	21	62
sY86-R	20	62
sY127-F	20	56
sY127-R	20	60
sY254-F	20	60
sY254-R	22	66
SRY	29	86
sY86	26	82
sY127	26	76
sY254	30	86

Primer and Probe Name	Lenght (bp)	Temprature (°C)
ZFX/Y	26	94
sY84	34	78
ZFX/Y-F	25	73
ZFX/Y-R	25	72
sY84-F	20	60
sY84-R	20	64
sY134-F	20	60
sY134-R	20	56
sY255-F	20	58
sY255-R	18	68

Table 3: Primer and probes Feature Tables

DNA ISOLATION

First, 6 patient's DNAs have been isolated . DNA concentrations were measured by NanoDrop 2000. Conentrations are shown in Table 4.

Patients	Concentration
A1	30.7 ng
A2	92ng
A3	44.9 ng
A4	135 ng
A5	76.1 ng
A6	33.0 ng

Table 4: Concentrations of DNAs

QUADRUPLEX REAL TIME PCR METHOD

Promega Plexor Kit was used for Real Time PCR reactions (Table5). MOPS/EDTA was used for dilution of all materials in the kit contents. It is required for the kit to work.

Quadruplex reaction includes 2 reaction which are Reaction A and Reaction B. Reactions were designed for 6 patients. In Reaction A, sY86, sY127, sY254 regions on AZF gene have been viewed for 6 infertile men (Table 1). And, in Reaction B, sY84, sY134, sY255 regions on AZF gene have been viewed for 6 infertile men (Table 2).

BioRad CFX was used for this method. PCR method is shown Table 6. The present method has been optimized to laboratory conditions.

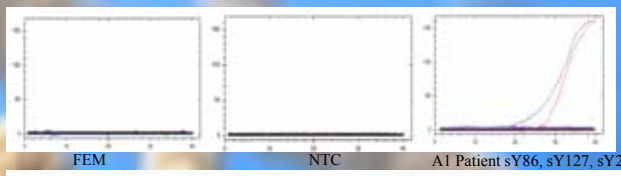
Reaction Mix	Quantity (µl)
Nuclease Free Water	6.5
Forward Primers	0.5
Reverse Primers	0.5
Sample DNA	4.0
Plexor Master Mix, 2X	12.5
Total Volume	25 µl

Table 5: Quadruplex Real Time PCR PCR Mix content

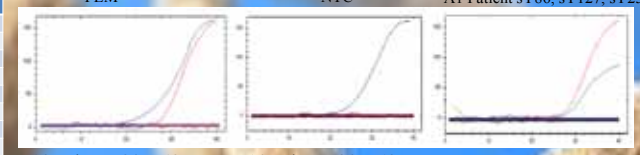
Process	Temprature	Time
First Denaturation	95°C	3 min.
Denaturation	95°C	5 sec.
Annealing	62°C	35 sec.
Elongation	72°C	5 min.

Table 6: Quadruplex Real Time Process

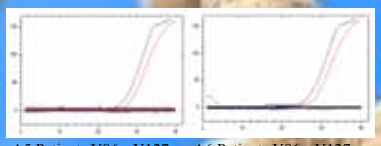
REACTION A



FEM NTC A1 Patient sY86, sY127, sY254

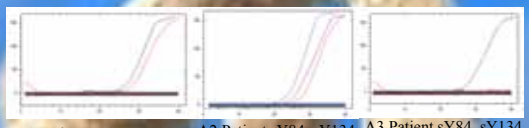


A2 Patient sY86, sY127, sY254 A3 Patient sY86, sY127, sY254 A4 Patient sY86, sY127, sY254

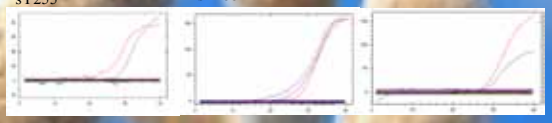


A5 Patient sY86, sY127, sY254 A6 Patient sY86, sY127, sY254

REACTION B



A1 Patient sY84, sY134, sY255 A2 Patient sY84, sY134, sY255 A3 Patient sY84, sY134, sY255



A4 Patient sY84, sY134, sY255 A5 Patient sY84, sY134, sY255 A6 Patient sY84, sY134, sY255

Pt. No	sY84	sY86	sY127	sY134	sY254	sY255
A1	37.4	37.6	26.0	23.1	38.9	20.3
A2	N/A	39.8	26.2	26.5	N/A	23.2
A3	N/A	N/A	39.8	38.7	N/A	N/A
A4	33.7	N/A	22.7	22.5	N/A	N/A
A5	N/A	N/A	25.4	25.2	N/A	N/A
A6	N/A	N/A	25.8	27.8	35.1	N/A

Table 7 : CT value table for six patients

RESULTS

This 6 infertile male patients have Y chromosome microdeletion on different regions of AZF gene. Patients with a CT value greater than 30 and N/A value have AZF gen microdeletions (Table 7).

A1 and A2 patients have AZFa microdeletion. AZFa microdeletions can cause the maturation arrest.

A3 patient has AZFa+b+c microdeletion. This type is associated with azoospermia.

A4, A5 and A6 patients have AZFa+c microdeletion. AZFc microdeletion can cause oligospermia, Sertoli Cell-Only Syndrome (SCOS). In this phenotype, girls can reproduce normally, while the boys are born vicious.

In this study, we used Quadruplex Real-Time PCR method to show Y chromosome microdeletion detection in a high confidence interval. We were able to optimized this method to laboratory conditions.

In light of my reflections as a result of the study, the method is fast, simple analysis with high precision and without the risk of contamination have reached the conclusion that we did.

We will try more number of patients with this method and we will expect tis method to work with the

CLINICAL AND GENETIC SPECTRUM OF LIMB-GIRDLE MUSCULAR DYSTROPHIES (LGMD) IN BULGARIA

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Introduction

Limb-girdle muscular dystrophies (LGMD) are heterogeneous group of genetically determined progressive disorders of skeletal muscles with primary or predominant involvement of the pelvic or shoulder-girdle musculature. To date over 50 genetic loci, related to LGMD have been identified.

Objective

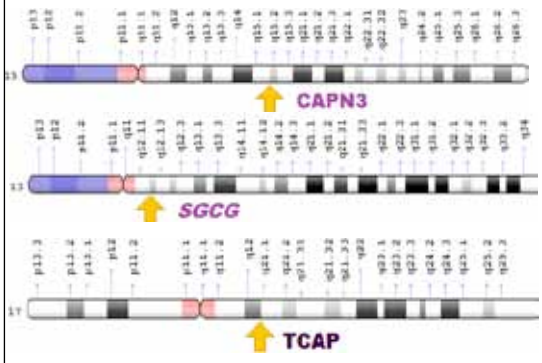
To determine the genetic and clinical spectrum of LGMD in the Bulgarian population.

Material and methods

One hundred and three affected individuals with LGMD were genetically verified. All the patients underwent clinical examination, including manual muscular testing (MRC), electromyography (EMG), Serum creatine kinase levels (CK) were measured in all of them. Respiratory (ventilator assessment) and cardiac (electrocardiography- ECG and echocardiography- echoCG) functions were tested. Magnetic resonance imaging (MRI) of the muscles of the lower limbs in was performed in 9 patients.

Results

One hundred and three patients with 6 sub-types of LGMD were diagnosed. The most common type was LGMD2C due to Cys283Tyr mutation in *Gamma-sarcoglycan gene* (founder for 57 Gypsy/Roma patients);The second most frequent was LGMD2A due to mutations in the *CAPN3* gene –found in 25 patients. 88% of them are homozygous for the550delA mutation. The third most frequent form was LGMD2G, with mutation in the *TCAP gene*- 17 patients, 16 of whom were homozygous for the c.75G>A, p.Trp25*mutation. They belonged to the religious minority of Bulgarian Muslims. Three patients with LGMD2Z due to mutation in the *POGLUT1 gene*, one patient with LGMD2L- due to mutation in *ANO5 gene*, and one with LGMD 2B, due to mutation in *DYSF gene*.The clinical spectrum is broad in terms of age at onset, rate of progression and age of loss of ambulation. The initial manifestations in most of the patients include proximal muscle weakness in the lower limbs, followed by involvement of the upper limbs (LGMD2A) or distal weakness in the legs (LGMD 2G). The age at onset is ranging between 5 (LGMD 2C with Duchenne-like progressive course), and around 50 years (LGMD 2L with very slow progression). Pulmonary and cardiac functions were affected later in the course of the disease only in few cases with LGMD2A.



Conclusion

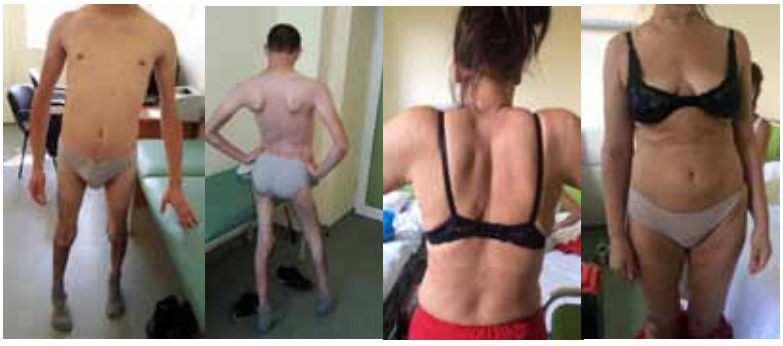
Our study present clinical and genetic variety of LGMD sub-types with autosomal recessive pattern of inheritance in Bulgaria. Several worldwide rare types of LGMD were determined in the country. While diagnosing such patients, it is necessary to consider the patients' ethnicity.

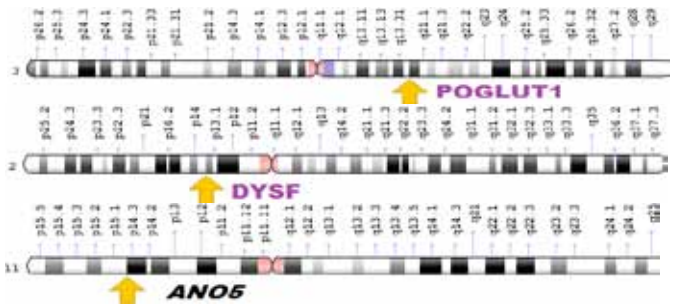
Table 1: Clinical and genetic presentation of the 6 sub-types LGMD in Bulgaria.

LGMD type	2C	2A	2G	2Z	2L	2B
genetic findings	Cys283Tyr mutation in *Gamma-sarcoglycan gene* located at 13. chromosome.	88% homozygous for the550delA mutation, 12% homozygous for c.1811_1812 delTC mutation in CAPN3 gene, located at 15. chromosome.	96% are homozygous for the c.75G>A, p.Trp25*mutation, 4 % (1 person) compound heterozygous, with second mutation in exon 2 of *TCAP (at 17. chromosome)*	homozygous for c.292C>T, p.Arg98Trp mutation in POGLUT1 gene, located at 3.chromosome	Compound heterozygous for c.1180+6t>C and c.1520delT,p.Phe507SerfsTer6 mutations in *ANO5* gene, located at 2.chromosome	Mutation in *DYSF gene , located at 11. chromosome*
ethnicity /religion	Gypsy/Roma	84% Bulgarian; 16% - Bulgarian muslims	Bulgarian Muslims (religious minority)	Bulgarian	Bulgarian	Bulgarian
age of onset (years)	Around 5	Around 14	Around 18	Around 25	Around 35	Around 26
clinical manifestation	Duchenne- like weakness, with additional involvement of the periscapular muscles causing scapular winging , with calf hypertrophy .	Proximal shoulder and pelvic girdle weakness with initial difficulty in standing from sitting position, with later involvement of the distal limb and axial muscles.	Proximal weakness in lower limbs, accompanied or followed by weakness of m. tibialis anterior, and later involvement of the upper limb muscles.	Proximal shoulder and pelvic girdle weakness with initial difficulty in standing from sitting position.	Late onset proximal lower limb weakness, with additional involvement of the shoulder limb muscles, with slow progression.	Initial proximal and axial muscle weakness . proximal parts of the arms becoming weak a long after.
CPK values	5 to 30 times above the norm	2 to 5 times above the norm	2 to 25 times above the norm ..	Normal	Over 5 times above the norm	around 50 times above the norm
cardiac and respiratory functions	Spared	Affected later in the course of disease	Spared	Spared	Spared	Spared
loss of ambulation	52.5% before 13, 27.5% between 13 and 16 years of age.	One patient around 32.	Around 34-40 years of age.	Walking is retained after 20 years disease progression.	Walking is retained.	Around 54 years of age.

Fig.1.Patient with LGMD 2G in advanced stage.

Fig.2. Patient with LGMD 2Z.





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Клиничен случай на пациент със синдром на Arnold-Chiari

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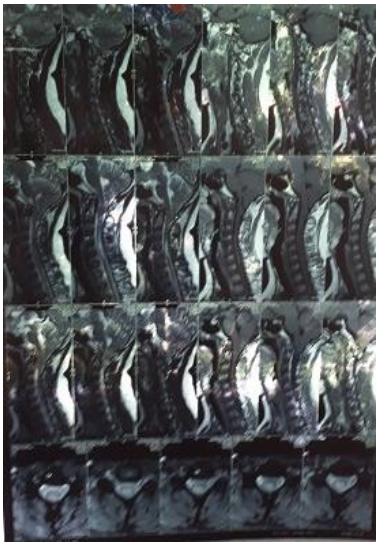
ВЪВЕДЕНИЕ:

Синдромът на Arnold-Chiari е рядко срещано заболяване с честота 1:1000 и представлява вродена малформация на мозъка [1]. Характеризира се с удължаване и протрузия на малкомозъчните тонзили през foramen occipitale magnum към шийния гръбначен мозък. Различават се 4 основни типа на синдрома. Те са кръстени на Hans Chiari, патологът, който пръв описва цялата група малформации [2]. Arnold - Chiari тип I малформация е най-честата и най-слабата от спектъра, често диагностицирана в зряла възраст. Прекъсването на нормалния поток на цереброспиналната течност (ЦСТ) през региона предизвиква клиничния синдром.

Симптоми при хора с Arnold-Chiari малформация тип I:

1. **Тежко главоболие - класически симптом, появява се след внезапна кашлица, кихане или напрежение;**
2. **Болка в шията;**
3. **Нестабилна походка (проблеми с равновесието);**
4. **Лоша координация на ръцете (фини двигателни умения);**
5. **Изтръпване на ръцете и краката;**
6. **Виене на свят;**
7. **Затруднено преглъщане, понякога придружено от зачервяване, задушаване и повръщане;**
8. **Проблеми със зрението (замъглено или двойно виждане);**
9. **Проблеми с речта, дрезгавост** [3].



Фиг. 1. МРТ на глава и шия от 2008 г.

КЛИНИЧЕН СЛУЧАЙ:

Представяме рядък случай на 25-годишен пациент със синдром на Arnold-Chiari - тип I (ORPHA:268882).

Начало: 16-годишна възраст

Диагноза:

1. Симптоми на заболяването (остри болки във врата, често придружени от главоболие, а понякога и с ирадиация към лявото рамо), без данни за предишна инфекция;
2. Физикален преглед и локален неврологичен статус: апсихозен, с лека ригидност в шийния сегмент на гръбначния мозък, без органична неврологична симптоматика;
3. МРТ скен на глава и шия: синдром на Arnold-Chiari с подчертано инкарцериране на малкомозъчните тонзили под foramen magnum и дъгата на C1; сирингомиелична кухина на нивото от C3 до C6; увеличен над граничните размери обем на хипофизата.

Параклинични данни:

Кръвна картина: левкоцитен брой – $7.3x10^9$/L; еритроцитен брой – $5.27x10^{12}$/L; HGB – 147g/L; HCT – 0.429 L/L; тромбоцитен брой – $351x10^9$/L;
Биохимия: кр. захар – 5.3 mmol/L ; урея – 4.7 mmol/L; креатинин – 64 μmol/L; K – 4.0 mmol/L; Na – 140 mmol/L; Cl – 101 mmol/L; АСАТ – 23 IU/l; АЛАТ – 29 IU/l;

Лечение: През 2008г. е насочен за операция след предварителна консултация с интернист и детски хирург. Извършена е субокципитална краниотомия, включваща и отстраняване на foramen magnum и ламинектомия на C1.

Следоперативен и възстановителен период: Протичат гладко и с подобрение. На контролно МРТ малкомозъчните тонзили не пролабират през големия тилен отвор каудално, няма компресия и влошаване на ликворния поток, който е значително подобрен. За това свидетелства редукцията на сирингомиелията.

ДИФЕРЕНЦИАЛНА ДИАГНОЗА:

Друг тип на Arnold-Chiari малформация:

- Chiari I малформацията няма миеломенингоцеле;
- Chiari III има тилно и / или високо цервикално енцефалоцеле.
- Chiari IV има тежка церебеларна хипоплазия без изместване на малкия мозък през форамен магнум [4].

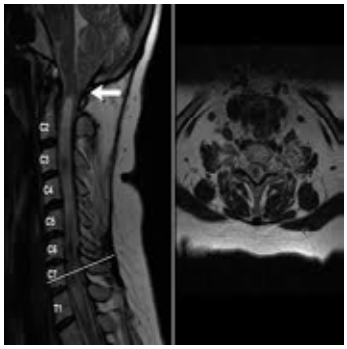
ОБСЪЖДАНЕ:

Няма данни за честотата на заболяването в България, както и регистър на пациенти с Arnold-Chiari. Последните проучвания са идентифицирали генни мутации в PI3K-AKT (Phosphatidylinositol-4,5-bisphosphate 3-kinase-Protein kinase B) сигнализиращия път, които могат да са причина за хидроцефалия, Arnold-Chiari и други мозъчни нарушения [5].

Причини за синдрома:

- Структурни дефекти на мозъка, липса на нутриенти от майката при феталното развитие
- По-рядка е появата след: контузия, инфекция, излагане на токсични вещества

Тип I е единственият тип малформация, която може да бъде придобита [6].



Фиг. 2. МРТ на глава и шия показващ Arnold-Chiari I

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Фамилен случай на псевдохипопаратиреоидизъм тип Ib

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УВОД:

Псевдохипопаратиреоидизмът (PHP) е хетерогенна група заболявания в резултат на резистентност към биологичното действие на паратхормона.
PHP исторически е първият синдром на хормонална резистентност, описан през 1942 г. от Fuller Albright. Той е предложил и термина „псевдохипопаратиреоидизъм“, за да опише пациентите, които са имали PTH-резистентна хипоСа и хиперР заедно с необичайна констелация от дефекти в развитието и скелета, наречена Хередитарна остеодистрофия на Albright.
Честота - 0,79 на 100 000 (доклад на Orphanet Series, ноември 2008 г.).
Възрастта на изява варира от детска до старческа с различна клиника-на хипокалциемия, гърчове, когнитивни нарушения, екстрапирамидна симптоматика, психоза. Това често затруднява диагнозата.

ГЕНЕТИКА:

Рецепторът за PTH е от групата на G-протеин свързаните рецептори. Такива са и рецепторите за TSH, GHRH, LH, FSH. Мутация в GNAS гена кодиращ $G\alpha_s$, разположен в 20q13.2. ***Схема 1.***

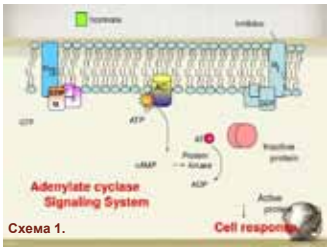


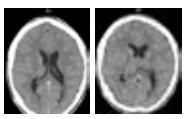
Схема 1.

Роля на геномен импринтинг - в някои тъкани е експресиран само майчиния алел на гена /бъбреци, хипфофиза/, а в други и двата /кости/.
Различна изява в зависимост от това дали е мутирал майчиния или бащиния алел на гена, което определя различните форми на PHP.

PHP	Костни промени	хипоСа	Ендокринен дефект	Генетика
Ia	Наследствена остеодистрофия на Олбрайт /АНО фенотип/	Да	Мултихормонална резистентност	Майчина инактивираща мутация в GNAS гена водеща до намалена функция на $G\alpha_s$
Псевдопсевдо-хипопара-тиреоидизъм	АНО фенотип	Не	Не	Бащина мутация
Ib	Брахидактилия при част от пациентите	Да	Резистентност към ПХ, частична резистентност към ТСХ при някои от случаите	АД-Делеция на STX16, NESP55 екзони или нарушения в метилирането на майчиния алел Спорадичен-бащина унипарентна дизомия или не се открива дефект
Ic	АНО фенотип	Да	Мултихормонална резистентност	Майчина инактивираща мутация в GNAS гена –нарушаваща свързването с аденилатциклазата на рецептора.
II	Не	Да	Резистентност към ПХ+вит Д дефицит	няма

КЛИНИЧЕН СЛУЧАЙ

- Момче на 14 години родено от първа бременност, протекла с контракции в 4 и 5 л.м.
- Проходил на 1г. 3м., сричкува от 1г.в.; говори от 5г.в.
- На 22.05, след прибиране от училище с оплаквания от главоболие - челно, стягащо, придружено от лек световъртеж. Вечерта с ГТКП с продължителност около 10 минути.
- Постъпва в задоволително общо състояние. Хиперпигментни петна в областта на дясна скапула и ляв среден коремен квадрант. ССС - РСД, ясни тонове, СЧ-80/мин, АН-110/60 mmHg. Нормален неврологичен статус.
- Дикретен лицев дисморфизъм. Малки длани с къси пръсти.
- Без прояви на латентна тетания.
- Нормални антропометрични показатели: Т-58 кг, Р-167 см, BMI-20.8 кг/м^2
- Стартирал пубертет
- Лош зъбен статус
- КТ на Глава:



Набелязани калциеви депозити фронтално, субкортикално, в базалните ядра, плексус хороидеус, гл. пинеалис.

- Рентгенография на китка:

Двустранно симетрично наличие на релативна хипоплазия на проксимална диафиза и основите на 4-ти метакарпални кости.

- ЕЕГ - Нормална основна активност. Генерализирани пароксизмални прояви от комплекси пика-бавна вълна 3 /сек.
- Консултация с психолог - Към момента функционира като дете с лека умствена изостаналост, IQ - 68 и дефицит в краткосрочната вербална памет. Затруднения в абстрактно-логическите операции.

По-малък брат на 6 г.в. от втора нормално протекла бременност и раждане, с тегло 3700 гр. Гладко протекъл неонатален период.

- Установена луксация на ТБС по повод, на което е бил с ортопедични гащета.
- Проходил на 1 год. 6 мес. възраст. Късно проговорил, като в момента казва не повече от 10 думи.
- Контролира микция, но не и дефекация.
- Обективно-Дискретен дисморфизъм - окръглено лице, къс нос, малки длани с по-къси пръсти. Бял дроб и ССС - б.о. Проби за латентна тетания - отр.
- Антропометрични показатели – ръст-124 см, тегло-33 кг - 25% над-нормено тегло за неговият ръст.
- Консултация с психолог - тежко УИ и аутистични прояви.

Майка - поставена диагноза Епилепсия на 10 год. възраст. Лекувана неизвестно докога. Неколкократно е регистриран ниско ниво на калций, но допълнителни изследвания не са правени.

	Калций	Йонизиран калций	Фосфор /1-1.8/	Алкална фосфатаза	Паратхормон /11-67/
Майка	1.34	0.56	2.02	98	322
Син на 14 г.в.	1.68 калциурия – 0.001 мг/кг/24 ч	0.7	2.87	369	437
Син на 6 г.в.	2.4	1.24	1.88	203	237

Диагноза: Псевдохипопаратиреоидизъм тип Ib-АД форма
Изчаква се резултатът от скрининга за микроделеции.

ОБСЪЖДАНЕ:

PHP тип Ib е по-рядката форма на едно рядко заболяване. За разлика от PHP тип Ia и Ic, тук костните промени са дискретни което затруднява диагнозата. Клиничната изява варира от гърчове водещо често до погрешна диагноза Епилепсия, през по-дискретна изява като когнитивни нарушения и латентна тетания, до психотични прояви при по-възрастни пациенти и поставяне на психиатрични диагнози.

Заболяването най-често се диагностицира около и след 10 г.в., което е и причината за липсата на хипокалциемия при по-малкото дете, но вече има завишен Паратхормон и горнограничен фосфор. Проследяването му в следващите години и своевременно започване на лечение с активен метаболит на вит. Д ще предотврати острата симптоматика наблюдавана при другите двама засегнати членове в семейството.

Целта на лечението на PHP е не само нормализиране на калциемията, което ще доведе до редуциране или пълно обратно развитие на симптоматиката, но и до нормализиране нивото на паратхормона. Това е особено важна при PHP тип Ib, където рецепторната резистентност е на ниво бъбреци, но не и на ниво кости. Задържането на висок паратхормон в случая ще доведе до развитие на остеопороза. При всички форми на PHP има риск от третичен хиперпаратиреоидизъм. Рискът от нефрокалциноза при PHP е нисък за разлика от хипопаратиреоидизма. Това е така защото рецепторната резистентност е на ниво проксимален тубул, докато дисталният не е засегнат.

ИЗВОДИ:

При всеки пациент с когнитивни нарушения, екстрапирамидна симптоматика, тикове и психотични прояви е уместно изследване на калциево-фосфорната обмяна.
Лечението на PHP е с активни метаболити на вит.Д с или без калциев препарат в доза достатъчна не само за нормализиране на калциемията, но и за поддържане на нива на паратхормон нормални или максимално близо до нормата без риск от нефрокалциноза.

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Пилотно изследване на феталния мозък чрез функционално магнитно-резонансна томография

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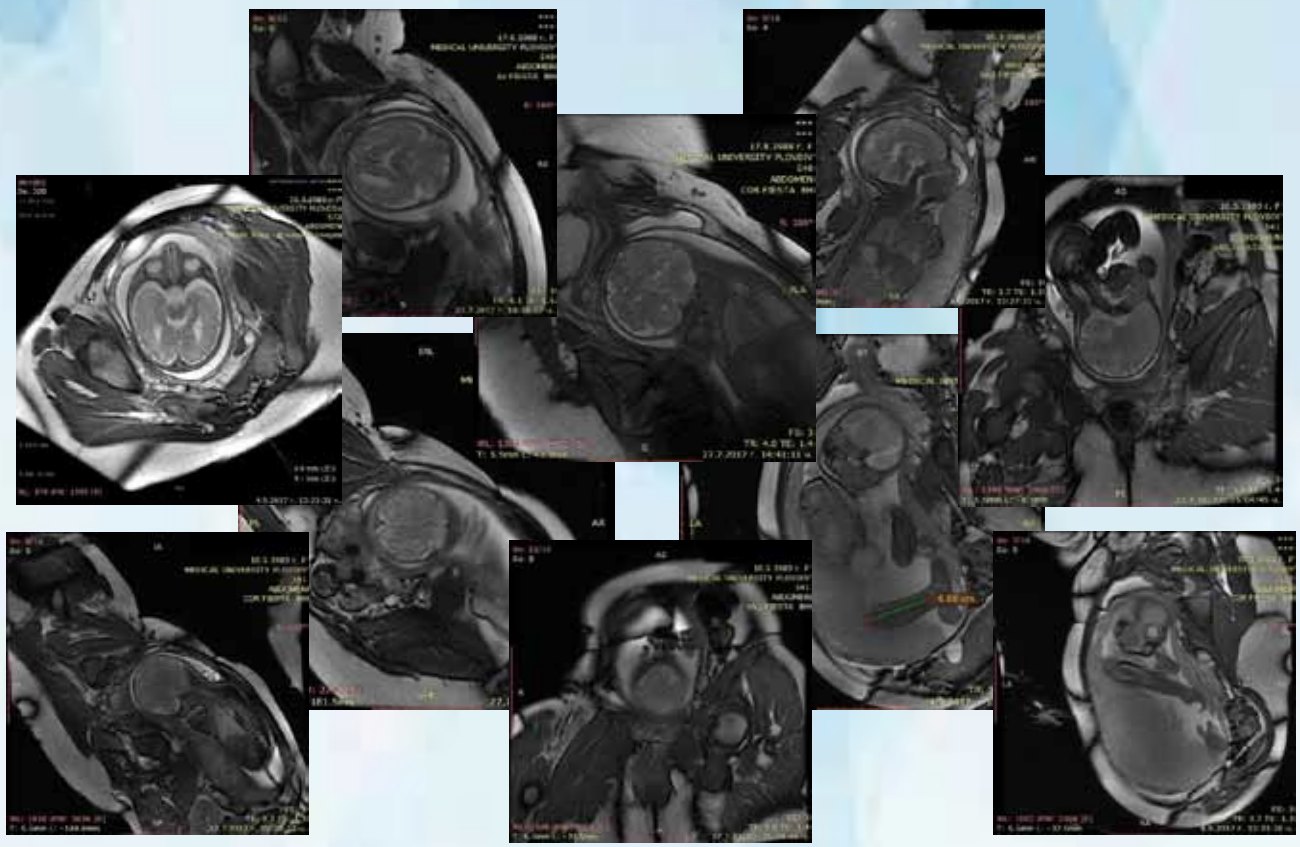
Магнитно-резонансната томография (МРТ) представлява неинвазивна компютър-асистирана изобразяваща техника. МРТ се базира на измерването на ефекта на ядрения магнитен резонанс на водородния атом, който се съдържа в различните тъкани. Тя използва силно магнитно поле, в съчетание с прилагането на радиочестотни импулси, за да генерира двуизмерни и триизмерни образи. Функционална МРТ е образно изследване, за което няма данни за увреждащо действие върху плода, но все още не е въведено като рутинно изследване на мозъка в клиничната практика. Изследването се провежда, когато резултатите от стандартните диагностични методи, като пренатално ехографско изследване и пренатален биохимичен скрининг не са достатъчно информативни за изследване крайната пренатална диагноза.

ЦЕЛ

Да бъдат представени резултати от пилотно проучване на феталния мозък с функционален МРТ по време на бременността.

МАТЕРИАЛ И МЕТОД

Изследването е проведено в Комплекс по Транслационна Невронаука към Медицински университет Пловдив, чрез Транслационно невроизобразяване с 3 Тесла ядрено-магнитен резонанс. Напревен е МРТ на три жени по време на 8 л.м. от бременността с цел търсене на патологични находки на различни органи и по-специално проследяване морфологията на развиващия се мозък. Изследвани са биометрични показатели - фронто-окципитален и бипариетален диаметър, напречен диаметър на малкия мозък, морфология на черепните и лицеви кости, гирификация на мозъка, вентрикуларна система, пространства, развитие на Corpus callosum, големина и фолиация на церебелума, състояние на нухалната гънка, сепарация на голямо мозъчните хемисфери, обем на задна черпна яма. Ширината на вентрикула е измерена в основата на Cornu occipitale.



ЗАКЛЮЧЕНИЕ

Магнитно-резонансната томография е от изключително значение за окончателната пренатална диагноза и за изхода на бременността. При суспектни бременности трябва да бъде задължителна част от мултидисциплинарните изследвания.

Екзацербация на болест на Whipple в резултат от лечение с метотрексат.

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ВЪВЕДЕНИЕ

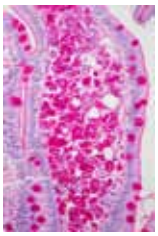
Болестта на *Whipple* е рядка, хронична, системна инфекция, **причинена** от *Tropheryma whipplei*, грам-положителен вътреклетъчен бацил. Засяга Мъже на средна възраст.
Може често да се **прояви** като хроничен серонегативен олигоартрит или полиартрит, които могат да имитират различни ставни заболявания (ревматоиден артрит или спондилоартрит).
Най-честите **симптоми** са: Диария, Малабсорбция, Фебрилни състояния, Артрит, ЦНС-симптоми, Лимфаденомегалия. Специфичен симптом от страна на Кардиоваскуларната система е културелно негативен ендокардит.
Лечението на серонегативен ревматоиден артрит с anti TNF alpha води до екзацербация на Болеста на Whipple. При не навременното лечение Болеста на Whipple може да доведе до екзитус леталис.

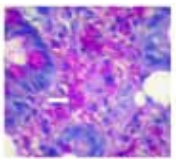
КЛИНИЧЕН СЛУЧАЙ

Касае се за мъж на 68 години. Получава диарийни дефекации от 2 седмици след прием на метотрексат. Наблюдавана е редукция на 7 килограма за 3 месеца. От 2010г. е с болки в ставите и периодично фебрилитет, понякога и до 40 °C. Хоспитализиран в клиника по ревматология кадето е приета Дг.Ревматоиден артрит, RF отрицателен. Запланувал за лечение с anti TNF-alpha. Придружаващи заболявания са: ХУХК, болест на Крон, Глутенова ентеропатия и СА на дебело черво.

Клиничен ход на заболяването

	Първа хоспитализация	Трета седмица от лечението	Един месец след дехоспитализация	6-ти месец от лечението	11 месец от лечението
Статус		Подобрение в локалния статус, афебрилен и без диарийни дефекации	Без ставни болки, фебрилитети и диария	Без ставни болки, фебрилитети и диария	добър клиничен и хистологичен отговор Следваща хоспитализация на 12 месец от лечението
Лаборатория	Анеминчен синдром Хгб – 100г/л; Fe – 5.5µmol/L Хипопротеинемия – общ белтък – 58;албумин-29 -завишени острофазови белтъци – СУЕ - 100mm/h, CRP – 135 Туморни маркери – в референтен интервал		В референтен интервал	В референтен интервал	
ВГС	дифузна чревна лимфангиектазия		Подобрение: нормална ендоскопска находка	Нормална ендоскопска находка	
ВКС	Нормална ендоскопска находка				
Други инструментални изследвания	УЗД – ХСМ, хемангиом на черен дроб				
Хистологичен отговор	в серийно изследвани фрагменти на тънкочревна мукоза включваща и мускулната лигавична пластинка, в ламина проприя са налице множество макрофаги чието съдържимо е PAS – позитивнии и диастаза резистентно. Установява се и мастни вакуоли.Находката насочва към болест на Whipple		Два фрагмента от тънкочревна лигавица, в единия от които се намира леко изразена хронична възпалителна реакция, а в другия - и наличие на пенести макрофаги, което е в корелация, при съответни клинични данни, с обсервираната диагноза.	Дванадесетопръстник със задабелени вси, леко скъсени, криптите са стеснени. Наличие на кръглоклетъчна възпалителна инфилтрация. При оцветяване с PAS реакция се виждат единични PAS позитивни гранули. При клинична находка хистологичната картина може да се интирпретира като болест на Wipple.	
Назначена терапия	Цефтриаксон 2г. i.v. за 2 седмици Sulfamethoxazole-trimethoprim (Бисептол) 80/400мг 2x2 tab per os			Продължава с приема на sulfamethoxazole-trimethoprim (Бисептол) 80/400мг 2x2 таб пер ос	





ЗАКЛЮЧЕНИЕ

Болеста на Whipple трябва да се подозира при всички пациенти, диагностицирани със серонегативен ревматоиден артрит, частично контролиран или неконтролиран с *antiTNF alpha*, чието състояние се влошава след лечението

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ФАРМАКОГЕНОМИКА НА АНТИХИПЕРТЕНЗИВНАТА ТЕРАПИЯ

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Хипертонията е глобален здравен проблем и важен модифицируем рисков фактор за развитието на сърдечно-съдови заболявания. Въпреки използването на лекарства, атакуващи различни патогенетични звена, контролът на хипертонията остава далеч под желаното ниво. Фармакогеномиката е подход, който може да подобри резултатите чрез въвеждане на генетични биомаркери за персонализирани и прецизирани терапевтични стратегии.

Настоящият обзор обобщава наличните данни за генетичните сигнали, свързани с терапевтичния отговор и нежеланите последствия от приложението на основните групи антихипертензивните лекарства.

Сред най-изследваните са гените за компонентите на ренин-ангиотензиновата система (RAS) – ренин, ангиотензиноген (AGT) с водещ вариант M235T, ангиотензин-конвертиращ ензим (ACE) с фокус върху вариациите инсерция/делеция (I/D), ангиотензин-1 (AT1) рецептори с два хаплотипа, свързани с терапията (H2, H3), ACE2 от „алтернативната” RAS и др. Тествано е влиянието на генните полиморфизми върху ефектите на ACE-инхибиторите и AT1-блокерите. Резултатите са интересни, но противоречиви и се нуждаят от доизясняване.

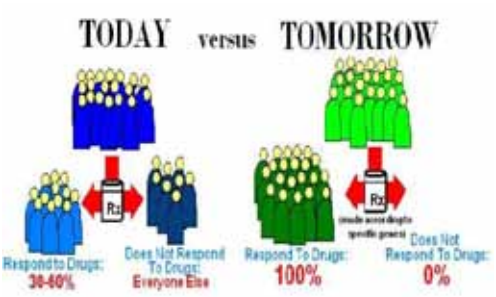
Понастоящем вниманието е привлечено от два гена, доказано свързани с ефектите на конкретни антихипертензивни лекарства - NEDD4L с тиазидните диуретици и ADRB1 с бета-блокерите, но все още няма готовност за широкото им практическо използване.

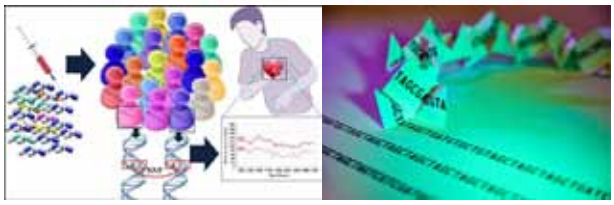
NEDD4L кодира белтък, свързан с натриевия транспорт в бъбрека (фиг.1). Генетичният полиморфизъм rs4149601G>A води до намалена отрицателна регулация на епителния натриев канал, повишена натриева задръжка, хипертония и повишен сърдечно-съдов риск. Има клинични доказателства, че такива пациенти се повлияват добре от тиазиди.

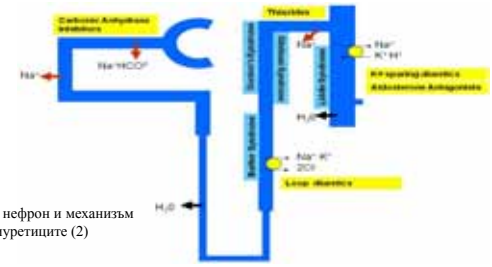
ADRB1 кодира бета1-адренергичния рецептор, който медиира физиологичния отговор към норадреналин и адреналин и е първичен таргет на бета-адренергичните блокери (фиг.2). Описани са два функционални полиморфизми, резултиращи във вариантите Ser49Gly и Arg89Gly, които осъществяват повишена нисходяща сигнализация от бета-рецептора чрез вторичния посредник аденилатциклаза. Те са свързани с развитието на хипертония, но също така и с по-изразен антихипертензивен ефект на бета-блокерите и с по-благоприятни резултати от прилагането им при предсърдно мъждене, камерни аритмии, сърдечна недостатъчност.

Фармакогеномиката на калциевите антагонисти и алфа-блокерите е много малко изследвана.

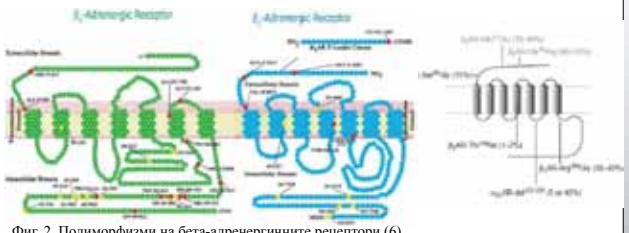
В кардиологията има генетично-базирани насоки за лечение с клопидогрел, статини, варфарин. За хипертонията такива за сега липсват. Този процес вече се ускорява чрез създаване на консорциуми между изследователските групи. С оглед недостатъчно големия самостоятелен ефект на отделните полиморфизми се работи върху дефинирането на комбинации от варианти за транслиране .







Фиг. 1. Схема на нефрон и механизъм на действие на диуретиците (2)



Фиг. 2. Полиморфизми на бета-адренергичните рецептори (6)

В обозримо бъдеще фармакогеномиката и други „-omics” подходи (metabolomics, transcriptomics) ще създадат възможности за по-добра превенция и оптимизирана терапия на хипертонията при минимален риск от нежелани реакции.

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Survey about Bulgarian pharmacists' opinion on the possibilities for implementation of pharmaceutical care for patients with rare diseases – a pilot study

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The current study is financed by Medical University-Sofia (Project: "Study of quality of life, ambulatory therapy of patients with rare diseases in Bulgaria as well as their access to treatment and to pharmaceutical care" Number 42/406/19.01.2016)

I. Introduction

Rare diseases are life-threating, seriously debilitating or serious chronic conditions affecting a very small number of patients. [1]

Pharmaceutical care is a complex of knowledge and skills applied by the pharmacists during the provision of drug therapy. [2]

Pharmaceutical care provides closely **monitoring**, timely **consultation** and **prevention** of various dangerous drug related problems for the patients with rare diseases. [3]

The specialist pharmacists can review patients' medication history in order to prevent the potential for serious adverse drug interactions, adverse drug reactions, wrong medicines. [4, 5]

A Serbian study (2014) concluded that the years of experience and age of the pharmacists do not influence their knowledge about rare diseases. The training programs are needed. [6 7]

II. Goal

The goal is to assess the pharmacists' opinion on the possibilities for implementation of pharmaceutical care for patients with rare diseases in Bulgaria.

III. Methods

- ✓ A pilot study was conducted among master pharmacists working in community pharmacies in Sofia;
- ✓ The survey included questions about:

1. *The **possibilities for implementation of pharmaceutical care (PhC)** for patients with rare diseases (RDs);*
2. ***The expected benefits** of implementation of PhC for patients with RDs;*
3. ***Willingness to pay for** PhC;*
4. ***The level of qualification of the pharmacists** in Bulgaria in the area of RDs;*

- ✓ **Descriptive statistics** has been applied using the statistical program STATISTICA Version 13.

IV. Results

- ✓ **48 master pharmacists** practicing in pharmacies serving the population **in Sofia** were interviewed;
- ✓ 88% of the respondents were women, with the predominance between 25-40 years old (47%), followed by 40-50 year (29%);

The possibilities for implementation of PhC

- ✓ 20% responded that patients with RD never ask for consultation and 75% said that giving consultations was rare;
- ✓ The most frequent questions towards the pharmacists were about drug therapy (60.42%) and legislative and administrative procedures (31.25%);
- ✓ **The main problems** were difficult communication (40%), lack of time and space (79.2%) and insufficient qualified staff in the pharmacy (66.6%).

The expected benefits

- ✓ Improving the **quality of life** of patients with RD (87.5%);
- ✓ **Increasing the confidence** in the pharmacist (81.3%);
- ✓ **Financial revenues** for the community pharmacies (21%).

The willingness to pay for PhC

- ✓ 30% think it is not necessary extra money for pharmaceutical care to be paid;
- ✓ 56.24% indicate an amount of over 5 euro per one consulted patient.

The level of qualification of the pharmacists

- ✓ **More than half (52%) were familiar** with orphan medicines definition;
- ✓ **89.6%** were **familiar with the pharmaceutical care concept** and more than 70% would invest in its implementation in the practice;
- ✓ Statistically significant more master pharmacists (98% versus 2%, $p < 0.05$) shared that they **do not have sufficient knowledge about rare diseases**;
- ✓ **96% were interested in continuing education** regarding rare diseases.

V. Conclusion

- ✓ The master pharmacists are the most accessible healthcare professionals who have the necessary knowledge and willingness to participate effectively in the overall care of patients with RD;
- ✓ The possibilities for implementation of PhC include conduction of ongoing trainings among the pharmacists and optimization of the duties of the pharmacists in the community pharmacy with a focus on the individualized patient care.

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Survey about the rare disease patients' attitude towards the quality of pharmaceutical service in Bulgaria – a pilot study

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The current study is financed by Medical University-Sofia (Project: "Study of quality of life, ambulatory therapy of patients with rare diseases in Bulgaria as well as their access to treatment and to pharmaceutical care" Number 42/406/19.01.2016)

I. Introduction

The patients with rare diseases (RDs) deserve the same access to **valuable medical information** as the other ones. [1, 2] The pharmacists are the most accessible medical specialist who can play a crucial role in ensuring relevant medical information.

Cross sectional survey assess whether patients with RDs use pharmacists for medical information. [3]

Only 41.4% of the patients perceived pharmacists as a credible source of medication information in comparison with physicians and Internet[3]

Both **dimensions of pharmaceutical services** are equally important and should be assessed: pharmacotherapy and socio-economic aspect. [4]

Nowadays **the quality of pharmaceutical service is crucial** and it should be periodically assessed and modified in order to satisfy the patients' needs. [5]

Provision of **pharmaceutical care** is an important element of the modern pharmacy practice.[5]

The quality of pharmaceutical services is a basis for building a strong relationship with patients with rare diseases who need specific care. [6]

Not only physical and financial access to treatment should be provided, but also highly professional medical advice and information about the diseases and treatment regimens.

II. Goal

The goal is to assess the level of satisfaction of patients with rare diseases (RDs) with the pharmaceutical services and their attitude towards the application of pharmaceutical care (PhC) in Bulgaria.

III. Methods

- ✓ **A pilot survey questionnaire** was conducted among patients diagnosed with RDs in Sofia;
- ✓ The questions are about patients' illness, their therapy satisfaction, the access to treatment, the accessibility and quality of pharmaceutical services;
- ✓ **Descriptive statistics** has been applied using the statistical program STATISTICA Version 13.

IV. Results

- ✓ 34 patients with RDs were included in the survey;
- ✓ 28 patients were with acromegaly, 5 with Cushing's disease and 1 with Pompe disease;
- ✓ The predominant respondents were female (74%);
- ✓ Mean time to diagnosis is 2.07 years (SD = 2.59), as the longest period was reported by the patient with Pompe disease - 11 years;
- ✓ **High level of satisfaction with the prescribed therapy** is indicated by about **57%** of the patients which demonstrates the effectiveness of drug treatment in real conditions;
- ✓ **Administrative obstacles** in obtaining the prescribed medication have been reported **by only 11% of respondents**, which confirms the adequate access to pharmacotherapy after changing of the payment procedure from Ministry of health to National Health Insurance Fund (NHIF);
- ✓ **The access to pharmaceutical services is not hampered**, probably because of the sufficient number of pharmacies and pharmacists in the major cities;
- ✓ Despite the high level of satisfaction with the quality of pharmaceutical services (93.3%), **about 60% of patients with RD are not inclined to consult with pharmacists** and to obtain advice due to distrust of their qualifications and knowledge about RD.

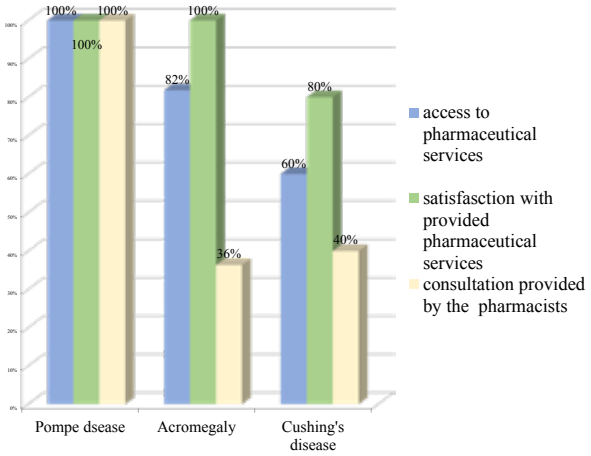

Figure 1 Percent of patients with access to pharmaceutical service, satisfied with provided care and the level of trust in provided consultation by the pharmacists

V. Conclusion

- ✓ The pilot study demonstrates **an adequate physical and financial access** for patients with RDs to treatment and their satisfaction with the pharmaceutical services as well;
- ✓ **The level of trust** the patients with RDs have with the pharmacists **remains low**;
- ✓ There is a **necessity to inform the public about the essential role of the pharmacists** and to demonstrate the value of providing pharmaceutical care not only for patients with common but also for rare diseases.

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Неврологични симптоми при пациент с перицентрична инверсия на 9-та хромозома

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ВЪВЕДЕНИЕ

Перицентричната инверсия на 9-та хромозома представлява структурен хромозомен вариант на нормалния кариотип. Според данните в литературата честотата в общата популация е от 0.8-1.2%. При хетерозиготни пациенти са описани спонтанни аборти и инфертилитет при мъжете. Установяват се речеви, моторни нарушения, очни проблеми, умствена изостаналост и шизофрения. Описани са редки асоциации със синдрома на Asperger, мускулна дистрофия на Walker – Warburg, субкортикалната лентовидна хетеротопия, церебрални кисти и остра левкемия.
Генетичната мутация се асоциира със синдрома на Goldenhar в 15-25% от случаите, както и с други вродени аномалии като: полидактилия, артрогрипоза, микротия, глухота, асиметрично лице, гигантски Мекелов дивертикул, дуоденална диафрагма, малротация на тънки черва, пулмонална стеноза, междупредсърден дефект, трикуспидна регургитация, кардиомиопатия, аритмия, интраутеринно изоставане в растежа и олигохидрамнион.

ЦЕЛ

Целта на настоящата работа е да се представи неврологична симптоматика при пациент с перицентрична инверсия на 9-та хромозома.

МАТЕРИАЛ И МЕТОДИ

Приложени са следните методи: клинични – анамнеза, фамилна анамнеза, неврологичен статус, консултация със специалисти: педиатър, детски невролог, детски психиатър, радиолог, клиничен генетик, детски хирург, детски офталмолог.
Генетични изследвания: кариограма, MLPA за микроделеции, субтеломерни делеции и дупликации, ДНК анализ за мутации в FMR1 гена
Биохимични изследвания: ПКК, СУЕ, АСАТ, АЛАТ, ГГТП, амоняк, лактат, МПЗ в урина, лактат, амоняк, капилярно зонова електрофореза на сиалотрансферини
Радиологични изследвания: КТ на глава, МРТ на главен мозък**:** ТЕХНИКА:1.5 Т, МРТ нативно, T1/mpr/sag, T2/tse/bl/sag, T2/spc/dark-fluid/iso/sag, dir/spc/sag/iso, T1/spc/ir/cor/iso, T2/tse/tra, ep2d/diff/b0-500-1000, ЕЕГ, ЕхоКГ, Ехография на коремни органи, Рентгенография на китка.
Психологични изследвания: Hawik-R, отделни проби за изследване на познавателните способности, наблюдение в структурирана среда.

ОПИСАНИЕ НА КЛИНИЧЕН СЛУЧАЙ

Анамнеза

Момче на 12 години от втора бременност, протекла с хиперемезис, първо нормално раждане в термин, данни за „зелени околоплодни води". Първата бременност на майката е завършила със спонтанен аборт през първия триместър. Тегло при раждане-3500 грама, дължина-53 см. В неонаталният период е установена пилорна стеноза. На 2 години е бил опериран по повод на конвергиращ страбизъм, а на 4 години - по повод умбиликална херния. Проходил на 1 година и 6 месеца. Първи думи на 3 годишна възраст. На 4 години и 6 месеца –фразеологичан реч, но с „бебешки говор". В последствие установена дизлексия, имал училищни затруднения и ползвал ресурсен учител. На 11 години и 6 месеца била проведена хоспитализация в УМБАЛ „Александровска", Клиника по детска психиатрия „Св.Никола", където бил установен **интелектуален дефицит** (IQ=59),

Обективно състояние

В запазено общо състояние. Кожа-бледо-розова, тургор и еластичност запазени, хипертрихоза на гърба, постоперативен цикатрикс в десен хипохондриум. Фациес-дискретно дизморфичен: ниско чело, дълбоко вложени очи, четковидни вежди, стърчащи ушни миди. Глава-правилна конфигурация (об. на глава-55 см). ДС-ДЧ-18/мин, нос-проходим, чисто везикуларно дишане двустранно. ССС-правилна ритмична сърдечна дейност, ясни тонове. Орофаринкс-спокоен, хипертрофични тонзили. Език-необложен. Корем на нивото на гръдния кош, без данни за ХСМ. ППС-пубертетно развитие-Танер-II ст. ОДС-правилно развит, хипермобилитет в лакетните стави, леко нарушена екстензия в коленните стави. НС-без данни за МРД, конвергентен страбизъм вляво. Нормална мускулна сила и тонус. СНР-живи, симетрични, патологични рефлекси не се установяват. Координация-дисдиадохокинезия двустранно, повече в ляво, Ромберг (-). Дислалия, дисграфия, дислексия. Контролира тазови резервоари.

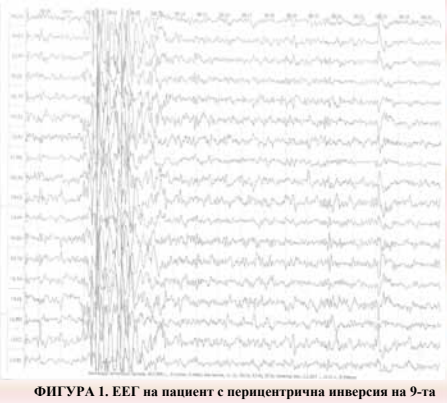
Генетични изследвания: MLPA за микроделеции, субтеломерни делеции и дупликации, кариограма-инверсия на 9-та хромозома. При изследването на двамата родители се установява носителство на частична инверзия на 9-та хромозома при майката. **МРТ на главен мозък** (16.06.2017 г.)**:** Не личат огнища на силни сигнали на Т2 образите в мозъчния паренхим. Не се откриват зони с ограничена дифузия в мозъка. ADC в целия мозък е в границите на нормата. Латерални, трети и четвърти вентрикул-в норма. Субарахноидните пространства по конвекситетните, медиалните и базалните повърхности на голямомозъчните хемисфери, интерфолиарните субарахноидни пространства и базалните цистерни не показват болестни промени. Не се откриват девиация на структурите по срединната линия или други феномени на херниране. Не се откриват интра и екстрааксиални Ту формации супра и инфратенториално. Диференциацията на сивото и бялото мозъчно вещество е запазена на всички образи. Не се откриват аномалии в гирацията или във формирането на палиума.Калвария и база-в норма. **ЕЕГ-** Двустранно фронтално и преднотемпорално с максимум в лява хемисфера се регистрират единични и на залпове нерегулярно **комплекси пика-бавна вълна с висока амплитуда, които спонтанно генерализират синхронно**. Находката се активира при ХВ на фона на общо забавяне на трасето. ФС-фотопароксизмален отговор. **Рентгенография на китка и пръсти-**костната възраст отговаря на календарната.

Лечение

Ноотропил, Конвулекс, Петинимид.

ОБСЪЖДАНЕ

Представеният от авторите пациент с перицентрична инверсия на 9-та хромозома се изявява със съчетание на очни, неврологични, психиатрични и гастроинтестинални симптоми, което досега не е описвано в литературата. Общото интелектуално функциониране е в рамките на лека степен на интелектуален дефицит, което обяснява занижените академични възможности, и по-ниската социална компетентност на детето. Подобни съобщения са направени от Scarinci R, и сътр., 1992 г. и Cora и сътр., 2000 г. (1,2). Установеният от нас конвергиращ страбизъм не е описван в литературата. Очни и мозъчни аномалии, характерни за синдрома на Walker-Warburg са описани от Baltaci V и сътр., 1999 г. при хомозиготни пациенти с перицентричната инверсия (3). Налице е асоциация между ранната дизлексия и епилепсията, подобно на описаните от Schachter S. и сътр., 1993 г., което налага проследяване с ЕЕГ (4). По анамнестични данни дизлексията при детето значително се подобрява от проведената терапия с Ноотропил. Въз основа на анамнезата и ЕЕГ, при пациента се установява ювенилна абсансна епилепсия. Нормалната находка от ЯМР на глава изключва наличието на структурни мозъчни промени или аномалии в гирацията като церебеларни кисти или субкортикална лентовидна хетеротопия, които са описвани при перицентричната инверсия на 9-та хромозома. След включване на терапия с валпроат за 3 месеца честотата на епилептичните пристъпи значително намалява, но се задържа находката в ЕЕГ (Фиг.1), което налага включването на втори антиконвулсант-етосукцимид. Клиничното наблюдение на пациента продължава.



ФИГУРА 1. ЕЕГ на пациент с перицентрична инверсия на 9-та хромозома.

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Mutational burden in Bulgarian schizophrenia and bipolar disorder patients: a bioinformatics analysis

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Background

Bipolar affective disorder (BAD) is characterized by a high genetic heterogeneity that leads to difficulties in the confirmation of discovered associations between the disease and specific genetic loci. In order to alleviate these difficulties there is a turn towards the selection of rarer variants with functional or regulatory consequence that have a much lower frequency, but a potentially larger effect.
In this study we examined the incidence of potentially functional variation in a preselected gene panel and looked for genes with increased variational burden.

Object

The number of observed potentially functional variations (missense, frameshift/indels, stop gain, splice site) in 187 genes from previous studies of schizophrenia, autism and psychotic spectrum disorders that have been found to harbour *de novo* loss-of-function or non-synonymous mutations. The group also includes calcium and sodium voltage-gated channels and genes found to have recurrent genetic variation in schizophrenia.

Samples

The cohort consisted of 377 Bulgarian BAD (240) and schizophrenia (137) patient samples collected in the Molecular Medicine Center as part of current and previous projects looking into the basis of psychiatric disorders, and 78 population controls.

Methods

The panel was sequenced on the Ion Proton platform and analyzed with the integrated Torrent Suite software and custom annotation and filtering scripts. The frequency of mutation per kilobase was combined with the percentile of the expected intolerance to variation of each gene based on the RVIS public database [1]. The final score was calculated according to the following formula:

$$(1) \qquad GeneScore = 1000 * \frac{var\_num}{gene\_len} * (1 - \frac{\%rvis}{100})$$

A normality test was performed using IBM SPSS Statistics 19. A Gene Ontology enrichment test [2] was performed to define specific pathways and processes linked to outlier genes that did not fit into the normal distribution.

Table 1. Variation characteristics of the outlier genes.

Gene	Total variants	Burden (var/kb)	RVIS %	Score
SCN1B	4	4.96	29.54	3.49
LPHN2	15	3.42	6.79	3.19
CYP17A1	5	3.27	12.18	2.88
CACNA2D3	10	3.05	11.26	2.71
GRIN2A	12	2.73	1.96	2.68
IQSEC1	9	2.69	6.95	2.50
ALDOC	4	3.65	32.61	2.46

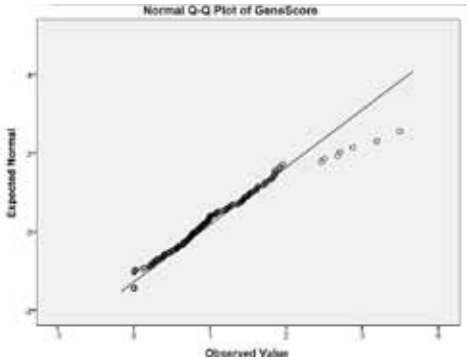

Figure 1. Q-Q plot of the scores versus the normal distribution.

Results and discussion

The Q-Q plot of the scores against the normal distribution showed a skew of the 7 highest scoring genes to higher than expected scores (**Figure 1**). These genes (**Table 1**) were used to define enriched molecular functions in GO, resulting in a single significant hit (aldehyde-lyase activity, 2 out of 9 possible hits, p=0.012). The gene products are characterized in **Table 2**.

The analysis suggests that the corrected number of different variations in the studied genes generally follows the normal distribution, but there are also some outliers linked to neurological, neoplasmic and psychiatric diseases. As expected, voltage-gated channels are common in this group with high heterogeneity of variation. On the other hand the LPHN2 (ADGRL2) gene that has no association with disease is a potential candidate for further study.

Table 2. Notable functions and disease associations of the products of the outlier genes.

Gene	Function	Disease
SCN1B	Voltage-gated sodium channel subunit	GEFS+ epilepsy, atrial fibrillation
LPHN2	GPCR, exocytosis regulation	-
CYP17A1	Steroidogenesis	adrenal hyperplasia
CACNA2D3	Voltage-gated calcium channel subunit	tobacco use disorder
GRIN2A	Glutamate-gated ion channel, NMDAr	focal epilepsy w/ speech dis.
IQSEC1	G-nucleotide exchange factor for ARFs	malignant neoplasms
ALDOC	Brain expressed fructose-bisphosphate aldolase	Schizophrenia

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Синдром на празната села-описание на клиничен случай

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ВЪВЕДЕНИЕ

Синдромът на празната села (СПС) е вроден дефект, при който села турцика е частично или напълно изпълнена с цереброспинална течност, а хипофизата е често притисната или деформирана. При първичния СПС се установява вроден дефект или липса на диафрагма селе. Вторичният СПС се установява при черепно-мозъчни травми, тумори на хипофизата, инфекции, инфаркти, лимфобластна левкемия, лъчетерапия или редки заболявания като синдромите на Sheehan, Alstrom, септо-оптична дизплазия, Wolfram, Robinof , GAPO, болест на Gitelman, мутации в PROP1 ген, спиноцеребарна атаксия и др.
Заболяването се среща по-често при жени със затлъстяване и хипертония. Няма данни в литературата за половата предилекция при деца.
Точната честота на заболяването в общата популация не може да бъде установена, тъй като СПС обикновено се установява като случайна неврорадиологична находка. В 1% от случаите се изявява с главоболие, зрителни нарушения, доброкачествена интракраниална хипертензия , ринорея, питуитарна хипофункция .
От ендокринните нарушения при децата най-често се описва дефицит на растежен хормон, преждевремен пубертет, хипогонадотропизъм, инсипиден диабет, множествен дефицит на хипофизни хормони, изоставане във физическото развитие.
Диагнозата се поставя въз основа на характерните симптоми, анамнеза и се потвърждава с невроизобразяващи изследвания КТ и МРТ на глава.
При наличие на хипопитуитаризъм се провежда хормон-заместваща терапия. Неврохирургично лечение се прилага при ринорея .

МАТЕРИАЛ И МЕТОДИ

Приложени са следните методи: клинични – анамнеза, фамилна анамнеза, неврологичен статус, консултация със специалисти, невроизобразяващи –ЯМР на глава и др., общ и селективен метаболитен скрининг на органични и аминокиселини в урина и кръв чрез GC/MS и LC-MS/MS методи, генетични изследвания - PCR-SBT метод за анализ на мДНК, кариограма, ДНК анализ на FMR1 ген, хормонални изследвания, алергологични тестове, изследвания на витамин B12.

ОПИСАНИЕ НА КЛИНИЧЕН СЛУЧАЙ

Анамнеза

Авторите представят момче на 6 години, роден от втора патологична бременност (артериална хипертония, медикаментозно лечение с допегит) и раждане 10 дни преди термин със секцио Цезареа. Тегло при раждане-3400 грама, дължина-50 см. Послеродовият период е протекъл без усложнения. По данни на родителите е неспокойно, „мрънкащо” бебе. Гукането и лепетът не са добре изразени. Около 1 годишна възраст използва звукосъчетания, но без ясна комуникативна цел. На 3 години и 11 месеца все още не е проговорил, но активно вокализира. Не реагира при повикване по име. Рядко изпълнява инструкции. Не е самостоятелен по отношение на обличане и събличане, но съдейства когато е обгрижен от възрастен. Може да си служи с прибори за хранене, но е подпомаган от близките си. В детската градина не изпълнява инструкции. Предпочита самостоятелни занимания. Обича да гледа детски клипчета по интернет. Страхува се от силни шумове. При фрустрация реагилра бурно-склонен да се удря и да посяга на майката.
Обективно състояние на 5 години и 6 месеца: Тегло-16 кг (SDS=-1.25), ръст 104 см (SDS=-2.0). Фациес-триъгълна брадичка, антимонголоидни очни цепки, ниско поставени ушни миди, хипертрихоза по гърба, къса шия. Нормален соматичен и неврологичен статус.

Психологичното изследване на 3 години и 11 месеца показва особености в общото функциониране. Регулацията на поведението като цяло е съществено нарушена, а изпълнението на инструкции е избирателно, отложено във времето. Предпочита дейности, които имат сензорен и стереотипен характер, както и това той да определя кога, какво и как да се случва. Не разбира и не проявява интерес към игра наужким. Нивата на инициране, отговор и поддържане на споделено внимание е социални интеракции са значително под възрастово съответните, като общото социо-комуникативно умение не надвишава 9-11 месеца. Очният контакт е беден, лицеизразът-недостатъчно информативен, жестове-основно подбудителни, за да достигне до желан от него предмет или резултат. Успеваемостта по реализираните тестови проби варира между 11м. (Зрително проследяване и постоянство на обекта) и 14-18 месеца (Развитие на операционалната причнност). Регистрираната спонтанна вокална продукция се ограничава до относително еднообразни като звуково съдържание и интонационни характеристики вокализации, които имат основно автостимулационен характер. В заключение се установяват затруднения в социо-комуникативното отношение, характерни за диагноза **Генерализирано разстройство на психичното развитие-детски аутизъм.** Наблюдават се двигателни стереотипии, дейности с автостимулационен произход. Когнитивните показатели съответстват на по ниска умствена възраст /11-18 месечна възраст/.

ФИГУРА 1. Пациент със Синдром на празната села.

Общ и селективен метаболитен скрининг на органични и аминокиселини в урина и кръв чрез GC/MS и LC-MS/MS методи е с данни за повишен аланин-404.48 (92.2-290 µmol/l), глутамат-318.65 (92-290 µmol/l), C14:1 (тетрадеценоилкарнитин)-0.18 (0-0.11 µmol/l). **Хормонални изследвания**: IGF1-63.3 ng/ml (50-233), IGFBP-3.49 µg/ml (1.3-5.6), TSH 4.44 µIU/ml [0.4-4], fT4 16.7 pmol/l [11.5-22.7], FT3 9.40 pmol/l [2.3-6.3], TAT <10 IU/ml [<35], MAT <20 IU/ml [<40], TSH-3.82 µIU/ml (0.27-4.2), fT4-13.97ng/l (9.3-17), LH <0.1 IU/l (0.1-1), FSH-0.44 IU/l (0.2-3.4), пролактин - 1120 µIU/ml (55.12-445,20), тестостерон <0.08 nmol/l (0.1-1.12), Vit.B12-412.8 pmol/l (145-569), ADH-4.6 ng/l – в норма, допамин - <20, при норма <85 ng/l, Кортизол 8h 494 nmol/l [107-662], Кортизол 24h 132 nmol/l, SHBP 135 nmol/l [39-146], функционални тестове: нощен ритъм на растежен хормон, тест с физическо натоварване-частичен дефицит на растежен хормон. **Генетични изследвания:** цитогенетично изследване-кариограма, митохондриална ДНК. **Рентгенография на китка и пръсти**-двукратно дисхармонична костна възраст, отговаряща на 3 години при календарна възраст 4 години и 11 месеца и 3 години при 6 години и 2 месеца. **МРТ на глава:** От нативното изследване – без огнищни изменения в мозъчния паренхим. Без данни за малформативни промени в церебралния кортекс. Разширени периваскуларни пространства на Вирхов-Робин в бялото мозъчно вещество на двете голямо-мозъчни хемисфери. Нормална конфигурация на параселарните структури**. Интраселарно херниране на супраселарната цистерна (т.нар. empty sella)**. Запазени субарахноидни пространства. Вентрикулна система-без деформационно-дислокационни промени. ЗЧЯ-без огнищни изменения в малкомозъчния паренхим и в ствола. Запазени субарахноидни пространства, нормална конфигурация на околостволовите цистерни, понто-церебеларните ъгли, четвърти вентрикул и вътрешните слухови канали. МРА-нормална конфигурация на компонентите на Вилизиевия кръг и проксималните сегменти на предна, средна и задна мозъчна артерии.

ОБСЪЖДАНЕ

Представя се момче на 6 год. и 2 мес. с рядка асоциация на детски аутизъм и СПС. Клиничен случай на дете със СПС и болест на Asperger е докладван от Raja M. и сътр, 1998 г. (1) . В описаният от авторите случай е възможно да се касае за вторичен СПС, дължащ се на инфаркт в областта на хипофизата, вследствие на повишено артериално налягане на майката и антихипертензивна терапия по време на бременността. Направените генетични и метаболитни изследвания до настоящия момент не установяват генетично заболяване, въпреки, че се описва лек лицев дизморфизъм. Представените данни от GC/MS и LC-MS/MS методи за увеличен аланин, най-вероятно се дължат на диетична грешка с предозиране на въглехидратите и са последствие от намалената глюконеогенеза, а леко повишеният глутамат допринася за изявата на хиперактивното поведение. При пациента се установяват хиперпролактинемия, подобно на описаните случаи от Gharib H. и сътр., 1983, Pallardo Sánchez LF и сътр., 1983 г. (2,3). Наблюдаваното разстройство на психичното развитие най-вероятно се дължи на вторични нарушения в допаминовия контрол, вследствие на хиперпролактинемията, подобно на описаните в литературата от Dornan J. и сътр, 1984 (4). Причините за двукратно отчетената дихармонична костна възраст са комплексни, като в етиопатогенезата участват повишения пролактин и частичния дефицит на растежен хормон, подобно на случаи, описани от Galli-Tsinopoulou A и Unsinn K, 1991 г..(5,6). Частичният дефицит на растежен хормон е описан при пациенти със СПС и в случая не налага терапевтична интервенция. При пациента е прилагано лечение със Седивитакс сироп с добър ефект върху хиперактивното поведение.. Клиничното наблюдение на случая продължава.

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A rare mutation of *SCN8A* gene in a patient with early infantile epileptic encephalopathy type 13

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Introduction

Early infantile epileptic encephalopathy type 13 (EIEE13) is a heterogeneous group of severe neurologic disorders with variable etiology. The disease affects newborns, usually within the first three months of life in the form of epileptic seizures. Those who live past the age of 2 years manifest with severe psychomotor deficits.

EIEE13 is caused by missense mutations in the *SCN8A* gene located on chromosome 12q13.13. *SCN8A* is a member of a gene family comprised of nine evolutionary related sodium channels with specific roles in neurons and skeletal muscle. The gene encodes one of the major voltage-gated sodium channels (VGSCs). Pathological variants in *SCN8A* in a heterozygous state are manifested phenotypically as an autosomal dominant form of EIEE13.

VGSCs play important role in initiation and propagation of action potentials. They consists of one pore-forming α-subunit (domains I-IV) and one or two β-subunits. The VGSCs structure consist of 9 α-subunits ($Na_v1.1$-$Na_v1.9$) (Figure 1). $Na_v1.6$ encoded by the gene *SCN8A* is major α-subunit in excitatory and inhibitory neurons in the brain and is highly concentrated in the distal half of the axon initial segments in many neurons and at nodes of Ranvier where it mediates action potential initiation and propagation (Figure 2). These channels regulate cellular excitability by controlling the flow of sodium ions across the cell membrane.

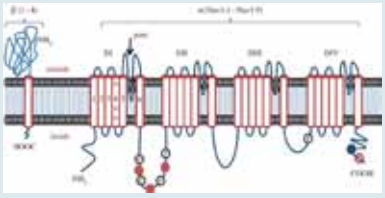

Figure 1: Schematic diagram of the structure and membrane topology of the VGSCs. Given α-subunits have four domains (DI-DIV) each composed of six transmembrane segments [Fraser et al. 2014].

Figure 2: Localization of $Na_v1.6$ channel [Larsen et al., 2015].

Hypoactivity and hyperactivity of $Na_v1.6$ are both pathogenic, but with different outcomes: haploinsufficiency is associated with impaired cognition while hyperactivity can result in epilepsy.

Methods

Our patient was diagnosed with early onset-epileptic encephalopathy followed by seizures, regression of speech, developmental delay, intellectual disability and difficulties with coordination and balance.

DNA from peripheral blood sample was extracted after obtaining written informed consent.

Through targeted next-generation sequencing we elucidated the genetic basis of early-onset epileptic encephalopathy in our patient. NGS libraries were prepared using the TruSight One gene panel, that provides comprehensive coverage of genes related with the EIEE. The library was sequenced on an Illumina MiSeq platform.

Data analysis and variant annotation were perfomed using the Variant Studio and the GenomeBrowse softwares.

Results

We identified an extremely rare heterozygous missense mutation in the *SCN8A* gene*:* NM_014191.3:c.5616G>A,NP_055006.1:p.Arg1872 Gln (Figure 3). The variant is a heterozygous nucleotide substitution of guanine with adenine at position 52200885 on chromosome 12. The change affects the coding sequence of the gene and results in the translation of a protein with an amino acid substitution (p.Arg1872Gln).

Amino acid replacement (Arg1872Gln) occurs in the cytoplasmic C-terminal domain (Figure 4). This residue was demonstrated to play a role in the inactivation gate of the channel, mediated by interaction between positively and negatively charged amino acids (Lee and Goldin 2008).

The mutation is known and has been observed in two patients also diagnosed with an early epileptic encephalopathy and developmental delay. Pathogenic variants in *SCN8A* are phenotypically expressed as an autosomal dominant form of EIEE-13 and thus affected individuals are heterozygous for the variant.

Figure 3. Pathological profile of 27 exon on the *SCN8A* gene in our patient. The replaced nucleotide is marked.

Results

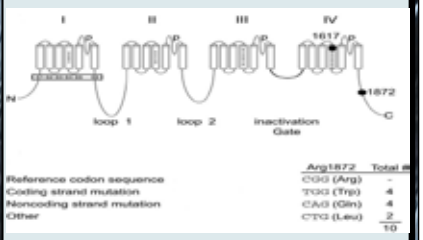

Figure 4. Recurrent mutation of Arg1872Gln in *SCN8A* gene. The positions of mutated amino acid is shown and also CpG hotspots in the codons for Arg1872 [Wagnon et al. 2015].

The variant identified by NGS was confirmed by Sanger sequencing.

Conclusion

Within the last few years *de novo* mutations in *SCN8A* detected by NGS, have revealed the role of $Na_v1.6$ in the pathogenesis of epilepsy and intellectual disability.

Considering the wide variability in severity and penetrance of the disorders from the epileptic spectrum, it is now obvious that there is large genetic heterogeneity in their inheritance.

Our finding demonstrates the application of the TruSight One gene panel in order to successfully detect rare defects in patients with EIEE. The identification of the causative mutation could deliver crucial insights into the etiology of the disease and potentially pave the way for a personalised treatment.

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ПЕРИФЕРНОСЪДОВИ И МИКРОЦИРКУЛАТОРНИ НАРУШЕНИЯ ПРИ ВИБРАЦИОННА БОЛЕСТ ОТ ЛОКАЛНО ВИБРОВЪЗДЕЙСТВИЕ

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Системното продължително въздействие на локални вибрации, генерирани от ръчни инструменти в условията на труда, обуславя редица неблагоприятни здравни ефекти предимно в горните крайници, като засяга съдови, периферонервни и мускулно-скелетни структури [1, 2]. Комплексът от симптоми и клинични прояви вследствие локално вибровъздействие е обобщен и международно признат като вибрационно обусловен „ръка-рамо" синдром [3] или вибрационна болест. Настоящото проучване акцентира върху съдовите нарушения в ръцете, които са водещи в клиничната картина на вибрационната болест от локално вибровъздействие (ВБ ЛВВ) най-често с асиметрични вазоспастични прояви в артериите на пръстите и типичните прояви на феномен на Raynaud [4], но съчетаните изследвания на кожната микроциркулация и периферните артерии при ВБ ЛВВ със съвременни неинвазивни диагностични методи са оскъдни [5].

ЦЕЛ:

Да се изследват периферносъдовите и микроциркулаторните нарушения в горните крайници при болни с вибрационна болест от локално вибровъздействие.

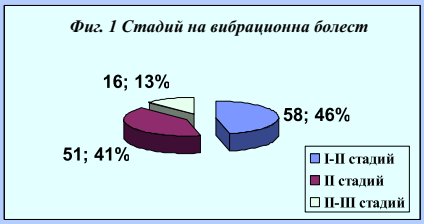
МАТЕРИАЛ И МЕТОДИ:

От хоспитализираните през последните 2 години 267 експонирани на вибрации болни 125 отговаряха на критериите за диагностициране на ВБ ЛВВ и бяха изследвани чрез дистална доплерова сонография (ДДС), лазерна доплерова флоуметрия (ЛДФ) и видеокапиляроскопия (ВКС).

РЕЗУЛТАТИ:

Изследваните 125 болни са от мъжки пол, на възраст 51.3±13.1 години и с продължителност на трудовия стаж с експозиция на локални вибрации 11.8±6.3 години. Относителният дял на болните в зависимост от стадия на заболяването е представен на Фиг. 1.

Фиг. 1 Стадий на вибрационна болест



Преобладават болните в първи към втори и втори стадий на вибрационна болест при изследвания контингент, съответстващ на степента на увреждане при обичайно хоспитализираните болни в Клиниката по професионални заболявания.

Данните от изследване на периферните артерии на ръцете чрез ДДС са представени на Фигура 2.

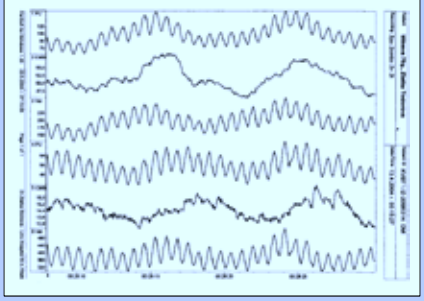
Фиг. 2 Дистална доплерова сонография

При 71.2 % се установява повишена съдова резистентност до липсващ сонографски отговор в aa. digitales palmares propriae, 12.8% – повишена съдова резистентност на arcus palmaris superficialis и 7.2% – на a. ulnaris. Единични са публикациите за установени съдови нарушения чрез доплерова сонография и тромбоза на периферни дистални съдове на ръцете [4, 6].

Изследването на глобалното кожно кръвообращение и на терморегулаторните, и на нутриционните кожни микросъдове чрез ЛДФ установява нарушен кожен кръвен ток в дисталните фаланги на пръстите на ръцете воларно и снижена кожна перфузия при 39.2% от болните. Нарушено кожно кръвообращение, регистрирано чрез лазер-доплерови изследвания описват и други автори [7, 8].

Илюстрация на мониторирана кожна перфузия, скорост на формените елементи на кръвта и техния брой чрез РДФ е показана на Фигура 3.

Фиг. 3 Лазерна доплерова флоуметрия



Данните от изследването на нутриционното кожно кръвообращение в епонихиума на пръстите на ръцете при болните от ВБ ЛВВ чрез видеокапиляроскопия са показани на Фигура 4.

Фиг. 4 Морфология на капилярите

При преобладаващия дял (98 болни) от изследваните се установяват функционални промени в нутриционните кожни микросъдове: дистонни с преобладаване на спастичните (38 болни), спастични (56 болни) и предимно атонични (4 болни) капилярни бримки. Структурно променени капиляри с аневризми, липсващи капилярни бримки, хеморагии в епонихиума са наблюдавани при 27 болни (Фиг. 5). Промени при видеокапиляроскопия описват и други автори [5, 9].

Фиг. 5. Видеокапиляроскопия при вибрационна болест от локално вибровъздействие

Периферносъдовите и микроциркулаторните нарушения са в положителна корелация със стадия на заболяването ($r = 0.461$, $p<0.001$).

ЗАКЛЮЧЕНИЕ:

Установяват се преобладаващо ангиоспастични промени в дисталните периферни съдове и кожните нутриционни и терморегулаторни микросъдове на пръстите на ръцете при ВБ ЛВВ. Ранното диагностициране на ВБ ЛВВ е от съществено значение за превенция на трофични нарушения в ръцете, подобряване на прогнозата и качеството на живот на болните и съхраняване на трудовия им капацитет. Необходими са достатъчно познания върху релевантните професии с вибрационна експозиция и основните клинични прояви на ВБ ЛВВ от страна на личните лекари и специалистите по ортопедия, ревматология, неврология, ангиология, обща медицина с оглед своевременно насочване за специализирани изследвания и консултация от специалистите по професионални болести и адекватно терапевтично и превантивно поведение.

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Blood biomarkers for risk prediction of aggressive prostate cancer patients – preliminary study of a Romanian cohort

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INTRODUCTION

Prostate cancer (PC) is the third most common oncologic disease in Romanian males, with an incidence of 37,9/100.000 and a mortality rate of 16,3/100.000 in 2012. It is acknowledged that PC is a multifactorial disease and despite the high prevalence, the disease etiology remains largely unknown. In addition to increasing age and ethnicity, genetic variation or polymorphisms existing in the human genome can confer genetic susceptibility to cancer and the detection of these modifications may provide useful molecular indicators of prognosis. Genome Wide Association (GWA) studies have yielded multiple single nucleotide polymorphisms (SNPs) associated with prostate cancer risk.

OBJECTIVE

To assess the impact of 5 SNPs (*KLK2-KLK3*, *SLCO2B1*, *SLCO1B3*, *HOXB13*, and 17p12) that are currently under debate as risk factors in Western Europe and American populations, in the genetic context of the Romanian male prostate cancer patients.

METHODOLOGY

A total of 53 patients were investigated. All recruited subjects were Romanian Caucasians.

The diagnosis of prostate carcinoma was confirmed by clinical and laboratory examination. Clinicopathological characteristics are presented in table 1.

Table 1. Clinic and pathological characteristics of patients with prostate carcinoma

	Cases (*n*=53)
Age	65,7±5,1[a]
<70	41
≥70	12
PSA values (ng/ml)	19±16,8[a]
<10	13
≥10	40
Tumor stage	
Early	4
Advanced	49
Gleason score	
<8	30
≥8	25

[a]Statistics were calculated as mean ± standard deviation (SD)

Genomic DNA was isolated from whole blood samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI).

Genotyping was performed by allelic discrimination with Taqman 5'-nuclease assays according to the manufacturer's recommended protocols. The following SNPs were determined: rs2735839 (*KLK2-KLK3*), rs12422149 (*SLCO2B1*), rs4149117 (*SLCO1B3*), rs138213197 (*HOXB13*), rs4054823 (17p12).

RESULTS

All 53 samples were genotyped.

The genotype distribution for each of the 5 SNPs are summarized in table 2.

Table 2. Genotype frequencies in the study group

SNPs	Genotype frequencies		
	Wild-type	Single-variant	Variant
rs2735839 (*KLK2-KLK3*)	6%	15%	79%
rs4054823 (17p12)	25%	43%	32%
rs12422149 (*SLCO2B1*)	6%	17%	77%
rs4149117 (*SLCO1B3*)	73%	23%	4%
rs138213197 (*HOXB13*)	100%	0%	0%

***KLK2-KLK3* (rs2735839)**

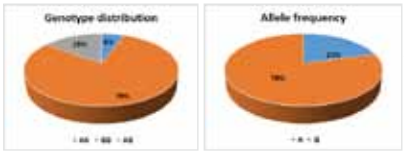

Figure 1. Genotype distribution and allele frequencies. AA/AG—high-risk for aggressive PC, GG—normal risk

79% of patients were GG homozygous and 21% were carriers of the high-risk allele (A).

17p12 (rs4054823)

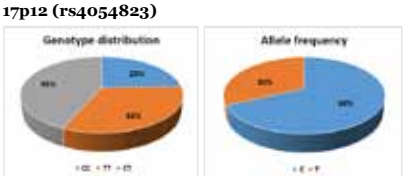

Figure 2. Genotype distribution and allele frequencies. CC/CT—normal risk, TT—high-risk for aggressive PC

68% of patients were CC/CT genotype carriers and 32% were carriers of the high-risk genotype (TT).

RESULTS

***SLCO2B1* (rs12422149)**

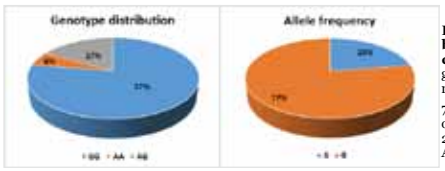

Figure 3. Genotype distribution and allele frequencies. GG—high-risk for aggressive PC, GA/AA—normal risk

77% of patients were carriers of the "at-risk" allele G and 23% were carriers of the GA/AA genotype.

***SLCO1B3* (rs4149117)**

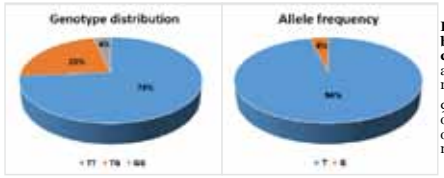

Figure 4. Genotype distribution and allele frequencies. TT/TG—high-risk for aggressive PC, GG—normal risk.

96% of patients were carriers of the high-risk allele T and only 4% were carriers of the normal risk genotype.

***HOXB13* (rs138213197)**

Genotypes: CC—normal risk, CT/TT—high-risk for prostate cancer.

All analyzed patients presented the normal risk genotype CC.

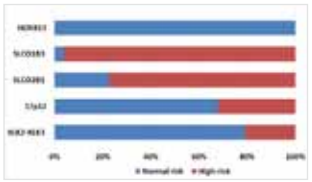

Figure 5. Allele frequencies in genes associated with aggressive PC.

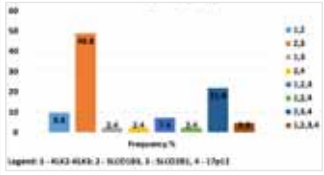

Figure 6. Combined high-risk genotypes.

In our case-only study, the high-risk genotype of *SLCO1B3* gene is the most frequent (96%) followed by "at-risk" allele of *SLCO2B1* (77%), and both variants were found to be the most frequent combined genotypes (48,8%) present in the study group.

A percentage of 21,9% from the analysed group was found to be carriers of 3 high-risk variants: *SLCO1B3*, *SLCO2B1* and 17p12.

DISCUSSIONS

In recent years, a large amount of information on PC risk associated SNPs is available for multiple case-control samples. Accumulated data suggest that many pathways are involved in the progression of prostate cancer.

In our study group, the most frequent gene variants were found in *SLCO1B3* and *SLCO2B1*. The frequency distribution of rs12422149 (*SLCO2B1*) and rs4149117 (*SLCO1B3*) were similar to previous reported data (Kim et al., 2013; Namgoong et al., 2015).

Previous studies (Xu et al., 2010) have identified that the frequency of SNP rs4054823 at 17p12 is significantly higher among more aggressive cases. Our data reveals that TT high-risk genotype of rs4054823 was not significantly associated with aggressive PC, although its frequency in our study group was high (32%).

In contrast to other studies (Ewing et al., 2012; Beebe-Dimmer et al., 2014) the PC susceptibility allele *HOXB13* was not found to be present in our PC group.

CONCLUSIONS

- In summary, the results of this study confirm the presence of 4 from the 5 analysed SNPs in prostate cancer patients.
- Our preliminary results in this small patient cohort show no significant association of the investigated SNPs with the biochemical markers and tumor stage.
- However, the increase in the frequency of this high-risk variants indicates that there is a strong association between genetic variants and prostate cancer.
- The observed genotype distribution of the studied gene polymorphisms and the differences in frequency of risk alleles may contribute to characterizing the genetic diversity of prostate cancer.

Acknowledgements: Paper supported by the Partnerships in Priority Domains program – PN II, implemented with the support of MEN – UEFISCDI, project code PN-II-PT-PCCA-2013-4-1851.

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Genetic markers for thrombophilia in patients with cerebrovascular accident and persistent foramen ovale

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Introduction

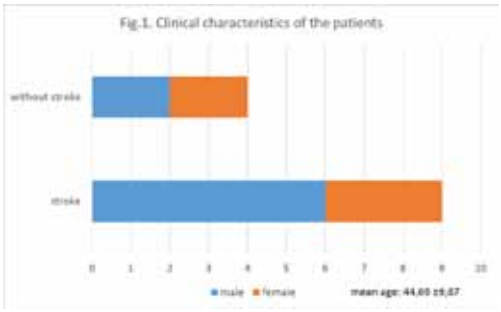
The incidence of persistent foramen ovale (PFO) is 25-30% of the population. Usually it has no adverse health consequences, but there is data, that it is found more often in patients with cryptogenic stroke, because of the possibility of paradoxical embolism through the defect. In the recent years research is ongoing to establish a correlation between PFO and ischemic stroke (IS).

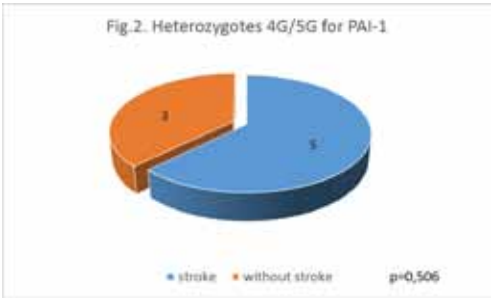
Aim

To assess the genotype profile of patients with PFO and to search for mutations associated with ischemic stroke.

Methods and participants

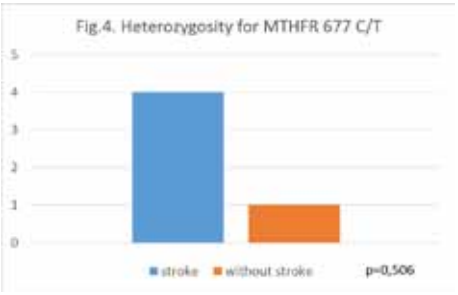
13 patients were referred from the dept. of Neurology - divided in two groups: who have survived ischemic stroke (9 patients) and who presented with a different neurologic pathology (4 patients) - fig.1.
All PFO patients presented with permanent or intermittent right to left shunt. Because of the history of cerebrovascular accident the patients underwent molecular genetic test for thrombophilia – factor V Leiden, factor II prothrombin, PAI, MTHFR.

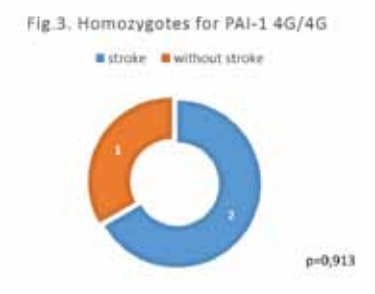




Results

1. 8 of the patients were heterozygotes 4G/5G for PAI-1 alone or in combination with another marker, 5 of them (55.5%) in the first group and 3 (75%) in the second group, p=0,506 – fig.2.
2. Homozygote genotype for PAI-1 4G/4G was found in three patients (2 in the first group (22%) and 1 in the second group (25%) (p=0,913) – fig.3.
3. One patient in the group with ischemic stroke was positive for 2 markers – heterozygote for Factor II prothrombin and homozygote for MTHFR 677 T/T.
4. Heterozygosity for MTHFR 677 C/T was found in 5 patients – 4 (44%) in the first group and 1 in the second group (25%), p=0,506 – fig.4.





Conclusions

Patients with PFO and stroke carry more often genetic mutations, associated with thrombophilia than patients with PFO without stroke, although we could not find statistical difference between the two groups. Genetic markers should be evaluated in patients with PFO and stroke and used as a guide for continuous antithrombotic treatment in combination with a proper lifestyle change for secondary prevention.

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УМБАЛ "СВ. ИВАН РИЛСКИ" СОФИЯ

Сравнителен анализ между здрави и пациенти с болест на Уилсън – оксидазна активност на церулоплазмин

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Въведение: Според съвременни проучвания ензимната активност на церулоплазмина (eCp) е потенциален неинвазивен маркер за диагноза на болест на Уилсън (БУ) **(1)**. Целта на настоящето проучване е да се сравнят концентрациите на церулоплазмин измерени чрез имунотурбидиметрия (iCp) и стойностите за eCp между здрави контроли и пациенти с БУ (от Българската популация) на дългогодишна терапия с пенициламинов препарат.

Материали и методи: В изследването са включени 41 здрави доброволци (м:ж=16:25; ср.възраст - 43±13 год.) и 28 пациенти с БУ (м:ж=14:14; ср. възраст 38±12 год.), които са на дългогодишно лечение с пеницилами със средна доза 1000 mg/24h. На всички лица са определени: серумна мед Cu(s) µmol/L с пламъкова атомно-абсорбционна спектрофотометрия, iCp g/L – турбидиметрично, eCp чрез ензимен метод с хромоген субстрат o-dianisidine и е изчислено съотношението eCp/iCp IU/g x 10-1.

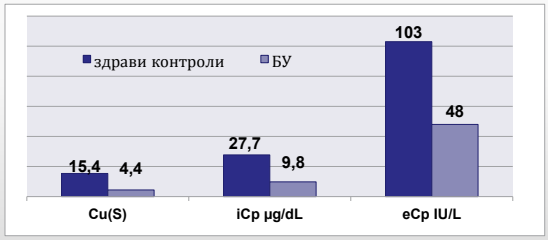
Резултати

Таблица 1 – резултатите са представени като x± SD и обхват

	Cu(s) µmol/L	iCp mg/dL	eCp IU/L	eCp/iCp IU/L
Контроли, n= 41	**15.4**±1.9	**27.7**±2.88	**103.4**±19.41	**4.1**
БУ, n= 28	**4.4**±5.2	**9.79**±10.6	**48.4**±31.9	**6**
P-values	<0.001	<0.001	<0.001	<0.003

eCp референтен интервал: 70 – 130 IU/L

Фигура 1 – сравнение между здрави и пациенти с БУ



Обсъждане: Изследването на eCp и eCp/iCp разширява характеризирането на медния статус в организма и дори се смята, че ензимната активност на Cp има по – висока диагностични възможности при БУ и други състояния с чернодробна патология – хепатална енцефалопатия (ХЕ) и вирусен хепатит Ц (ВХС) и други хронични чернодробни заболявания **(1, 2)**.

Cp се синтезира в апарата на Голджи в хепатоцитите, където чрез посредничеството на протеина ATP7B се натоварва с 6 атома Cu. Така неактивната форма на апо-Cp преминава във функционално активна форма - холо–Cp **(2)**. Регулацията на този процес включва самия синетз на Cp, бионаличността на Cu, състояние на черен дроб, функция на ATP7B протеинът и съответно ATP7B генът.

При БУ мутация в ATP7B генът е причина за нарушено вмъкване на Cu в Cp и следствие на това се синтезира апо–Cp формата, която освен, че е нефункционална е още нестабилна и се разгражда по-бързо в сравнение с холо–Cp. Измерването на eCp може да подкрепи резултатите за iCp при диагностика на БУ и в допълнение би могло да бъде показател при проследяване на понижаващата медта терапия **(2)**.

Проучвания съобщават данни за стойности на eCp под 55 IU/L **(1)** като cut-off стойност за диагностика на БУ. Установените в нашето проучване стойности попадат в тази очаквана граница – 48,4 (от 12 до 134,5) IU/L.

Специфичната активност eCp/iCp дава информация колко точно от общата протеинова молекула на Cp всъщност е активна **(2)**. Това съотношение не се повлиява от фактори на биологична вариация като пол, възраст, хормонален баланс, което е още едно предимство за обективното му използване.

ЗАКЛЮЧЕНИЕ

По данни от литературата като диагностичен критерий при пациенти с новооткрита БУ, eCp има по-висока диагностична специфичност в сравнение с iCp (100% vs. 78.8%;). При новодиагностицирани пациенти с БУ корелационната зависимост между iCp и eCp е по-ниска в сравнение със здрави (R 0.70 vs. 0.94). Получените от нас резултати показват обратното, което дава основание да се заключи, че определянето на eCp, към рутинните маркери за охарактеризирането на медния статус, би могло да бъде обещаващ показател и при проследяване хода на терапията с пеницилами при БУ.

Проучването е осъществено с продкрепата на МУ СОФИЯ, Grant Project 60/2016 – "Референтни граници за оксидазна активност на церулоплазмин за българската популация. Меден статус – нови лабораторни аспекти при пациенти с болест на Уилсън."

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Ортодонтско лечение при пациент със синдром на Търнър: представяне на клиничен случай

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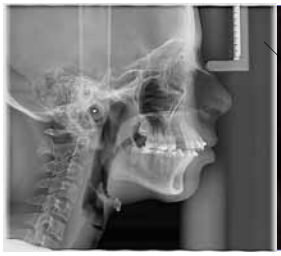
[2]Ортодонт, гр. Пловдив

Въведение

Синдром на Търнър (монозомия Х) е генетично заболяване, причинено от числена или структурна аберация на едната или и двете хромозоми Х. Най-често срещана е Х-хромозомна монозомия (45 Х) засяга 1/2000-2500 живо родени момичета. Поставянето на диагнозата може да стане веднага след раждането.
Пациентите със Синдром на Търнър проявяват, както зъбни, така и скелетни отклонения, изискващи дентална намеса:

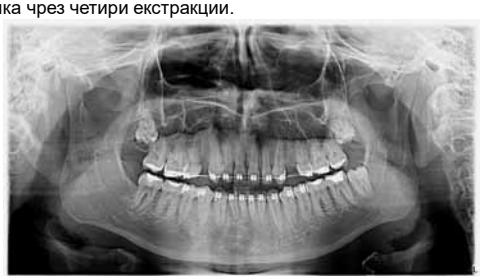
- Закъсняло прорязване на постоянните зъби
- Абнормална форма на коронката и корена на зъбите с редуциран размер
- Повишен риск от коренова резорбция- увеличава възможността от рецидив след ортодонтското лечение
- Периодът на растеж при децата е по-продължителен от средния
- Костната възраст при пациенти със синдром на Търнър не отговаря на хронологическата, поради което на пациентите се предписва терапия с растежен хормон. Времето на терапията съвпада с подходящото време за започване на ортодонтско лечение между десет и четиринадесет годишна възраст, когато обикновено пациентите търсят ортодонтска консултация. Интерсептивното лечение трябва да бъде отложено поради непредсказуеми резултати от провежданата по същото време хормонална терапия. Ортодонтското лечение може да се започне едва когато приключи хормоналната терапия, растежът завърши и настъпи стабилизиране в кранио-фациалните съотношения.


Фиг.1 Телегентгенография


Фиг.2 Екстраорална снимка преди лечение

Клиничен случай

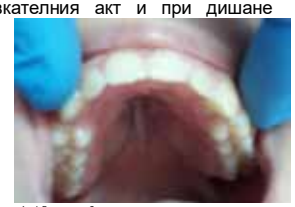
Шестнадесет годишна пациентка със синдром на Търнър след преминало три годишно интерсептивно ортодонтско лечение идва за консултация поради незадоволителен резултат. Денталната история включва рутинни посещения при детски дентален лекар и проведено фиксирано ортодонтско лечение, несъобразено с клиничните особености на придружаващото заболяване. Направен е анализ на ортодонтски модели, анализ на телерентгенография и ортопантомография. (Фиг.1, Фиг.3)
Единствената възможност да се коригира диагнозата отворена захапка (Фиг.2) е с късно ортодонтско лечение с фиксирана техника чрез четири екстракции.


Фиг.3 Ортопантомография

План на лечение

Основна патология

- Горните фронтални зъби не припокриват долните фронтални зъби с разстояние между тях три до четири милиметра, което създава неудобство при дъвкателния акт и при дишане (Фиг.5-8)
- По-къси корени на зъбите
- Готическо небце (Фиг.4)
- Позитивна устна стълбица
- Изпъднати назо-лабиални гънки
- Увиснали устни ъгли


Фиг.4 Готическо небце


Фиг.5-6 Екстраорални снимки преди лечение


Фиг.6


Фиг.7-8 Интраорални снимки преди лечение


Фиг.8

План за лечение

- Корекция на зъбите на горна челюст, която включва деротиране, нивелиране и дистализиране на зъбите.
- Като втори етап следва корекция на зъбите на долна челюст, включваща деротиране, нивелиране и дистализиране на зъбите.
- Синхронизиране на горна и долна зъбна редица. Корекция на зъбен клас II в зъбен клас I оклузия със симетрия и съвпадане на горна и долна централни линии.
- Постигане на баланс в профила на устните за подобряване екстраоралния вид на лицето.

Дискусия

При пациенти със синдром на Търнър зъбно-челюстните деформации се наблюдават по-често и са по-тежки, поради което се препоръчва фиксирано ортодонтско лечение с използване на леки сили за преместване на зъбите. От особено значение е ранното диагностициране и навременно лечение на денталните заболявания със стриктен пародонтален контрол насочен към лична и професионална орална хигиена. Преди всичко трябва да се преценят усложненията на общото заболяване и да се изработи план на лечение съобразен с придружаващата обща патология.

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CRITICALLY ILL MSUD PATIENT - CHALLENGES, WAYS TO PREVENT AND OUTCOME

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KEYWORDS: MAPLE SYRUP URINE DISEASE (MSUD), BRANCH AMINO ACIDS (BCAA), INTENSIVE CARE, HAEMODIALISIS

Maple Syrup Urine Disease (MSUD) is an autosomal recessive disorder caused by abnormal oxidative decarboxylation of branched amino acids – leucine, isoleucine and valine(fg.1). The toxic components in this metabolism are leucine and the ketoacids. Their and the corresponding ketoacids accumulation leads to encephalopathy and progressive neurodegeneration. Brain edema is probably a result of high levels of osmotically active amino acids enteraped in brain cells by high leucine levels. At same time increased intracellular osmolarity and low extracellular sodium levels act together to cause movement of water into the cells . Catabolic events such as severe infections require timely management in an intensive care setting and monitoring of amino acid concentration.

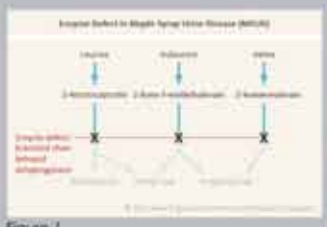

Figure 1

Figure 2

The classical form present in the first week of life. Affected newborns appear normal at birth with symptoms developing between 4 -7 days. The characteristic sweet of maple syrup-like odour in the urine and earwax is one of the first symptoms (fg.2). Other initial signs are lethargy, poor suck, episodic ataxia, cyclic vomiting and lack of interest in feeding. The other variants have initial symptoms by age of 2 years.

Biochemical diagnosis relies on the increasing levels of BCAA and a-keto acids in blood, plasma or urine. In Bulgaria BCAA can be measured using MS/MS analysis (Tandem mass spectrometry) in National genetic laboratory. The urine dinitrophenylhydrazine(DNPH) test detect a-keto acids and can be use for preliminary screening, but is less sensitive than amino acid screening.

We present a MSUD patient, diagnosed and genetically confirmed right after birth. Enteral therapy with metabolic formula and appropriate diet were started immediately and the child was doing well ever since. At age of 4 years the child developed acute respiratory infection during family travel. Proper feeding was interrupted and despite the family instructions to return to the treating unit in cases of emergency if possible, the patient was admitted at the local pediatric ward on the second day of the disease debut. Recurrent episodes of ataxia, lethargy and vomiting followed and 3 days later the child was transferred to us. He presented with GCS – 7 points, brain edema, ketonemia and progressive acidosis.

Three short courses of continuous hemodialysis were performed in 5 consecutive days, full calorie intake was restored parenterally (Intralipid, Dextrose, protein solutions). Using Medical Protocol for Diagnosis, Treatment and Monitoring Maple Syrup Urine Disease of the Department of Paediatrics, King Abdulaziz Medical City, Riyadh and other published protocols, the ketoacidosis and plasma amino acid profile were stabilized. On the day pre-dialysis plasma valine was 487.49 umol/L (24.3-243.2), and leucine – 1567.61 umol/L (50.3-304), but we were only able to see the results after the dialysis launch. In the next days the level of leucine decreased to 626.38 umol/L and the patient started normal enteral feeding. After stabilization the child presented a new gastraintestinal infection, without a moment of decompensation. Table 1 show levels of amino acids - leucine and valine during the decompensation moment, testing with MS/MS analysis. Level of ammonia was 74.218-72nmcl/l,and Na was 137 mmol/l (132 – 146mmol/l) K – 3.5mmol/l (3.5 - 6.0mmol/l) at admission.

Table 1	Before: Acute episode	During: Acute episode	After: 1-st hemodialysis	After 2-nd hemodialysis	After: Acute episode	Reference levels umol/L
Leucine	84.15	1567.61 umol/L	1257.59 umol/L	1033.48 umol/L	626.38 umol/L	50.3-304 umol/L
Valine	180.13	487.49 umol/L	442.11 umol/L	329.13 umol/L	158.16 umol/L	24.3-243.2 umol/L

The purpose of this paper is to describe applicability, safety and efficacy of clinical protocols of acute MSUD treatment from international centers for rare diseases with similar characteristics and resources to ours. In moments of acute decompensation we recommend to:

1.Identify the main reasons for decompensation.
2.Stop all sources of protein central and parenteral intake.
3.Rise the total caloric intake to 125% of normal energy needs for older children.
- Use Dextrose solution 10mg/kg/min.
- Start Intralipid 20 %, 40% of calories can be achieved through 2-3gram/kg/day.
- If tolerated, begin enteral metabolic formula, containing all amino acids except for limited BCAA- home formula supplies include BCAA (free power Ketonex 1 and Ketonex 2).
4.In period of vomiting, give Zofran 0.15 mg/kg/day / Granisteron 10-40 microgram/kg/day .
5.Add Sodium to keep serum Na concentration >140m Eq/l.
6.Try to start isoleucine and valine solutions supplements when the levels approach lower levels of normal; use doses 20mg/kg/day.
7.Control brain edema using Furosemide/Mannitol.
8.Goals of treatment – plasma Leucine level 50.3-304 umol/L, plasma Valine level 24.3-243.2 umol/L.
9.In cases when plasma Leucine is more than 750 umol/L, short courses of continuous hemodialysis must be applied.

GENETIC DIAGNOSTIC SURVEY ON CHILDREN WITH CONGENITAL AND HEREDITARY DISEASES FOR A PERIOD OF TWO YEARS

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INTRODUCTION:

This study is focused on the routine work of the Medical Genetic Service in the Varna region with pediatric patients for a two years period.
Genetic counselling and testing can play an important role in the care and treatment of children and aims better management of a child's condition.

PURPOSE:

To evaluate the activity of the Genetics counselling unit to pediatric patients by indications for referral and verified diagnosis.

METHODOLOGY:

We performed a retrospective analysis of genetic registries for a 2015-2016 year period in the Genetics Unit of the University Hospital St. Marina –Varna.
A survey was conducted for all pediatric patients(0-18 age) who have been consulted or tested in the genetic laboratory.
Clinical phenotyping, imaging examinations, literature review and specialized computer programs /dysmorphology databases were as well applied.
The children were classified according to indications for referral, indicative unit and verified diagnosis.

RESULTS:

The total number of children was 560 (45%) out of 1246 genetically consulted patients for this period (the number of women for biochemical screening excluded).
The majority of the children were referred by pediatric clinics of the UH St.Marina – Varna: 227 (40.5%) from First Pediatric Clinic, 217(38.7%) from Second Pediatric Clinic; 34(6%) from other hospital pediatrics clinics and divisions and 82(14,8%) were outpatients. (Figure1)

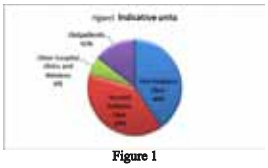


Figure 1

Diagnostic genetic and metabolic investigations were performed in 536 children in our laboratory and in other genetic laboratories in Bulgaria and abroad.
The spectrum of all genetic diagnoses is being presented and analyzed.
235 (42%) pediatric patients were consulted for possible single gene disorders. This group includes 109 children (mostly boys), which were cytogenetically screened for FRA X syndrome for autistic behavior with/or without intellectual disability (Table1)

Suspected single gene disorders		Pediatric patients	Verified diagnoses
Inborn errors of metabolism (IEM)	Wilson disease	5	1 – *homozygote H1069Q/ H1069Q*
	Gilbert's syndrome	4	2 – *homozygote (TA)7-UGT1A1*
	MPS	2	1 - *(positive metabolic screening)*
	Niemann-Pick	2	2 – *homozygotes for mutation c.1177T>G in SMPD1*
	Other IEM	16	1 - Metachromatic leukodystrophy *(enzyme method)*
			1- Congenital neonatal hyperplasia *(neonatal screening)*
			2 - Alkaptonuria
			1- Glycogen storage disease type 9A *(DNA analysis)*
Cystic fibrosis		40	2 - homozygotes - *F508del/F508del*
			6 - heterozygotes	*10.774delTA/N and 5 -F508del/N)*
FRA X syndrome (included children with autistic behavior and/or intellectual disability)		109	1 - *46,fra(X)(27.3)[4]/46,XY)[96] and DNA confirmed FRA X*
			1- 46,XX[2]/46,XY[48]
			1 - 46,XY,del9(q32 -33)
Neurological and neurodegenerative diseases		20	2 - Rett syndrome *(deletion in exons 3 and 4 in MECP2, c.848C>G (MECP2)*
			1 – SMA *(heterozygous deletion in exons 7 and 8 in SMN1)*
			1 - Pontocelebellar hypoplasia *(homozygous mutation c.G31A in EXOSC3)*
			1 - CCFDN *(homozygous mutation ivs 8+389C>T)*
			1- Congenital myasthenia *(homozygous mutation 1267delA2)*
			1- DMD *(deletion of exon 39)*
			2 - Joubert Syndrome *(clinically diagnosed)*
			2 - NF1 *(clinically diagnosed)*
Beta - thalassemia		6	1- compound herozygote *IVS46/IVS 1-100 in HBB)*
			1 - heterozygote *(IVS 1-100N in HBB)*

Table 1

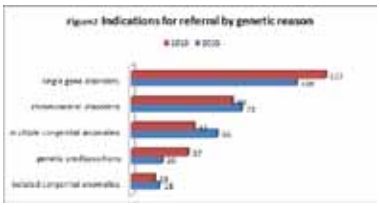
97(17.3%) children were genetically consulted for a diagnosis of multiple congenital anomalies. The diagnoses of 17 children were verified (17.5%).(Table 3)

Multiple congenital anomalies	Pediatric patients	Verified diagnoses
Contiguous gene syndromes	15	1- Silver –Russel syndrome *(abnormal methylation in H19DMR)*
		1- DiGeorge syndrome(*deletion 22q11.21)*
		2- Prader-Willi syndrome
Children with dysmorphic phenotype with or without mental retardation	59	1- Beare-Stevenson cutis gyrata syndrome *(c.1124A>G in exon 8 of FGFR2)*
		1 - Kabuki syndrome *(heterozygote for KMT2D c.12028delT)*
		Diagnoses based on clinical phenotyping, imaging examinations and specialized computer programs:
		3- Cornelia de Lange syndrome
		1 - Sotos syndrome
		1- Aarskog syndrome
		1- Marshall syndrome
		1- Thanatophoric dysplasia
Disorders of sex development (DSD)	23	1- Mixed gonadal dysgenesis :45,X[42]/46,XY[8]
		1- Kallmann syndrome
		1- Denys-Drash syndrome *(clinically diagnosed)*
		1- Swyer syndrome *(cytogenetically and clinically diagnosed)*

Table 3

Figure 2

The indications for referral to genetic counselling service varied, but we divided them into five main groups: single gene disorders – 235 (42%), possible chromosomal disorders – 138 (24.6%), multiple congenital anomalies – 97 (17.3), genetic predispositions - 57 (10%) and isolated congenital anomalies – 33 (5.8%). (Figure 2)



A cytogenetic analysis was performed in 138 suspected for chromosomal disorders patients. Pathological findings were established in 48 children (34.7%).(Table 2)

Possible chromosomal disorders	Pediatric patients	Verified diagnoses
Down syndrome	49	25 – free trisomy 21
		3 - translocational variants
		1 – mosaic variant
Turner syndrome	19	4 - 45,X
		2 - mosaic variants
Klinefelter syndrome	20	1- 47,XXY
Other disorders suspected for chromosomal etiology	50	46,XX,der(18)(9:18)(p22;p11.2)pat
		47,XY,+8[46]/46,XX[4]
		46,XX,add(15)(p11)
		46,XX,del(4)(p15.2)
		47,XX,+mar
		46,XX,+13,rob(13:14)(q10;q10)
		46,XY,del(8)(q11.2 -q13)
		47,XY,+idic(15)(pter -q12::q12pter)[47]/46,XY[3]
		46,XX,t(5;11)(p15.2;q23.2)
		46,XY,del(1)(p34 -35)
		45,X[21]/46,X,der(X;?)(p10;q10)+?[29]
		46,XX,add(19)p

Table 2

57 (10%) pediatric patients were consulted and examined for genetic predispositions:

Thirty three (5.8%) children were referred for genetic counseling with isolated congenital anomalies. These were 17 cases with congenital heart defects, 7 with neural tube defects and 9 with other anomalies (facial clefts, hypospadias, clubfoot).

CONCLUSIONS:

1. Most of the children were referred by University specialized pediatrics clinics (85%).
2. The diagnosis was identified in 30% of the referred children.
3. The survey shows the importance of genetic counselling in childhood, when congenital or hereditary pathology is suspected. Genetics laboratories are centres for highly specialized health care and are focused on rare diseases in children, which need appropriate genetic investigations and long lasting follow up.

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Comparative study of the effect of total extract from Haberlea Rhodopensis leaves and vitamin C on the cell viability of lymphocytes isolated from peripheral human blood

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Introduction

Haberlea Rhodopensis Frisv. (HR) is an evergreen plant of the family Gesneriaceae, the subfamily Didymocarpeae. What is characteristic of it is that it is a poikilohydric biological type, as well as a Balkan endemic species and a relic of the pre-glacial era. In Bulgaria it is mainly found in the Rhodopes and in some parts of Sredna Gora and Central Stara Planina. In the recent years, it has been found that the total extract of HR has an antibacterial, anticlastogenic and antioxidant activity.

Aim

The aim of the research was to perform a comparative study of the effect of the extract of leaves from Haberlea Rhodopensis and L-Ascorbic acid on the cell viability of mononuclear cells isolated from peripheral human blood by an MTT assay and by a test with trypan blue staining.

Materials and methods

Plant materials

Dried leaves of HR, gathered in different locations in the region of Plovdiv, were kindly provided by associate professor B. Popov, from Department of Molecular Biology, Immunology and Medical Genetics. The leaves of the plants were dried and roughly pulverised. A total extract from HR leaves macerated for 72 hours in 70% water-ethanolic solution with subsequent distillation of the ethanol in vacuum evaporizer to a drug/liquid phase proportion of 1:1, was used.

Peripheral blood mononuclear cells isolation, cell cultures and treatment

Venous blood was taken from 10 healthy volunteers with subsequent isolation of the mononuclear cell fraction. Cell cultures were made containing RPMI-1640 medium supplemented with L-glutamine, 10% fetal bovine serum , 1% by volume of a mixture of antibiotics and antimycotics, a part of the cell mixture, the plant extract at different concentrations (0.1, 2, 5, 10 and 20 µl / ml) or L-Ascorbic acid (0.1mM and 10mM). The cell cultures were cultured for 12 hours at 37 ° C, after which the cell viability was assessed by MTT assay and the trypan blue staining test.

Statistical analysis

Data are expressed as mean ± standard deviation. Kruskal-Wallis test was used to determine the statistical differences between mean values. Differences were considered significant when the p value was less than 0.05.

Results and Discussion

Figure 1. Photomicrographs of human lymphocytes prepared by using Trypan blue exclusion technique (A, B and C).

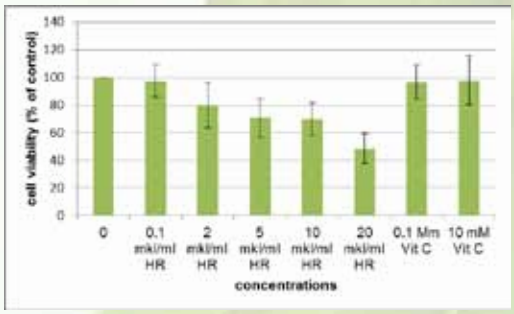

Figure 2. Trypan blue staining test in lymphocytes after an 12 h overnight incubation with various concentrations of extract of HR or L-Ascorbic acid .

The results of the trypan blue staining test show that the administration of a 0.1 mM or a 10 mM solution of L-ascorbic acid or a 0.1mkl/ml HR extract does not result in a statistically significant decrease in cell viability, whereas administration of the HR extract at concentrations of 2 µl / ml to 20 µl / ml significantly reduces the cell viability of the cell cultures studied.

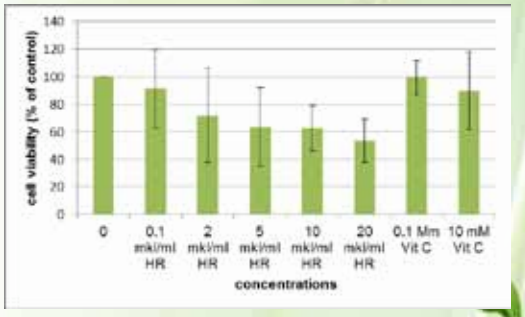

Figure 3. MTT assay in lymphocytes after an 12 h overnight incubation with various concentrations of extract of HR or Vit C.

In the MTT test, the values obtained vary considerably, requiring more extensive research for their accurate interpretation / analysis.

Our team has performed similar studies of comparative influence on cellular viability with another plant extract, received from Lemna Minor L.

Conclusion

The plant extract from dried leaves of HR, applied in concentration of 0.1 µl/ml on lymphocytes isolated from peripheral human blood, has an effect similar to that of vitamin C when applied in a physiologic concentration (0.1mM).

The application of the extract from HR in a concentration of o.1µl/ml is particularly suitable for evaluation of a predicted anticlastogenic effect in human lymphocyte cultures under the influence of γ radiation or antineoplastic medicines which have proven clastogenic effect.

The studies were performed under scientific research projects №7/2017 and №14/2017, Trakia University, Stara Zagora, Bulgaria.

Rare syndromes with unusual dental manifestations (review)

Nina Musurlieva; Medical University – Plovdiv, Faculty of Public Health, Department of Social Medicine, and Public Health
Keti Yovcheva; Medical University – Plovdiv, Faculty of Dental Medicine, Department of Orthodontics

The aim of this poster is to draw attention to rare syndromes with unusual dental manifestations and jaws abnormalities. Craniofacial anomalies account for approximately one-third of all congenital birth defects reflecting the complexity of head, facial and dental development. Craniofacial development is dependent upon a multipotent, migratory population of neural crest cells, which generate most of the bone and cartilage of the head and face.

Methods and materials: A systematic review of available literature in Pub Med was provided.

Results: Table 1 presents the most common orofacial manifestations, features and inheritance of various rare syndromes. Several syndromes of craniofacial region have their origin in mutations in fibroblast growth factor (FGF) receptor genes, two transcription factors, MSX2, core binding factor 1 gene (CBFA1), etc. It is now understood through advance research on molecular genetics that in cleidocranial dysplasia, mutations in the core binding factor 1 gene (CBFA1), results in defects in the membranous bones of the cranial vault and clavicles due to deficiencies in signaling between the periosteum and chondrocytes essential for endochondral bone formation.

Oculo-facio-cardio-dental (OFD I and II) syndrome is a X-linked rare congenital anomaly, with incidence of less than 1 in 1 million people and it might be lethal in males. Heterogeneous clinical features characterize it such as dental radiculomegaly (extremely long roots), particularly of the canines and occasionally of other teeth including premolars and incisors, congenital cataract, facial dimorphism, and congenital heart disease.

Proteus syndrome is an extremely rare congenital disorder with progressive asymmetric overgrowth of multiple tissues. Oral manifestations of Proteus syndrome may include gingival overgrowth and malposition of teeth, as well as unilateral enamel hypoplasia.

Ellis-van Creveld syndrome also called chondroectodermal dysplasia is an autosomal recessive disease. A typical case of this syndrome exhibits the following tetrad-disproportionate dwarfism due to chondrodysplasia of long bones, bilateral postaxial polydactyly of the hands, ectodermal dysplasia (nails are small and dystrophic, generalized hypodontia and abnormally formed teeth), congenital heart malformations. Other oral symptoms are: labiogingival frenulum hypertrophy, accessory labiogingival frenula, diastema, enamel hypoplasia, teeth may show premature eruption at birth or premature exfoliation. Supernumerary teeth may also be present.

Dental abnormalities are present in around 30% of patients with Gardner syndrome, and may include supernumerary teeth, compound odontomas, hypodontia, abnormal tooth morphology and impacted or unerupted teeth. The highest incidence of dental abnormalities is found in patients with multiple osteomas, but dental changes may be determined in the absence of skeletal lesions, and the dental anomalies are not secondary to bony changes.

Treacher-Collins syndrome is defined clinically by bilaterally symmetrical features that include hypoplasia (underdevelopment) of the facial bones, in particular the mandible (lower jaw) and zygomatic complex (cheek bones) (Figure 1) and micrognathia (small lower jaw) with or without cleft and/or high-arched palate.

ORGAN	FEATURE	SYNDROME	INHERITANCE
Tongue	Macroglossia	Beckwith-Wiedemann	Sporadic
	Microglossia-aglossia	Aglossia-adactyloia,	Sporadic
		Möbius	Sporadic
	Multilobulated	OFD I	Dominant, X-linked, or autosomal
	Bifid	OFD II	A.R.
Palate	Cleft lip and palate	Associated with several syndromes: EEC syndrome, OFD I and II, Trisomy 18, Van der Woude	A.D. Chromosomal A.D.
	High-Arched	Apert	A.D.
		Cleidocranial dysplasia	A.D.
		Crouzon	A.D.
	Isolated cleft palate	Apert	A.D.
		Robin	Sporadic
		Stickler	A.D.
		OFD I	(see above)
		Treacher-Collins	A.D.
Soft tissue of mouth	Frenulae	OFD I and II	(see above)
		Ellis van Creveld	A.R.
	Gingivae	Ehlers-Danlos	
		Papillon Lefèvre	A.R
		Cowder	A.D.
Mandible	Micrognathia	First arch	Various
		Hypoglossia-hypodactylia	Sporadic
		Robin	Sporadic
		Fetal methadone	Sporadic
		Hallermann-Streiff	A.D.
		Chromosomal (13, 18 trisomies)	--
	Asymmeteric	Facioauriculovertebral	Sporadic
		Klippel-Trenaunay-Weber	Sporadic
	Prognathism	Acrodysostosis	Sporadic
Maxilla	Hypoplsaia	Maxillonasal dysplasia	Sporadic
		Stickler	A.D.
		Crouzon	A.D.
		Trisomy 18	--
		de Lange	Sporadic
Oral Opening	Microstomia	Otopalatodigital	X-linked dominant
		Robinow	A.D.
		Hellermann-Streiff	A.D.- Sporadic
	Macrostomia	Facioauriculovertebral	Sporadic
		Mucopolysaccharidoses	Various
		Trisomy 18	--

Table 1. Orofacial considerations in the diagnosis of Craniofacial syndromes

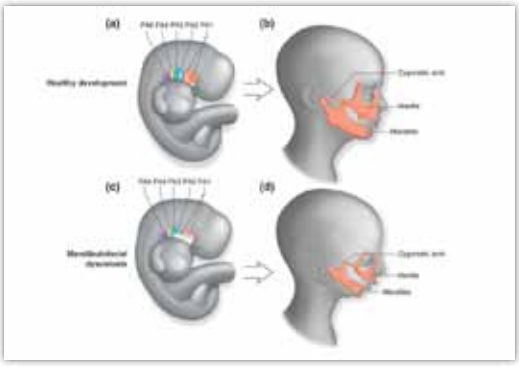

Figure 1. Mandibulofacial dysostosis. (a) Schematic of the pharyngeal arches of a healthy human embryo. (b) Maxilla and mandible bone structures derived from neural crest cells that colonize the first pharyngeal arch. (c) Schematic of the pharyngeal arches of a human embryo with mandibulofacial dysostosis which arises as a consequence of hypoplastic first and second pharyngeal arches. (d) Hypoplastic maxilla and mandible bone structures observed in mandibulofacial dysostoses. (in:WIREs Dev Biol 2017, e263. doi: 10.1002/wdev.263)

Children with congenital malformations and syndromes involving craniofacial structures are at a significant risk for dental and jaws abnormalities. The number of syndromes and isolated malformations is great. Syndromes are one such pathology which arises from different etiologies resulting in mild to fatal consequences both with respect to morphology and function of the stomatognathic system. Craniofacial abnormalities are a significant part of syndrome and dental professionals have an essential role to play in diagnosis of syndromes.

Захарен диабет и лош апетит – каква е диагнозата?

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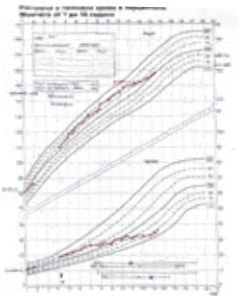
Увод: Захарният диабет е 2ро по честота хронично заболяване в детска възраст. Често той е "маска", под която се крият генетични синдроми. Представяме пациент с клинични симптоми на *Wolfram (DIDMOAD) синдром* и първоначални оплаквания от лош апетит и ненаддаване на тегло. При проследяването са установени аномалия на отделителната система, оптична атрофия и инсипиден диабет. *Wolfram* синдром е с честота 1:770 000 автозомно рецесивно невроендокриннно дегенеративно заболяване. Причинява се от мутация на гена *WFS1,* който кодира трансмембранен протеин валфарин от ендоплазматичния ретикулум. Допуска се, че около 1% от общата популация са носители на мутацията и хетерозиготите са сигнификантно рискови за психиатрични заболявания.

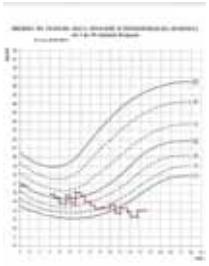
Анамнеза: Юноша на 13 г. 6 м. с давност на захарния диабет от 4 г.в. (фиг.1) открит по повод полидипсо-полиуричен синдром без данни за кетоацидоза и липсваща собствена инсулинова секреция (C-peptid 0.167 ng/ml). Роден от 2ра нормална бременност и раждане, Тр 3 400 г, Рр 52 см. Гладък перинатален период без данни за продължителен иктер, кърмен до 6 м.в. Редовно имунизиран, липсват данни за алергични реакции, спортувал до 11 г.в.



Фиг. 2. Растежна крива

Минали заболявания: Пиелонефрит на 2 г.в, когато е обективизирана аномалия на отделителната система (десен бъбрек с пиелектазии, ляв хидронефроза 1ва ст., неврогенен пикочен мехур).

Фамилна анамнеза: ЗД2Т баба по майчина линия.
По-голям брат без ендокринни и психични заболявания, суицид
Социално-икономически статус: много добър



Фиг. 3. Индекс на телесна маса

Ауксология (фиг. 2, 3):
- Тегло: до 7 г.в. на 75ти р, задръжка на кривата до 10 г.в. с последваща децелерация до 25ти р.
- Ръст: в горните граници на генетичната прогноза ($SDS_{акт.ръст}$ = +0.14 v SDS-MPH = -0.52)
- Костна възраст на 12 г.в., изостава с около 2 г.
- Начало на пубертетното развитие от 13 г.в., но все още липсва "пубертетен скок"

Фиг. 1. Пациентът на 5 г.в.

Лечение:
- Трайна и продължителна ремисия в рамките на 3 г., наложила 2-кратен инсулинов режим.
- Гликемичен контрол НвА1с м/у 6.6 – 7.7%
- На 7 г.в. по повод лош апетит и децелерация на тегловна крива, ИТМ 15.7 kg/m^2 v 14 kg/m^2 (съответно 50ти -10ти р) е инициирано лечение с инсулинови аналози.

Табл. 1. Проследяване на състоянието

Възраст	Основен проблем	Отхвърлена Дг	Потвърдена Дг
11г.в	Лош апетит Failure to thrive астено-адинамичен см	Хипогликемии Инфекциозен см Хипотиреоидизъм	Анемичен см
11 9/12	+ негативно отношение към храната, дисфагия, периоди на диария	ГЕРБ цьолиакия	Психогенни причини за намаляване на апетита
12	+ стрес + отказ от храна		Мегавезика, мегауретер, хидронефроза поради пасивен ВУР
12 6/12	+ главоболие, запек, влошен успех в училище, затруднено зрение	Хипогликемии Инфекциозен см Хипотиреоидизъм Анемичен см	"тапеторетинална дегенерация със съпътстваща оптична атрофия"
13 5/12	+ "липса на сили"		Субклиничен инсипиден диабет Намаление на слуха от сензорен тип

Табл. 2. Критерии за диагноза *Wolfram синдром*

Критерии за диагноза *Wolfram синдром*	При пациента
Големи: ·Захарен диабет <16 г.в. ·Оптична атрофия <16 г.в.	+ +
Малки: ·Инсипиден диабет ·Захарен диабет >16 г.в. ·Оптична атрофия >16 г.в. ·Намален слух от сензорен тип ·Невралогични с-ми (атаксия, епилепсия, когнитивни нарушения) ·Аномалии на отделителната с-ма (структурни или функционални) ·Мутация в гена *WFS1/CISD2* и/или фамилна анамнеза за *Wolfram см*	 + +
Други съмнителни състояния: ·Хипогонадизъм у мъже ·Липса на автоантитела при ЗД1Т ·Билатерална катаракта ·Психиатрични нарушения ·Гастроинтестинални заболявания	
Критерии за Дг *Wolfram см:* *2 големи или 1 голям+2 малки или* *Идентифициране на 2 патологични мутации в WFS1 или CISD2*	

Проблеми:
- Предложено е генетично изследване
- От страна на пациента отказ:
  - за консултации с психолог
  - посещения при лекар/хоспитализациии
  - за прием на лекарства
  - осигурено е индивидуално обучение в училище
- Разговаряно е със семейството за:
  - същността и прогнозата на заболяването
  - възможности за включване в програмата EURO-WABB
  - наличните проучвания с валпроат върху подобряване на психиатричните и зрителни проблеми.
    Валпроата потиска стреса на ендоплзматиния ретикулум чрез регулиране на WFS1.

Дискусия:

Възможно ли е самоубийственото поведение на брата също е да проява на *Wolfram* синдрома?

Как да се организира хранителния режим?

Изводи:
- Лошият апетит е често срещан симптом в детската възраст.
- Захарният диабет е "маска", под която може да се крият различни заболявания и синдроми.
- Подробната анамнеза и системното проследяване на пациента, доверието м/у семейството и лекарските екипи са в основата за своевременна диагностика и адекватно лечение.
- *Wolfram (DIDMOAD)* синдром е рядко срещано заболяване с начало в детската възраст, изискващ отлична комуникация между системата на здравеопазване, образование и социални служби.

Авторът изказва благодарности за съвместната работа с д-р В. Атанасова (семеен лекар), д-р М. Панайотова (детски гастроентеролог, Болница "Тракия"), д-р Драгоева (офталмолог), мед. сестра Вичева (ДГ "Железник"), д-р Момчилов (УМБАЛСМ "Пирогов"), Клиника по Диабет при СБАЛДБ, Очна Клиника при МА София

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Комплексно лечение при пациент с болест на Wilson

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Въведение: Болестта на Уилсън е описана за първи път от д-р Samuel Alexander Kinnier Wilson, британски невролог, през 1912 година, като той я нарича „хепатолентикуларна дегенерация“.Тя е наследствено автозомно-рецесивно заболяване, причинено от мутация на АТР 7В гена, който е мембранно-свързана мед-транспортираща АТФ-аза. Нарушената екскреция на мед повишава серумната й концентрация и води до натрупването й в организма. Наблюдават се мултиорганни прояви от страна на черен дроб, нервна система, бъбреци, очи, сърце.Болестта на Уилсън е много рядко заболяване с честота 1 на 30 000-40 000 от населението с по-висока честота в Северна Индия и Сицилия.

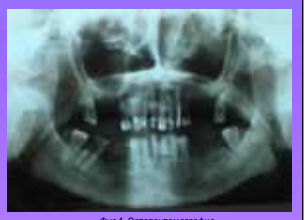
Началото на заболяването е в ранна детска възраст като тогава се наблюдават симптоми от страна на черния дроб. Първите изяви на засягане на нервната система се проявяват с интелектуален дефицит. Намалява се концентрацията в училище, пациентите се депресират от забавянето на говора и некоординираните движения на ръцете. Мускулната ригидност и брадикинезията водят до развитието на клинична картина, наподобяваща ювенилен Паркинсонизъм. Останалите клинични промени настъпват около 5-та декада. Наличието на златисто-кафяв пръстен на пигментация по външния ръб на корнеята, наречен пръстен на Keyser-Fleischer, се наблюдава при засягането на нервната система и наличието на неврологична симптоматика.

Клиничен случай: Представяме 46-годишна жена на видима възраст, неотговаряща на действителната.Анамнестично пациентката съобщава за болест на Уилсън, която е потвърдена през месец юни 2015 и е започнато лечение с Купренил. Оплакванията започват от 2000 год. с появата на тремор на глава, слабост при ходене и залитане. Проведен е КАТ на глава с данни за исхемична зона в лява голямо-мозъчна хемисфера на нивото на путамена, като е приета с диагнозата исхемичен инсулт с десностранна хемипареза. През 2002 год. от ЯМР изследване се установява двустранна некроза на базалните, мезенцефалните и церебеларните ядра. Поради прогресиращ дискоординационен синдром и хемипареза, както и новопоявили се смущения в гълтане особено на твърда храна, промени в говора, апатия, залежаване, хепатоспленомегалия, при пациентката е проведено изследване на обмяната на медта и е потвърдена диагнозата на болест на Уилсън. От общия статус се установява астеничен хабитус, кифосколиоза на торакален и лумбален отдел. Жената е брадипсихична с дизартрия, говорът е сакадиран, завален на моменти. Установява се тремор в покой на горните крайници, по-изразен в дясна ръка с контрактура в десните метакарпофалангеални стави, ригидно повишен тонус на четирите крайници, по-изразен в дясно. Спастично-атактична походка. Наблюдава се типичният пръстен на Кейзър –Флейшър.

Фиг.1 Екстраорален статус

Фиг.2 Пръстен на Кейзър-Флейшър

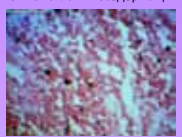
Фиг.3 Интраорален статус



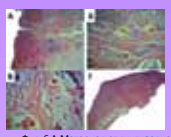
Фиг.4 Ортопантомография

От локалния интраорален статус се установи частично обеззъбена горна и долна челюст, обилно отложен зъбен камък, хроничен генерализиран пародонтит II-III степен. Липсва пигментация по гингивата, езикът е необложен. Обширни кариозни лезии на моларите и големи обтурации с вторичен кариес по фронталните зъби. Обтурациите са маргинално оцветени

Материали и методи: Комплексното лечение започна с поетапна екстракция на зъби 18, 48, 47, 41, 42, 28, 38. Поради увреждане на ч.дроб се използваха местни анестетици от амидната група- артикаин, които съдържат тиофенов пръстен, като имат два пътя на елиминация – през черния дроб и плазмата, така че се намалява токсичния ефект от кумулацията им.Намаленото производство на коагулационни фактори наложи изследването на протромбиновото време и INR.Приложени бяха местни хемостатици - стерилна желатинова гъба, както и хирургична обработка на екстракционните рани-сутура. Направено бе хистологично изследване на лигавица и кост, при което се установи наличие на мед в тези тъкани.Вторият етап от комплексното лечение включваше обработка на наличните кариозни лезии и възстановяване на разрушените зъбни повърхности. Протезирането на пациента се осъществи, чрез изработване на снемаеми и неснемаеми конструкции, възстановяващи говорна, дъвкателна функция и естетика.Металните елементи бяха направени от сплави несъдържащи мед.



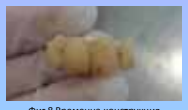
Фиг. 5 Сред еритроцити са разположени макрофаги с наличие на черно-зелени гранули в цитоплазмата (оцветяване с рубеанова киселина, х200).



Фиг .6 А.Материал от гингивална лигавица с хиперплазия на многослоен плоъск епител и наличие на възпаление и кръвоизливи в субепителна съединителна тъкан (ХЕ, х40), Б. Сред възпалителния инфилтрат се намират остеокластподобни гигантски многоядрени клетки (жълти кръгове) (ХЕ, х100). В. Субгингивално разположени макрофаги с наличие на кафеникава гранули в цитоплазмата (жълти кръгове) (ХЕ. х200). Д. Материал от кост с умерена редукция на костно вещество (ХЕ, х40).

Фиг.7 Поставяне на терминална анестезия със Septanest 1:200 000 за екстракция на 18 зъб.

Неснемаема конструкция обхващаща фронтална област в горна челюст.(фиг.7,8)



Фиг.8 Временна конструкция

Фиг.9 Постоянна конструкция

Неснемаема конструкция обхващаща фронтална област в долна челюст.(Фиг.9)

Фиг.10 Постоянна конструкция

Фиг.11 – Преди лечението

Фиг. 12– След лечението

Заключение: Заболяването е изключително рядко, приблизително 1 на 40 000, със сериозно увреждане на черен дроб и нервна система, което изисква задълбочен анализ и обсъждане, както на клиниката, така и на параклиничната ситуация с цел преодоляване на усложненията и постигане на желания резултат.

The MLL rearrangements with additional chromosomal aberrations in infant patients with AML.
2 cases report

Authors: **Lesia Mazurenko**, Yana Muratova,
Tatiana Ponomarenko, Tamara Smolenska, Alexandra Sharamok, Tatiana Didyk, Elena Kreminska and Svetlana Donska

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INTRODUCTION

Pediatric acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disease. In addition to the patient's initial response to treatment its prognosis is largely determined by the presence of cytogenetic abnormalities and genetic lesions. Several recurrent cytogenetic abnormalities, such as 11q23/MLL-rearrangements, predict outcome in myeloid neoplasms and acute leukemia. MLL gene rearrangements are found in more than 70% of the cases of infant leukemia, both acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML), but are less frequent in leukemia from older children (1). Different translocation partners have been identified, and new partners are still being reported to add to the diversity of MLL-rearranged leukemia. Recent international studies highlighted the heterogeneity of 11q23/MLL-rearranged pediatric AML by demonstrating that outcome is dependent on translocation partners. The study of 733 de novo pediatric 11q23/MLL-rearrangements AML patients showed that specific additional cytogenetic aberrations (ACAs) were an independent adverse prognostic factor(2). 2 new cases of the 11q23/MLL-rearranged AML, associated with ACAs and complex karyotype in infants can supplement knowledge about this type of leukemia.

METHODS

1. Karyotyping: the diagnostic bone marrow samples were cultured for 24 h in RPMI 1640 medium with L-Glutamine and 25mM HEPES (Gibco by Life Technology, UK) supplemented with 20% fetal bovine serum (Gibco by Life Technology, UK). Chromosome preparations were made by standard methods and banded by trypsin (Gibco by Life Technology, UK). Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (Shaffer et al., 2013).
2. Nuclear and metaphase FISH (Fluorescence in situ hybridization) performed using locus specific and enumeration probes (Abbot Molecular/Vysis,USA) .

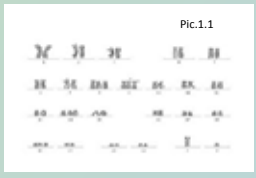
Case 1
REPORT

11-month old boy with white blood cell count 5,6x10^9/L, Hgb – 126 g/L, plt – 94x10^9/L, hepatosplenomegaly, CNS, skin and gonads involvement with preceding immunodeficiency and septic complications for two month. Morphology and immunophenotyping of bone marrow and peripheral blood further confirmed AML (FAB-5a) with monoclonal blast population (31%) which showed the monoblastic phenotype with aberrant coexpressions: CD45+,CD34-,CD38+,HLA-DR+,Anti-MPO-,CD33+,CD65+,CD64+,CD4+,**CD56+**,CD11c+.

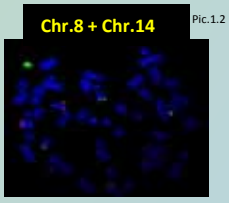
RESULTS

To investigate this case, we started from nuclear FISH using LCI and enuveration probes panel for AML cases as a screening, speed and high informative method. The MLL-rearrangement probe showed the presence of the break in 11q23 and also the presence of an additional orange-colored material containing 3' part (nuc ish (5' MLL×2,3' MLL×3) (5' MLLcon3'MLL×1)). Enuveration probes showed +8 and +14 (Pic.1.2). GTG-banded karyotyping of the bone marrow were perfomed and showed the presence of additional chromosomes 8,14,19 and translocation t(9;11) as well as the presence of another derivative chromosome 9 (Pic.1.1). The next step we made was FISH on metapheses using mixed 2 probes: LSI **BCR/ABL**, Dual Color, Dual Fusion Translocation Probe and LSI **MLL** Dual-Color, Break-Apart Probe (Abbot Molecular/Vysis). This step confirmed the presence of two derivative chromosomes **9** - der(9)(9;11). Picture 1.3 demonstrate normal chromosome 9 with orange signal on q34, both chromosomes 22 with green signals, normal chromosome 11 with fusion signal on q23, derivative chromosome 11 with small green signal containing 5' part and 2 derivative chrpomosomes 9 - der (9)(9;11) - with orange signal on q34 and smaller orange on short arms containg 3' part.

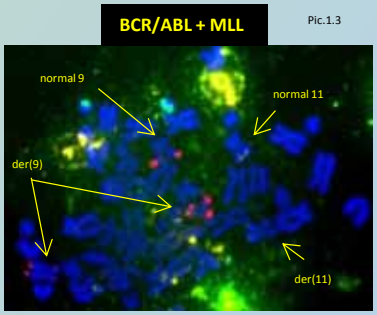
Pic.1.1



Pic.1.2



Pic.1.3



Case 2
REPORT

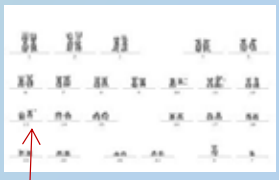
In a 2,5-month infant with suspected congenital immunodeficiency monoblasts were detected in the study of peripheral blood lymphocytes subpopulations. White blood cell count was 10,7x10$_9$/L, Hgb – 79 g/L, plt – 165x10^9/L, significant hepatosplenomegaly, CNS, skin and gonads involvement. Morphology and immunophenotyping of bone marrow and peripheral blood further confirmed AML (FAB-5a) with monoclonal blast population (37%) which showed the monoblastic phenotype with aberrant coexpressions: CD45+,**CD34+**,CD38+,HLA-DR+,Anti-MPO-,CD33+,CD65+,CD15+,CD64+,CD4+, **CD56+**,CD11c+.

RESULTS

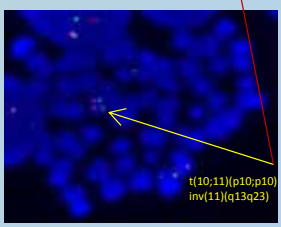
The study performed by using fluorescence in situ hybridization (FISH) and convertional karyotyping of bone marrow. FISH using the MLL Dual Color, Break Apart Rearrangement Probe (Vysis/Abbot, Des Plaines, IL,USA) on metaphes confirmed the MLL gene rearrangement showing the 5' probe high and 3' probe lower on the long arm of the der(11). ish inv(11)(q13)(5'MLL+)(q23)(3'MLL+) (Pic.2.2). Convertional karyotyping showed the presence of 46 chromosomes with two isochromosomes 10 and 11 over the short and long arms (46XY,t(10;11)(p10;p10)inv(11)(q13q23),inv(13)(p11.2-13q32)), invertion 13 chromosome (Pic.2.1.,2.3) that also was confirmed by metaphase FISH analysis (ish inv(13)(p11.2)(D13S1020+)(q34)(D13S1020-)) (Pic.2.4).

Pic.2.1

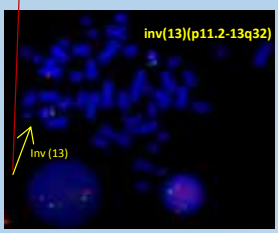
Pic.2.3



Pic.2.2



Pic.2.4



CONCLUSIONS

1.The nuclear FISH is still the fastest and most highly informative method for MLL-rearragement search.
2. Standard karyotyping gives additional information about other aberrations such as the presence of other chromosome rearrangements, additional chromosomes, possible chromosome 11 partner´s in MLL-rearrangements.
3. Metaphase FISH analysis is very useful and often necessary to confirm the results of karyotyping and identifying a MLL-rearrangement partner chromosome or even a partner gene.

ACKNOWLEGEMENTS

Department of Intensive Chemotherapy, head dr.I.Stetsyuk.
Department of BMT, head dr.O.Ryzhak.

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2. Prognostic significance of additional cytogenetic aberrations in 733 de novo pediatric 11q23/MLL-rearranged AML patients: results of an international study. Coenen EA, Raimondi SC, Harbott J, Zimmermann M, Alonzo TA, Auvrignon A, Beverloo HB, Chang M, Creutzig U, Dworzak MN, Forestier E, Gibson B, Hasle H, Harrison CJ, Heerema NA, Kaspers GJ, Leszl A, Litvinko N, Lo Nigro L, Morimoto A, Perot C, Reinhardt D, Rubnitz JE, Smith FO, Stary J, Stasevich I, Strehl S, Taga T, Tomizawa D, Webb D, Zemanova Z, Pieters R, Zwaan CM, van den Heuvel-Eibrink MM. Blood. 2011 Jun 30;117(26):7102-11.

MOLECULAR DETECTION OF VIRUS HERPES SIMPLEX TYPE 1 IN PATIENTS WITH PERIODONTAL DISEASE

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Introduction

Periodontal disease is an inflammatory-destructive disease of the supporting tissues of the teeth caused by periopathogens microorganisms.

The aim of this study was to analyze the presence of herpes simplex virus type 1 (HSV-1) in the dental plaque (supra- and subgingival), to examine the possible association with the stage of periodontal disease.

Materials and methods

Supragingival and subgingival dental plaque samples were taken from a total of 89 patients with periodontitis, and DNA was extracted.

Fifty-four (60.7%) of the patients had a moderate clinical stage, while 35 (39.3%) of them had an advanced clinical stage of the periodontal disease.

Molecular detection of HSV-1 was performed using the primers H1P32 (5'-TGGGACACATGCCTTCTTGG-3') and H1M32 (5'-ACCCTTAGTCAGACTCTGTTACTTACCC-3'), by PCR method. Presence of a 147 bp fragment confirmed the presence of HSV-1 virus in the analyzed sample (Figure 1).

Angiotensinogen was used as a control gene for monitoring the success of HSV-1 PCR amplification by using the pair of primers oligo25/oligo26, resulting in PCR product of 165 bp (Figure 1).

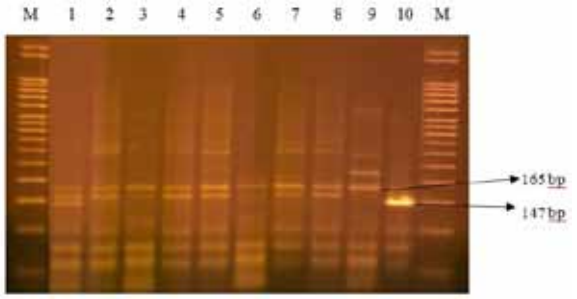

Fig. 1. Molecular detection of HSV-1 virus in patients with periodontal disease (PCR product of 147 bp) - line 1, 10. PCR product of control gene (165 bp) - lines 1-10; M-marker (50 bp)

Results and discussion

HSV-1 virus was detected in 22/89 (24.7%) patients with periodontal disease.

HSV-1 was observed in 12/54 (22.2%) patients with moderate stage of the disease, of which in all of them in the supragingival and in only 2/12 (16.7%) patients in the subgingival plaque samples, $p<0.05$.

In 10/35 (28.6%) patients with advanced stage of the disease the HSV-1 virus was detected, of which in 6/10 (60%) patients in supragingival and 6/10 (60%) in subgingival plaque samples, $p>0.05$.

There was not statistically significant difference in the frequency of detected HSV-1 virus between the patients with moderate and advanced periodontal disease ($p=0.49$).

Opposite, the frequency of detected HSV-1 virus was significantly higher in the subgingival plaque of the patients with advanced disease compared to patients with moderate periodontal disease ($p>0.05$).

Conclusion

HSV-1 was present in patients with periodontal disease in both supragingival and subgingival plaque samples.

The significantly higher frequency of HSV-1 detected in the subgingival plaque in patients with advanced disease compared to patients with moderate periodontal disease might suggested that the presence of HSV-1 is related with the different degree of periodontal destruction.

MOLECULAR DIAGNOSTIC APPROACH IN NEURO-ONCOLOGY

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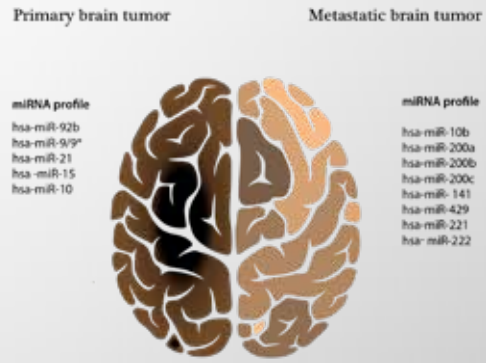
INTRODUCTION

Brain tumors are characterized by invasive nature and difficulties to resect surgically all neoplastic cells with frequent relapses. Secondary or metastatic brain tumors (MBTs) begin as tumors in other parts of the body (primary tumors), which eventually metastasize to the brain. MBTs are the most common brain tumors. Differentiation between primary and metastatic brain cancer, as well as its origin may improve prognosis of the patients by giving them a chance for adequate anticancer therapy. Metastatic brain cancer is usually a very aggressive disease with a poor prognosis. Despite the current options for diagnosis, even after extensive and costly examination, in approximately 11 % of patients with MBTs, the primary tumor site remains unknown.
The role of epigenetic mechanisms and post-transcriptional gene regulation in neuro-oncology has been widely investigated. Among many possibilities, the potential role of the non-coding RNAs, in particular microRNAs, as a non-invasive biomarker in the diagnosis and therapy of normal and malignant tissue, as well primary and metastatic brain tumors, has recently received increased attention.

METHODS

qRT–PCR is used for miRNAs expression profiling analysis and differentiation of normal brain tissue, primary and metastatic brain tumors. The following miRNA signature has been selected:

- hsa-miR-21 - together with miR-15 markers in CSF for glioma that can distinguish from normal individuals and from patients with brain lymphoma and metastatic brain tumors
- hsa-miR-10b - undetectable in normal brain, while highly expressed in the most aggressive form – glioblastoma multiforme - GBM; promotes cell migration
- hsa-miR-92b and hsa-miR-9/9* are over-expressed in primary brain tumors, as compared to primary tumors from other tissues and their metastases to the brain
- members of the hsa-miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) are highly elevated in patients with brain metastases but not with any other pathologic conditions, allowing discrimination between glioblastoma and metastatic brain tumors
- metastasis-promoting miRNAs miR-221/222



RESULTS

In the present study, different patients' samples like tissues, plasma and/or CSF have been collected for microRNA analysis. The determination of the unique miRNA signature is ongoing, as well biobank gathering of such samples.

CONCLUSIONS

Dysregulation of miRNAs in human cancer has raised possibilities for their clinical application as a potential source of diagnostic and prognostic biomarkers. Selecting a specific miRNAs signature has an opportunity for differentiation between normal and cancerous cells, primary and metastatic tumors, and in the future, recurrence probability and therapy efficacy.

Investigation of noninvasive urine specimens with molecular profile fluctuations for a presence of high-risk Human Papilloma Viruses as an inflammatory cofactor in the complicated origin of prostate cancer

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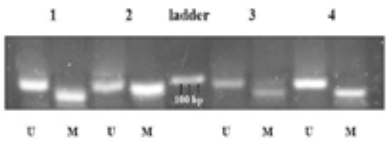
AIM OF WORK

- Investigation of a high specific molecular panel (*PCA3*, *TMPRSS2-ERG* fusions and *GSTP1* promoter hypermethylation) for early diagnostics in suspected PCa patients by noninvasive urine specimens.
- Analysis of the presence of high-risk *Human Papilloma Viruses* (HPV) as an inflammatory cofactor in the complicated origin of PCa on urine specimens, obtained after digital rectal examination (DRE).
- **Materials and Methods**: RNA and DNA isolation; Reverse transcription; Real-time PCR; DNA sequencing; Bisulfite conversion of DNA; Methylation-specific PCR; Cytological preparations and staining

MOLECULAR RESULTS

✓ **Neoplastic *GSTP1* allele, *PCA3* strongly elevated expression or hyperexpression were registered in most of the patients.**
✓ **Only in 4 cases a positive *TMPRSS2-ERG* status was detected.**

Methylation profile of GSTP1 gene promoter

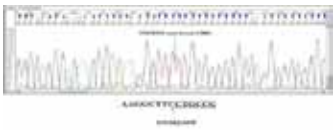


Methylation-specific PCR of *GSTP1* gene promoter U= unmethylated; M = methylated *GSTP1* alleles.

PCA3 expression profile

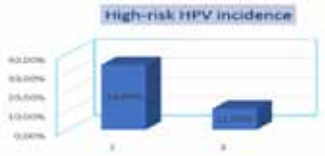
Elevated levels of *PCA3* expression in noninvasive urines in suspected PCa patients.

Sequencing profile of TMPRSS2-ERG fusion in urine of patient with PCa



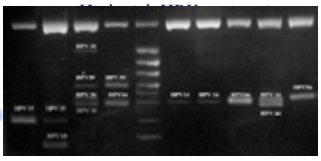
T2 exon1-ERGexon 4 fusion transcript-the most often detected splice variant.

VIRAL ANALYSIS



1. Positive HPV in PCa suspected patients, based on their molecular profile.
2. Positive HPV in a verifying control group.

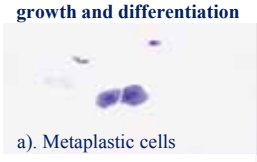
96% of detected high-risk HPVs are: 16, 33, 35, 31, distributed in the subgroup with highest oncogenic potential !!!



High-risk HPV types, detected in urines of suspected PCa patients.

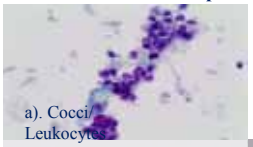
CYTOLOGYCAL RESULTS

A. Adaptations of cellular growth and differentiation

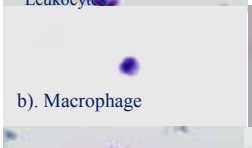


a). Metaplastic cells

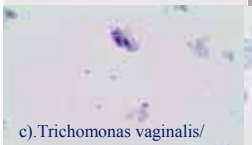
B. Inflammation and repair



a). Cocci/ Leukocytes

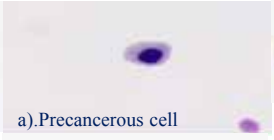


b). Macrophage



c).Trichomonas vaginalis/ Spermatozoon

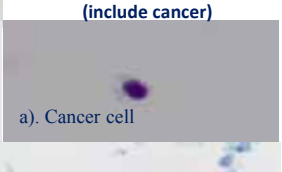
C. Precancerous changes



a).Precancerous cell

b).Precancerous cells

D. High grade alterations (include cancer)



a). Cancer cell

b). Cancer cells with a tendency for adenostructure formation

c).Cancer cell with macronucleoli

d). Tumor Diathesis

CONCLUSIONS:

- The chosen molecular panel definitely contributes to better and earlier PCa diagnostics and distinguishing of high risk PCa probands.
- High-risk HPVs mainly (16, 33, 35, 31) were determined as a cofactor in PCa on the base of our molecular and virological data for higher frequency and partially observed cytopathic effect.
- Normal findings and alterations, associated with an inflammatory process and response were registered cytologically.

-In a proportion of patients with molecular PCa disturbed profile the following cytological findings were found: **precancerous condition (increased primitive cells with disturbed maturation; enlarged hyperchromatic nucleus and condensed chromatin).**

- Our molecular findings, indicating PCa condition were confirmed on the cellular level and correlated with cytological findings of high grade alterations, such as: **coarse distributed chromatin texture with nuclear membrane irregularity and thickening; high N:C Ratio; prominence of nucleoli and irregularity in shape thereof; identical monotonous nucleoli present in all cells in a group (i.e. "Clonal" pattern); Tumor diathesis.**

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Role of the *FTO* gene polymorphism rs9939609 in Bulgarian obese adults in the development of prediabetes

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INTRODUCTION

While factors such as age, sex, diet and other environmental variables play a role in the development of type 2 diabetes, genome-wide association studies have shown that there are genetic factors which influence an individual's risk of becoming a diabetic.

The FTO gene is mainly expressed in the hypothalamus and encodes a 2-oxoglutarate-dependent nucleic acid demethylase. This expression of FTO suggests a potential role in the control of food intake and whole body metabolism. Our study was designed to determine the effect of FTO rs9939609 in a group with established high risk for T2D (based on obesity), and to follow the progression of prediabetes to diabetes and possibly relate that to a specific genotype.

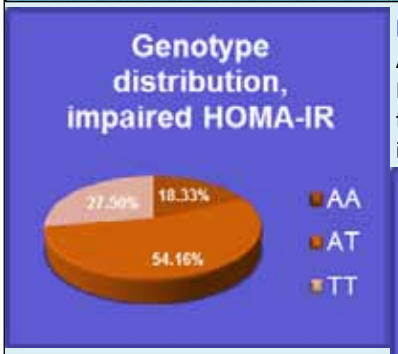
MATERIALS AND METHODS

Patient data was collected with the assistance of three medical universities in Bulgaria to form a randomized cohort. The final study selected individuals who had a confirmed risk of type 2 diabetes and a BMI>25. Genomic DNA was amplified by PCR and directly sequenced.

Each individual was tested for the following:

- Allelic variants of *FTO*
- Total weight, waist-to-hip ratio and body composition (using Tanita's Body Fat Monitor), as established by WHO standards
- Blood pressure, arterial pressure, resting heart rate and blood tests for increased risk of cardiovascular disease (lipids, liver enzymes and C-reactive protein)
- Glucose tolerance test and blood test for increased risk of type-2 diabetes measuring glucose, insulin, C-peptide, HbA1c, Matsuda Index and Beta-cell disposition
- Hormonal analysis by blood tests for insulin, ghrelin, leptin and adiponectin.
- TNF-α and IL-1 cytokine blood levels

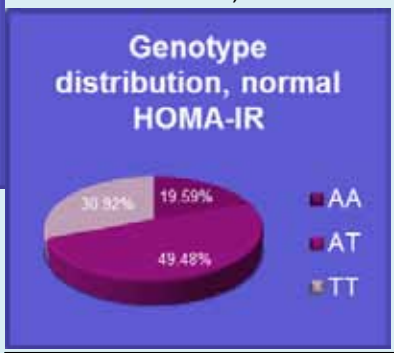
The measurements were performed at the beginning of the study and as a follow-up over the course of three months.



Genotype and allelic distributions in patients presenting increased HOMA-IR (AA: 18.33%, AT: 54.16%, TT: 27.5%) and in patients with normal HOMA-IR (AA: 19.59%, AT: 49.48%, TT: 30.92%) provided small, but no significant difference ($p > 0.5$) between the two groups due to the higher percentage of TT homozygous in the group with increased HOMA-IR index.

RESULTS

Although carrying the risk allele was expected to result in increased initial levels of the HOMA-IR index, no association was found for any of the examined genotypes in the 218 tested individuals. No correlation was found between the minor allele frequency and initial HOMA-IR levels, both in normal and impaired HOMA-IR groups (p=0.63-0.77).



No correlation was discovered when HOMA-IR levels were used as an absolute value across all tested individuals for any of the allele frequencies (p=0.38-0.82). None of the alleles of the examined genes was associated with changes in HOMA levels. Index dynamics remained uninfluenced (no statistical significance was found) even when comparing the alterations in HOMA levels to the initial ones over the course of the 3-month study.

CONCLUSIONS:

Our study shows that the FTO T-allele can be an indicator for the development of a prediabetic condition as an intermediate step to developing type 2 diabetes. While no direct connection could be established between the risk allele and an impaired HOMA-IR index, the addition of other factors such as BMI helps to reveal that the implicated risk of rs9939609 can be traced to the prediabetic status of individuals at risk. We would like to encourage further study on the topic in order to fully establish the role of FTO in prediabetes.

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ARSA gene testing: metachromatic leukodystrophy or ARSA pseudodeficiency?

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INTRODUCTION

Metachromatic leukodystrophy disorder (MLD) is a rare autosomal recessive lysosomal storage disorder leading to severe neurological symptoms and an early death.

MLD occurs due to the deficiency of enzyme arylsulfatase A (ARSA) in leukocytes that is less than 10% of normal controls. Assay of ARSA enzymatic activity cannot distinguish between MLD and ARSA pseudodeficiency (ARSA-PD), in which ARSA enzyme activity varies between 5% and 20% of the normal control values and does not cause MLD.

Thus, the diagnosis of MLD must be confirmed by one or more of the following additional tests: molecular genetic testing of *ARSA*, urinary excretion of sulfatides, and/or finding of metachromatic lipid deposits in nervous system tissue.

METHODS

➢**The *ARSA* mutation screening**: PCR amplification of all exons and exon-intron boundaries of the *ARSA* gene, followed by direct sequencing using BigDye Terminator3.1 Cycle Sequencing kit.

RESULTS

We analyzed for germline mutations in *ARSA* gene a three index patients with suspected diagnosis MLD (see Figure).

The molecular genetic testing showed already reported :

- ARSA deficiency mutations: c.684+1G>A; c.763G>A; p.Glu255Lys; c.1150G>A, p.Glu384Lys;
- ARSA-PD variants: c.*96A>G; c.1055A>G, p.Asn352Ser; c.1178C>G, p.Thr393Ser.

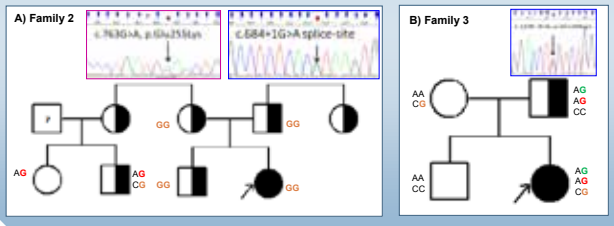

Figure: *The pedigree of the families and chromatograms of ARSA MLD mutations of all members. The arrow shows the position of mutated nucleotide.*
*ARSA-PD variants: c.*96A>G; c.1055A>G, p.Asn352Ser; c.1178C>G, p.Thr393Ser*
A) Family 2, B) Family 3

DISCUSION

➢The first MLD patient (family 2) was compound heterozygous for the following pathogenic variants p.Glu255Lys and c.684+1G>A, each being inherited from one of the healthy carrier parents. All family members were homozygous for p.Thr393Ser ARSA-PD variant. Her first cousin was heterozygous for the p.Glu255Lys mutation and p.Thr393Ser PD variant inherited from his mother and second PD variant inherited probably from his father. The child is not expected to develop the MLD disease.

➢In the second patient (family 3) were found the genetic ARSA-MLD variant p.Glu384Lys and two ARSA-PD variants (p.Asn352Ser, and c.*96A>G) situated on one allele inherited from the father and another ARSA-PD variant (p.Thr393Ser) inherited from the mother. Due to the absence of second pathogenic mutation, the MLD diagnosis of the patient must be revised.

➢The third patient was heterozygous only for the p.Asn352Ser ARSA-PD variant and MLD was not genetically confirmed.

CONCLUSIONS

➢ In the present patients we detected known MLD and PD variants in *ARSA* gene.

- Because of the high prevalence of the *ARSA*-PD alleles, low ARSA enzyme activity caused by arylsulfatase pseudodeficiency can be found in association with many disorders.
- Therefore for accurate diagnosis of MLD the results from ARSA enzyme activity, genetic testing and urinary excretion of sulfatides should be combined.

Disclosure statement – All authors declare that they have no conflict of interest.

8-МА НАЦИОНАЛНА КОНФЕРЕНЦИЯ ЗА РЕДКИ БОЛЕСТИ И ЛЕКАРСТВА СИРАЦИ
12-ТИ БАЛКАНСКИ КОНГРЕС ПО ГЕНЕТИКА НА ЧОВЕКА

8-11 септември 2017, Гранд хотел Пловдив

РЯДЪК СЛУЧАЙ НА ПАЦИЕНТ С ПРОТРАХИРАНО ПРОТИЧАЩА УРТИКАРИЯ И ИНЦИДЕНТНО ДИАГНОСТИЦИРАНА РАННА ХИВ-ИНФЕКЦИЯ

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РЕЗЮМЕ

Представяме случай на болен насочен в Клиниката по токсикология от инфекциозното отделение на областна болница, поради трудно повлияващ се на лечение уртикариален обрив на фона на антибиотична и симптоматична терапия по повод суспекция за Лаймска болест. Анамнеза за професионален контакт с животни и извършвани дейности в селскостопански район. Болният пролежа в клиниката 8 дни с тенденция за протрахирано рецидивиращо протичане на заболяването. Изписан е със значително подобрение. При последващото контролно микробиологично изследване за потвърждаване на борелиоза, инцидентно болният позитивира ХИВ-антитяло тест. Установен висок вирусен товар при RNA PCR-тест и започнато лечение в Инфекциозна клиника. Настоящият случай следва да бъде използван като алгоритъм за диагностично уточняване при пациенти с неясни фебрилни състояния и протрахирано протичащ уртикариален обрив, което налага снемане на щателна анамнеза за рисково сексуално поведение и злоупотреба с интравенозни психотропни медикаменти и прецизно интерпретиране на клиничните и лабораторни резултати. В клиниката по Токсикология следва да бъде изграден работен алгоритъм за стратификация на подобни рискови болни с цел ранна диагностика и превенция на разпространение на ХИВ с използването на последна генерация p24/Anti HIV1/2 тестове, които да стеснят пространство диагностичен период до няколко седмици след инфектирането.

ВЪВЕДЕНИЕ

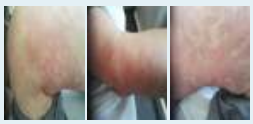
СПИН е болестната изява на латентна вирусна инфекция причинява се от ХИВ1 и ХИВ2-лентивируси от групата на ретровирусите, водещо до имунен дефицит и развитие на животозастрашаващи опортюнистични инфекции и канцерози в късния стадии на еволюцията си. Към 2016 г. диагностицираните с ХИВ в световен мащаб са над 36 000 000, въпреки намаляването на годишния темп на новооткрити случаи от около 3,1 млн през 2001 до 1,8 млн през 2016 г.[1a] България е в групата страни с все още ниско разпространение на болестта, като по статистически данни около 0,1% от полово активното население в групата 15-59 г. могат да бъдат неразпознати носители на вируса. Преобладава инфектирането основно при непротектирани хомосексуални контакти, с най висок риск за рецептивния партньор при участие в анален секс и последвани от хетеросексуални контакти и кръвен път при ползване на заразени с телесни течности медицински изделия и консумативи [1b]. Въпреки развитата национална мрежа от кабинети за тестуване, разработените профилактични, скринингови и терапевтични програми към РИОКОЗ и инфекциозните клиники, много случаи остават недиагностицирани поради липсата на клинична изява на ранната инфекция при част от заразените или поради неспецифичността на симптомите при настъпване на сероконверсията [2]. При симптоматичните пациенти от няколко дни до седмици след експозицията се наблюдава неспецифичен симптомокомплекс най-често включващ фебрилитет, фибромиалгия, адинамия, болки в гърлото, изпотявания, лимфаденопатия, повръщане, диария, уртикариален обрив, лезии по лигавиците на половите органи/орофаринкс, които могат да протичат по- протрахирано, но рядко навеждат ОПЛ или други медицински лица към диагнозата ХИВ-инфекция. След навлизане в латентния стадии на болестта могат да изминат до средно 11 г. преди разгръщане на СПИН с последващите клинични прояви на заболяването. В описания от нас случай представяме пациент с водещ симптом протрахирана с тенденция за резисистеност на лечението с антихистамини и кортикостероиди уртикария, който при инцидентно тестуване месец след пролежаването е серопозитивен за ХИВ-антитела, с висок вирусен товар и насочен за лечение в инфекциозна клиника. Това още един път намомня за високия риск от неразпознаването на такива болни поради разминаване във времето на изявата на симптомите, тяхната неспецифичност и серопозитивирането, което налага стесняване на прозоречния период с внедряване на последни генерации комбинирани тестове, след внимателна оценка на риска. Това би скъсило периода до започване на лечение, би предотвратило по-нататъшно разпространение на болестта и би довело до намаляване на риска за медицинския персонал.

Фиг.1 Разпространение на ХИВ при възрастно население, 2005 г.

Таб.1 Честота на изява на симптоми при ранна ХИВ-инфекция

КЛИНИЧЕН СЛУЧАЙ

Представяме пациент на 46 години, който търси медицинска помощ в инфекциозната клиника на областната болница поради изява на макулопапулозен обрив, фебрилитет, отпадналост около 30 дни след ухапване от кърлеж. Първоначалното серологично изследване за Рикетсия конори като предполагаем инфекциозен агент е негативно, но е предприето емпирично антибиотично и симптоматично лечение поради обсервираната инфекция. Обривът авансира, конфлуира на плаки, приложените антихистамини и кортикостероиди към този момент оказват минимален терапевтичен ефект. След консултация с дежурния лекар пациентът е насочен за хоспитализация към Клиника по клинична токсикология на УМБАЛ "Свети Георги"ЕАД - Пловдив. Клиничен статус при постъпването на 20.06.2016 г. Пациент с обезитет, телесно тегло 110 кг, субфебрилен 37,3°С. Генерализиран сърбящ макулопапулозен обрив, конфлуиращ по торса и крайниците в плаки, на места ливиден с централно изсветляване (Фиг. 2), без епидермолиза и лезии по лигавиците на орофаринкса, носна кухина, конюнктиви, ушен канал. Не се палпират увеличени периферни лимфни възли. Двустранно везикулярно дишане без прибавени хрипове. Ясни сърдечни тонове без шумове. Стабилна хемодинамика. Корем – над нивото на гръдния кош, мек и респираторно подвижен. Не се обективизират периферни отоци и неврологичен дефицит. Приемащият лекар отбелязва в документацията изключително трудния венозен достъп наложил многократни венепункции по време на престоя. Параклиничните изследвания показват нормален левкоцитен брой, но прави впечатление стойността близо до долната референтна граница с лимфоцитопения. Данни за повишение на чернодробните ензими при анамнеза за редовна употреба на алкохол, но без коагулационни нарушения или диспротеинемия.



Фиг. 2 Макулопапулозен обрив при постъпването

ЛЕЧЕНИЕ И ИЗХОД ОТ ЗАБОЛЯВАНЕТО

Предприета терапия с комбинация от антихистамини и кортикостероиди в намаляващи дози, но поради неколкократните екзацербации на симптомите и авансиране на обрива се налагат допълнителни апликации. Пациентът е изписан на 8-ия ден афебрилен и със значителна редукция на екзантема (Фиг. 3). Назначена е профилактична противорецидивна терапия с антихистамини. Насочен за амбулаторно проследяване на стойностите на чернодробните ензими и от инфекционист за повторна серология за "Марсилска треска". Около месец по-късно бяхме уведомени от инфекциозната клиника където пациентът се наблюдава, че при обработването на кръвната проба, последната инцидентно и непреднамерено е тестувана, при което се позитивират анти - ХИВ антитела. Пациентът е уведомен и насочен към Инфекциозна клиника за уточняване. След тестуване в националната потвърдителна лаборатория и RNA PCR е установен висок вирусен товар и започната антиретровирусна терапия. Не разполагаме с информация за състоянието на пациента на по-късен етап, както и за проведено епидемиологично обследване. Няколко лекари и медицински сестри са били контактни на телесни течности/кръв от пациента при венозни манипулации, без данни за убождане или попадане на секрети в кожни наранявания. Пациентът беше обсъден на клиничен съвет и се взе решение за изработване на алгоритъм за поведение, превенция и ранна диагностика при последващи случаи.

Табл. 2 Лабораторни показатели

Показател	Стойности	Показател	Стойности
Левкоцити	3,65 долно гранични	Еритроцити	4,48 T/l
Лимфоцити	0,57/15,6% L	Хемоглобин	146 g/l
Неутрофили	2,68/73,3% H	Хематокрит	0,387 L
Моноцити	0,17/4,8%	Тромбоцити	162 G/l
Еозинофили	0,03/1%	АсАТ	107 H U/l
Базофили	0,02/0,5%	АлАТ	230 H U/l
Моноцити	–	Общ.белтък	62 g/l

Фиг. 3 Значителна редукция на обрива на 8-ми ден от започване на терапията

ОБСЪЖДАНЕ

Клиничното протичане на ранната ХИВ инфекция е в пряка зависимост от фактори като тип на заразяване, статус на преносителя на инфекцията, индивидуални бариерни механизми, наличие на съпътстващи полово предавани инфекции. Най-ранната изява на симптомите е 3-5 дни след експозицията, но може да се наблюдава и към 4-5 седмица. Симптомите се дължат на активиране на имунни механизми по време на бързото размножаване на вируса и настъпващата виремия с продукция на антитела и имуномодулатори, с което се обясняват неспецифичните грипоподобни симптоми като фебрилитет, фибромиалгия, адинамия, болки в гърлото, изпотявания, лимфаденопатия, повръщане, диария, уртикариален обрив, лезии по лигавиците на половите органи/орофаринкс [3] [4] [5] [6] [7]. С напредване на сероконверсията и увеличаване на количеството антитела симптомите се самоограничават, което може да отнеме общо от 3-5 дни до няколко седмици при по-протрахираните случаи. В диагностично отношение първия маркер, наличен при нарастваща виремия е вирусния товар, който се потвърждава с доказване на вирусната РНК с RNAT-PCR тест, с възможна детекция от около 1 до 4 седмици след експозицията. Методът обаче е скъп, не се прилага рутинно, а позитивния резултат не е потвърдителен за наличие на ХИВ- инфекция до настъпване на сероконверсия. Използва се проследяване на ефекта от лечението с антиретровирусни препарати или за прогресия на болестта в по-късни стадии. Следващия маркер, който може да бъде открит преди настъпване на сероконверсията е белтък, част от капсида на вируса – p24, като при повечето пациенти той започва да се позитивира от 10дни с пик около 3-4 седмица, последващ спад и изчезване от кръвта при увеличаване на нивото на антитела, което го прави надежден ранен маркер. Отсъствието му, обаче, може да провокира фалшиво отрицателни резултати, когато концентрацията спадне при свързването му в антитяло-антиген комплекс. Прилагането на кисели субстрати към серум на такива болни разрушава комплекса и прави възможна детекцията в лабораторни условия. Въпреки това методът е скъп и недифинитивен. Първите антитела срещу вируса започват да се образуват около 2 седмица, 95% от болните са с вече настъпила сероконверсия към 6-та седмица а 99,7% на 12-та седмица след експозицията. Комбинацията от тестове с откриване на антиген-антитяло значително намалява възможността за негативно фалшив резултат при отделното им приложение при пациенти в прозоречния период при ниско ниво на p24 и недостатъчен титър на антитела между 2-6 седмица [8] [9] [10]. Първоначално снетата анамнеза не е била насочена към уточняване поведението на пациента в посока вероятен сексуален риск или такъв от страна на партньорите му. Поради това в диференциалната диагноза не беше взета предвид възможността за ХИВ инфекция. На този етап тя най-вероятно щеше да се окаже неразпозната. Предизвикателство за клиницита в случая се оказа съвпадението между самоограничаването на симптомите във времето и тяхната неспецифичност при вече предприета терапия, както и преморбидната анамнеза за възможна кърлежова трансмисивна инфекция. Въпросите, които си зададохме бяха: следва ли да провеждаме рутинно тестуване за ХИВ при пациенти с подобна констелация имайки предвид особеностите на инфекцията и евентуалната реакция/отказ от даване на информирано съгласие за тестуване, както и ефективността и икономическата оправданост на подобни скринингови тестове. Поради естеството на лекуваните пациенти в клиниката – работа с рискови групи като венозни наркомани, хора без постоянен адрес с рисково сексуално поведение, както и честите ситуации в които персоналът е изложен на риск от експозиция, дали е необходимо тестуване на тези болни, особено ако са в критично състояние, неспособни са да дадат съгласие за провеждане на тест при налични данни за висок риск от половопредавани/кръвни инфекции, между които и ХИВ.

ИЗВОДИ

На фона па пандемията в която се е превърнало разпространението на ХИВ в световен мащаб, в България ранната диагностика на заболяването все още създава проблеми. Не съществува здравна култура сред населението за превенция и своевременно откриване на инфекцията поради неоправдания страх от тестуване и стигматизацията на ХИВ-позитивните. Липсата на патогномонични симптоми на заболяването, протрахираното му протичане,особено при болни с рисково сексуално поведение или злоупотреби с психотропни медикаменти прави трудно епидемиологичното обследване и снемането на щателна анамнеза. Много често такива болни се лекуват симптоматично от ОПЛ, УНГ, дерматолози, пулмолози, токсиколози. За да се избегнат рисковете за персонала и да се ограничи разпространението на заболяването се налагат следните няколко извода, във връзка с които е умесно да се предприемат конкретни мероприятия:

1. Увеличаване нивото на информираност и обема от знания сред рисковите категории медицински персонал чрез запознаване както с типичните, така и с нехарактерните форми на клинично протичане на болестта, с наличието на големи вариации в инкубационния период /времетраенето на симптомите - от няколко дни до седмици, както и тяхната неспецифичност. Уместно е всяко протрахирано неясно фебрилно състояние, особено съчетано с трудноповлияващ се от медикация обрив да бъде последвано от по-задълбочено снемане на анамнеза, включително за рисков полов живот и наркотична злоупотреба.

2. Придобиване на знания и умения за адекватно интерпретиране на клиниколабораторните стадии в развитието на болестта и наличието на прозоречен период ,в който диагностицирането е затруднено и пациентите могат да бъдат фалшиво негативни, но силно контагиозни. Негативните за Анти-ХИВ антитяло пациенти могат да бъдат във фаза на активна репликация на вируса при което рискът за предаването му при необезопасен полов контакт или друга рискова ситуация може да нарасне до 12 пъти.

3. Внедряване на 4-та генерация комбинирани тестове,които правят възможно диагностицирането на инфекцията само няколко седмици след експозицията, изискват минимално обучение и консумативи за извършване при леглото на болния за кратко време.

4. Използване на подобни тестове за скрининг на лица и персонал, контактни на болните и изложени на висок риск не по-рано от 4-та седмица и последващи контроли на 12 седмица и 24 седмица от експозицията. Изготвяне на схеми за оценка на риска, особено при инциденти с остри предмети, замърсени с телесни течности от инфектирания пациент.

5.Повишено внимание за спазване на правилата на добра медицинска практика и лична хигиена, използване на лични предпазни средства при обслужване на пациенти с подобна суспектна констелация от симптоми.

6. Запознаване на персонала с етично-деонтологичните проблеми при работа с подобен тип пациенти с оглед преосмисляне начина на снемане на анамнеза, получаване на информирано съгласие за извършване на изследвания или отказ при наличие на капацитет у пациента за вземане на решения, уведомяване на болния и семейството му, съхраняване на конфиденциалността паралелно с приоритизирането на здравето и безопасността на персонала.

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Прояви и лечение при неврофиброматоза

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ЕМБЛЕМА

ЕТИОЛОГИЯ

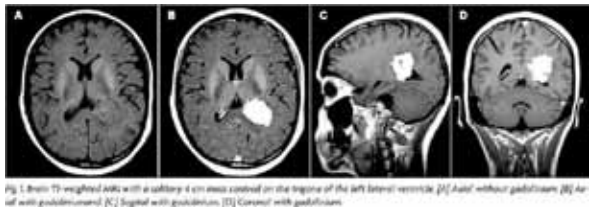
Неврофиброматоза е генетично заболяване,, което води до образуване на тумори. Тя обикновено се диагностицира още в детска възраст. Има 2 типа:

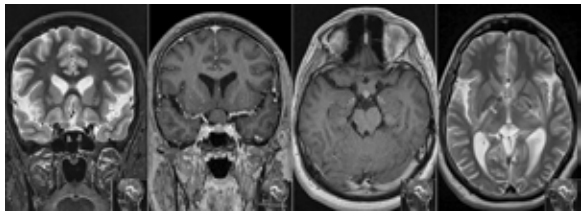
Неврофиброматоза тип 1 (Болест на Реклинкхаузен) се асоциира с голям ген в 17q11.2. Той кодира протеин, наречен неврофибромин, който действа като туморосупресорен ген.

Честота: 1 : 2 500-3 300

Неврофиброматоза тип 2 - AD мутация на NF2 гена, който е разположен в дългото рамо на 22 хромозома и се приема, че притежава туморно супресивно действие.

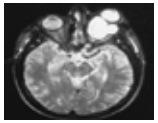
Честота :1 :35-45 000

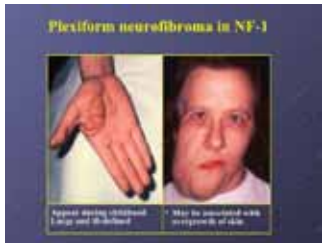


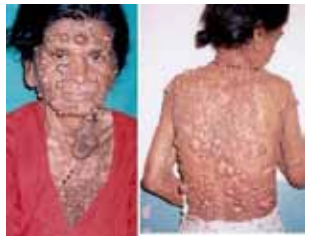


БОЛЕСТ НА РЕКЛИНКХАУСЕН

- наличие на шест или повече петна, с бледожълт до кафеникав цвят (café-au-lait), с големина около1,5 см
- два или повече фиброма или поне един плексиформен неврофибром;
- лунички в областта на axilla или слабините
- глиом на n. opticus
- два или повече възела на Lisch (доброкачествен хамартом на ириса)
- дисплазия на сфеноидната кост или дисплазия или изтъняване на кората на дългите кости







НЕВРОФИБРОМАТОЗА ТИП 2

Двустранните шваноми (VS) на вестибуларните нерви се считат за отличителен белег Впоследствие пациентите развиват шваноми на други черепно-мозъчни и периферни нерви, както и на дорзалните коренчета на гръбначния мозък. Приблизително 40% от пациентите са с множествени менингиоми. Срещат се и тумори на ЦНС от невро-ектодермален произход (епендимоми, глиоми). Те растат бавно, причинявайки прогресивна компресия на нервните и съдови структури в съседство.

Симптоми: помътняване на лещата,катаракта,множествени подкожни шваноми, полиневропатии, повишено интракраниално налягане

ЛЕЧЕНИЕ

Лечението до голяма степен зависи от възрастта на пациента,проявените симптоми и коморбидност.Процесът на мониторинг на състоянието може да продължи години преди да се пристъпи към по-инвазивни методи като лъчетерапия,химиотерапия и хирургически намеси.

За овладяване на болката се използват Gabapentin, Amitriptyline, SNRI като Duloxetine ,антиконвулсанти - Topiramate ,Carbamazepine.

Химиотерапия прилага при злокачествени засягания ПНС или метастази недостъпни за отстраняване. Използват се комбинации от медикаменти,като най-новите in vitro проучвания таргетират RAS пътя.

Farnesyl в комбинация с lovastatin има инхибиращ ефект малигнените тумори на периферните нерви.

Carboplatin и Vincristine се използват при контрол на глиоми на оптичните нерви.

ХИРУРГИЧНО ЛЕЧЕНИЕ

Цели се отстраняване на туморните формации,които при притискане на съседни структури,например нервни плексуси ,могат да причинят болка,промяна в зрителната острота или слуха и дискомфорт.Последният е харектерна находка при повърхностно разположени формации,които подлежат на механично триене от дрехи.В други случаи интервенциите имат чисто козметична насоченост. Плексиформената неврофиброматоза преставлява проблем при опитите за оперативна намеса поради по-дълбоката локализация на туморите и риска от голяма кръвозагуба по време на интервенцията. Туморите обхващащи гръбначния мозък са обект на резекция като част от превенция срещу прогресивна параплегия и квадриплегия.
Радиохирургия може да се приложи при пациенти с следоперативна прогресия на туморите или при субтотално отстраняване на последните.

Източници- www. pubmed.com ; Неврология – проф Пенко Шотеков ; www. Medicalnews.bg

Budd-Chiari syndrome in a patient with mutations in JAK-2, factor V and MTHFR

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Introduction

Budd-Chiari syndrome (BCS) represents a rare hepatic condition characterized by vascular obstruction of the efferent hepatic flow at a site that may vary from the small hepatic veins up to the place where the inferior vena cava enters the right atrium. Budd-Chiari syndrome (BCS) is associated with thrombosis of hepatic veins or the terminal portion of the inferior vena cava. The etiology of the disease is complex as genetic, acquired, and local factors interact within the pathogenesis. Most patients with primary Budd-Chiari syndrome are also affected by myeloproliferative neoplasm (MPN). The current classification of the World Health Organization for MPN is based on the presence of a somatic V617F mutation in the JAK-2 gene, which is present in 40-60% of patients with BCS.

Case Report

We present a 37 years old woman with thrombosis of hepatic veins and the inferior vena cava, ascites and erythrocytosis. The diagnosis of Budd-Chiari's syndrome was based on ultrasound and MRI of the abdominal organs. The patient was found to be a heterozygous carrier of a Factor V mutation, a JAK-2 V617F mutant heterozygote, a double heterozygote of mutations 677C> T and 1298A> C in the MTHFR gene, and a wild-type homozygote of the factor II.

Discussion

The importance of the role of different aetiologic and risk factors among patients with Budd-Chiari syndrome has been reported previously in several studies. In our case, the patient presented with several risk factors for Budd-Chiari syndrome: *JAK-2* V617F mutation, an inherited disorder of blood clotting caused by Factor V Leiden mutation and a double heterozygote of mutations 677C> T and 1298A> C in the MTHFR gene.

Identification of factor V Leiden mutation as a causative mutation for activated protein C resistance and consequently to thrombosis has been one of the major advances in the understanding of the pathogenesis of thrombotic disorders including BCS. The prevalence of this mutation in the general population and venous thrombosis patients have been found to be in the range of 10-37% making it the commonest thrombophilia marker identified today. The prevalence of factor V Leiden mutation in BCS cases has been found around 51% in the literature.

The identification of JAK2 V617F mutation has led to new approaches to diagnosis and classification of MPN. The mutation produces constitutive activation of signal transduction resulting hypersensitivity to growth factors and has been detected in most cases of polycythemia vera and in about half of the patients with essential thrombocythaemia or primary myelofibrosis. The JAK2 V617F mutation has been detected in 40-60% of patients with BCS.

Hyperhomocystenemia is a relatively weak risk factor for both arterial and venous thrombosis. The mutations in MTHFR have also shown a weak association in cases of venous thrombosis, though a stronger association has been reported for arterial thrombosis.

The combination of these mutations is rare, with only a few patients reported in the literature.

Conclusion

Budd-Chiari syndrome is an uncommon disorder. Outcome is poor in many cases. Therefore, a successful diagnostic and therapeutic approach is of vital importance. The present case illustrates the need to perform a complete thrombophilia screening in all patients diagnosed with Budd-Chiari syndrome in order to improve diagnosis and therapy.

De novo c.721A>C, p.(Thr241Pro) mutation in BRAF gene in patient with RASopathy

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Introduction

The RASopathies are a genetically heterogeneous group of conditions with overlapping genotypes and phenotypes, consisting of Noonan syndrome, cardio-facio-cutaneous (CFC) syndrome, Noonan syndrome with multiple lentigines, Costello syndrome, Neurofibromatosis type 1, and Legius syndrome. Clinical features of the RASopathies include short stature, cardiovascular defects (pulmonary valve stenosis and hypertrophic cardiomyopathy being the most common), cutaneous findings, and characteristic facial dysmorphism. Skeletal, hematological, and developmental delays/intellectual disabilities can also be associated with the RASopathies.

Case Report

We present a 1-year-old boy with supravalvular pulmonary stenosis, mild hypertrophic cardiomyopathy, short stature, excess skin on the back of the neck, triangular face shape, large head with broad forehead and low-set ears, decreased appetite and delayed motor development. During pregnancy because of a cystic hygroma that was seen in 16 g.w., amniocentesis and karyotyping was performed and it showed normal karyotype. He was born in 37 gestational week by seccion cesarean. Target sequencing revealed that the patient has a de novo missense heterozygous, mutation c.721A> C p.(Thr241Pro) in the BRAF gene.

Discussion

The *BRAF* p.(Thr241Pro) variant has been reported in at least six patients with clinical features of cardio-facio-cutaneous syndrome, LEOPARD or Costello syndrome (PMID 17704260, 18042262, 19206169, 23950000). There are other missense variants affecting the same codon, p.(Thr241Met) and p.(Thr241Arg), found as *de novo* in patients with RASopathy (PMID 19206169). Dysregulation of the RAS-MAPK signaling pathway has been recognized as the molecular cause underlying this group of clinically related developmental disorders with features including reduced growth, facial dysmorphism, cardiac defects, ectodermal anomalies, variable cognitive deficits and susceptibility to certain malignancies. These Mendelian traits are caused by mutations in genes encoding RAS proteins (*KRAS* and *HRAS*), downstream transducers (*RAF1*, *BRAF*, *MEK1* and *MEK2*), or pathway regulators (*PTPN11*, *SOS1*, *NF1* and *SPRED1*)(fig. 1).

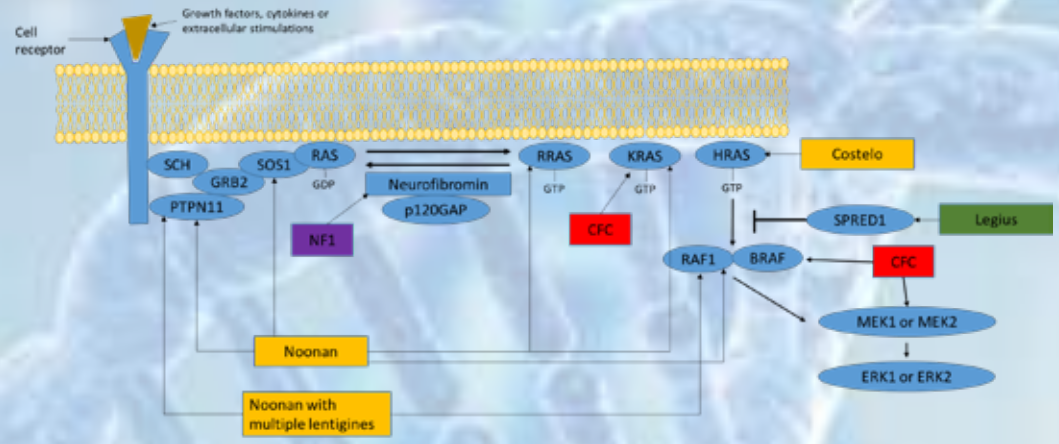

Figure 1. The syndrome (or syndromes) associated with each gene (or genes) is indicated arrows in the figure. CFC- cardio-facio-cutaneous syndrome

Heterozygous BRAF mutations are found in ~75% of mutation-positive CFC individuals. According to Rauen (Author of CFCS – GeneReviews), pathogenic mutations in BRAF molecularly define cardio-facio-cutaneous syndrome. Most BRAF mutations are missense and found in exons 6 and 12 and confer an activation of the oncoprotein. Few genotype–phenotype studies of CFC have been performed to date. BRAF mutations may be more commonly associated with hypertrophic cardiomyopathy (HCM) and pulmonic stenosis, moderate to severe intellectual disability, and significant feeding difficulties.

Table 1. Management Recommendations for CFC

Risks	Management
At risk for pulmonary stenosis, HCM, septal defects	cardiology follow-up if cardiac disease found at diagnosis
At risk for keratosis pilaris, eczema, progressive multiple pigmented nevilymphedema, hemangiomas, hyperkeratosis, and generalized hyperpigmentation	frequent dermatology visits
At risk for infantile spasms, seizures, hydrocephalus and other structural brain anomalies	Continued follow-up with neurologist
At risk for intellectual disability, delayed motor skills, emotional and behavioral problems, and speech/language impairments	Continued evaluation and services by early childhood intervention programs
At risk for feeding and/or swallowing difficulties, FTT, constipation and gastroesophageal reflux	Regular follow-up to monitor growth and nutrition
At risk for failure to thrive, short stature, GH deficiency, GH resistance, and delayed puberty.	Monitor growth carefully and refer to appropriate specialists if significant change in growth curves
At risk for ptosis, amblyopia, refractive errors, strabismus, cataracts, optic nerve hypoplasia, optic atrophy, cortical visual impairment, delayed visual maturation	Follow-up every 6–12 months or more frequently as recommended by ophthalmologist.
At risk for hypotonia with decreased muscle, scoliosis, pes planus, joint contractures, hip dysplasia and pectus deformities.	Continued follow-up with pediatric orthopedist

Conclusion

The introduction into clinical practice of target sequencing allows accurate genetic diagnosis and elucidating in more detail genotype phenotypic correlation. The presented case confirms that in all cases of dysmorphism and cardiovascular abnormalities, competent genetic counseling and the use of appropriate genetic tests allow early diagnosis. The early diagnosis allows for prognosis of the disease progression, adequate management and prophylaxis in affected families.

UPH
Prof. Ivan Mitev

NCIPD

RESULTS FROM CYTOGENETIC STUDIES IN PATIENTS WITH REPRODUCTIVE FAILURE (A 20-YEAR EXPERIENCE)

***Radoslava Emilova[1,4], *Mihaela Mladenova[1,2,3], Albena Todorova[2,3] and Iliana Boneva[1]**

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***These authors contributed equally to this work**

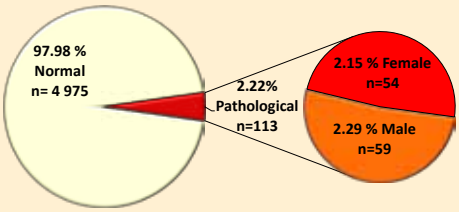
INTRODUCTION:
Among many other physiological, immunological and environmental factors, chromosomal abnormalities contribute to reproductive failure (*RF*).

AIM:
To determine the incidence, prevalence and types of chromosomal abnormalities in patients with *RF.*

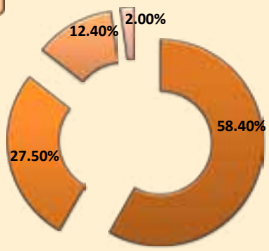
MATERIAL&METHODS:
Cytogenetic analyses were performed in 5088 patients aged 17-62 (mean 31) years - 2580 men (aged 17-62, mean 32) and 2508 women (aged 17- 49 mean 29) with unexplained RF (recurrent spontaneous miscarriage, missed abortion or infertility). Karyotype was established on GTG-banded metaphases on stimulated with PHA cultures of peripheral blood T-lymphocytes

RESULTS:

A



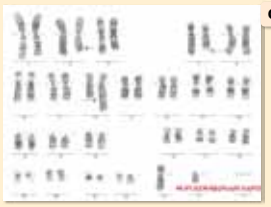
B



Translocations Gonosomal abnormality Inversions Mosaic

7 Cases for disscussion

Case 1

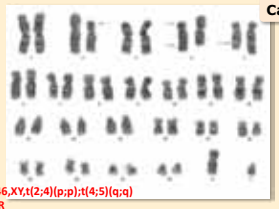


Case 2

Case 3

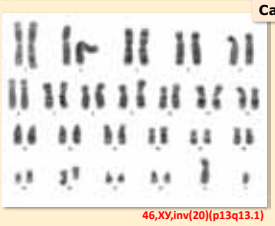
46,XX,t(3;6;7)(q13,2;q21;p22)

Case 4



? 46,XY,t(2;4)(p;p);t(4;5)(q;q)
OR
? 46,XY,t(2;5)(p21;q32),inv(4)(p15.1q27)

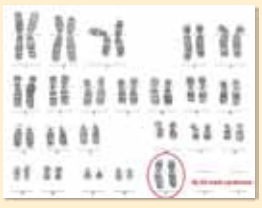
Case 5

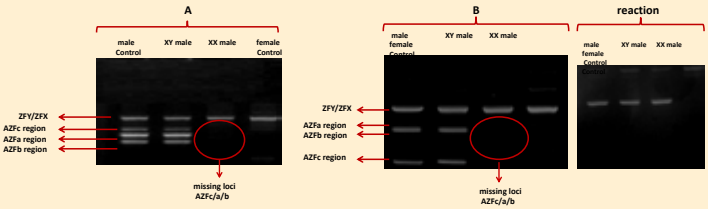


46,XY,inv(20)(p13q13.1)
Y-deletion

Case 6

SRY 46,XY,t(19;22)(q13.4;q11.1)





Case 7 Karyogramm and gel electrophoresis of patient with XX male syndrome - SRY positive

CONCLUSIONS
The incidence of chromosome abnormalities in our study (2.22%) was similar to most of the studies carried out in the last 20 years, varying from 1.28 to 4.5%. Cytogenetic analyses are an important and necessary part of the etiological research in individuals with unexplained reproductive failure and should be an integral part of diagnostic tool, especially those undergoing assisted reproductive procedures.

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RESULTS FROM CYTOGENETIC ANALYSIS IN CHILDREN WITH SUSPECTED CHROMOSOMAL DISORDERS (A 10-YEAR EXPERIENCE) THREE CYTOGENETICS CASE REPORTS

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[2] Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria;
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INTRODUCTION

Chromosomal abnormalities are result from changes in the number or in the structure or deletion of the part of the chromosomes causing significant human morbidity and mortality.

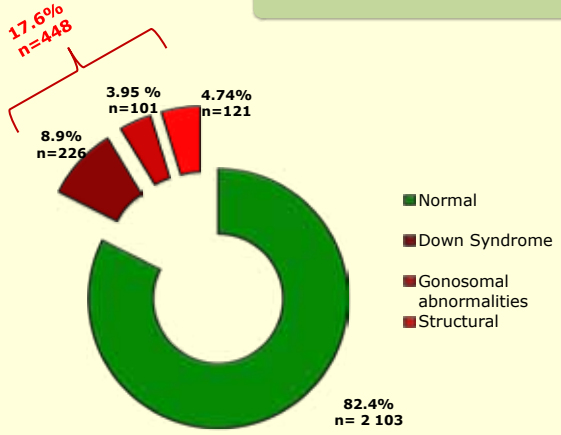
AIM

Retrospective study was carried out to identify the frequency and pattern of chromosomal aberrations among patients referred to the Cytogenetic Laboratory.

Material&Methods

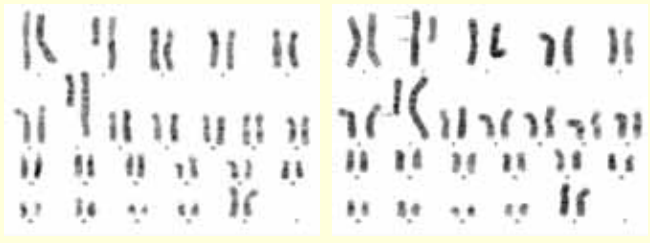
For 10 year period 2551 patients, ranging from newborn to 18 years of age were referred for cytogenetic analysis because of variety of clinical disorders. Karyotype was established on GTG-banded metaphases on stimulated with PHA cultures of peripheral blood T-lymphocytes according to standard protocol

RESULTS FOR DISCUSSION



CASE 1- mos46,XX,del(16)(q22)[9]/47,XX,fra(16)(q22),+mar[1]/47,XX,del(16)(q22),+mar[1]/46,XX [39]

CASE 2- 46,XX,t(2;7)(q13;q36)inv(2)(p23q13)dup(2)(p15p21)mat



First result - 46,XX,t(2;7)(q14;q35)

Mother` s karyotype - 46,XX,t(2;7)(q13;q36)inv(2)(p23q13)

CASE 3- 46,XY,?inv(15)(q14q22.2) mat

aCGH 4q22.3q25(95,663,991-107,880,122)x3,15q13.3q14(31,772,427-38,274,449)x1

FISH: ish 4q23 (RP11-147P21x3),15q14 (RP11-429A19x1

CONCLUSIONS

Our data suggest that cytogenetic analysis is an important part in the evaluation of genetic disorders and helps clinicians to provide accurate diagnosis and proper genetic counseling.

CLINICAL VARIABILITY OF CONGENITAL MYASTHENIC SYNDROME TYPE IA DUE TO MUTATION 1267DELG IN 100 CASES

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[3]Clinic of Neurology, USBAL "Prof. Dr. Ivan Mitev", Department of Pediatrics, Medical University-Sofia, Bulgaria
Iiatrics and Genetic Diseases, UMBAL "St. Georgi", Department of Pediatrics and Medical Genetics, Medical University-Plovdiv, Plovdiv, Bulgaria
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[7]Clinic of Neurology, University Hospital Sofiamed, Sofia, Bulgaria
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INTRODUCTION:

Congenital myasthenic syndromes (CMS) are a group of genetically determined disorders characterized by fatigable weakness of skeletal muscles (e.g. ocular, bulbar, limb muscles) with onset at or shortly after birth or in early childhood. Severity and course of disease are highly variable, ranging from minor symptoms to progressive disabling weakness. The most common type of congenital myasthenic syndrome in Bulgaria is type Ia caused due to 1267delG mutation in exon 12 in the gene for the εAChR subunit.

PURPOSE:

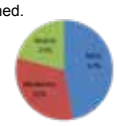
The purpose of the study is to determine the variety of clinical features in patients with congenital myasthenic syndrome type Ia.

METHODS:

Physical examination, neurostatus, myometry, spirometry (seated and laid back position), estimation of the level of myasthenia.

RESULTS:

CMS type Ia Clinical Course



One hundred affected with genetically confirmed disease were examined. All the patients are from Roma ethnicity and are aged between 1 year and 11 months and 64 years old, mean age 26 years. All patients have neonatal onset of the disease with bilateral fluctuating eyelid ptosis, ophtalmoparesis, feeding difficulties, poor cry. In the course of the disease the most common symptoms were ophtalmoparesis without diplopia, fluctuating eyelid ptosis, bulbar weakness and weakness in the limbs.

- Mild clinical course: 47% (47/100) of the patients with eyelid ptosis and ophtalmoparesis with or without mild bulbar weakness and weakness in the proximal muscles of the limbs;
- Moderate clinical course: 32% (32/100) of the patients with weakness in the proximal muscles of the limbs and bulbar weakness;
- Severe clinical course: 21% (21/100) of the patients with severe weakness in the proximal muscles of the limbs leading to gait disturbances or loss of ambulation, bulbar weakness;

Clinical Course	FVC average (seated position)	FVC median (seated position)	FVC average (laid back)	FVC median (laid back)
Mild	85.40%	80.50%	77.50%	76%
Moderate	78%	80%	75.40%	78.20%
Severe	62.80%	64%	59.50%	60%

Chart No1: *FVC depending on clinical course and position*

Spirometry was performed in a seated and laid back position. Spirometry in a seated position shows results for forced vital capacity (FVC) between 36 and 151%, FVC average is 77.92% (SD 21.15). Spirometry in a laid back position shows results for FVC between 15 and 130%, FVC average is 71.95% (SD 19.7). Chart No1 presents values for FVC in the different groups. FVC in a seated and laid back position in 14 patients shows more than 10% difference due to diaphragm weakness. Nine of these 14 patients have mild clinical course, 3 – moderate clinical course, 2 – severe clinical course.

Only one of all 100 patients used a tracheostomy for a month. He has a severe clinical course and had one respiratory crisis during pneumonia. None of the other patients used non-invasive or invasive ventilation, or ever had respiratory crises.

Swallowing was estimated according to the following scale:

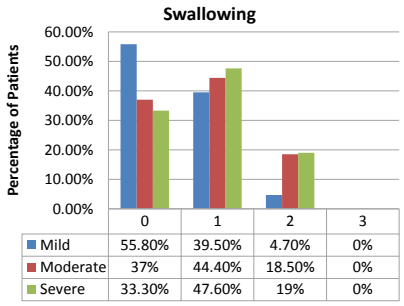
0 – never has problems when swallowing and never chokes on food/drink

1 – may experience occasional (< 1 per month) problems swallowing certain types of food or occasionally chokes

2 – has regular trouble swallowing food/drink or chokes on food/drink (>1 per month)

3 – has trouble swallowing saliva or secretions

Chart No2 presents the results depending on the clinical course.



	0	1	2	3
Mild	55.80%	39.50%	4.70%	0%
Moderate	37%	44.40%	18.50%	0%
Severe	33.30%	47.60%	19%	0%

Chart No2: *Swallow difficulties depending on clinical course*

Chart No2 shows that the patients in our study that have bulbar weakness leading to swallowing difficulties and choking on food/drink have mostly moderate or severe clinical course. Bulbar weakness in patients with CMS type Ia is not so severe to cause trouble swallowing own saliva.

CONCLUSION:

Congenital myasthenic syndrome type Ia is a genetically determined disease characterized by a neonatal onset and variety of the clinical course despite the genetic homogeneity of the study group. The clinical course varies from mild ptosis and ophtalmoparesis to severe muscle weakness, difficulties walking and swallowing. Although this type of myasthenia could also lead to a severe clinical course, respiratory insufficiency is not common among these patients and they do not need non-invasive or invasive ventilation like those patients who have mutation in the RAPSN gene, CHAT gene or slow channel CMS. Further examinations are needed to determine modifying genes, which are responsible for the pathogenesis of the disease.

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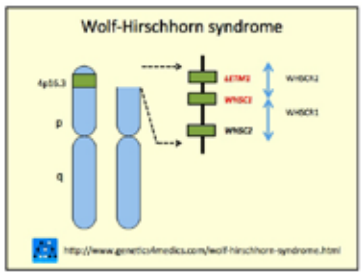
CASES OF WOLF-HIRSCHHORN SYNDROME DIAGNOSED IN THE LABORATORY OF MEDICAL GENETICS-VARNA FOR THE PERIOD 2008-2017

Tsvetkova M., M. Stoyanova, D. Konstantinova, Ts. Ruseva, M. Levkova, L. Angelova,

Department of Medical Genetics, MU-Varna



INTRODUCTION

The Wolf-Hirschhorn syndrome (WHS) is a chromosome disorder due to a deletion of the distal part of the short (p) arm of chromosome 4, with critically deleted region in 4p16.3, which includes the Wolf-Hirschhorn Syndrome Candidate genes –WHSC1 and WHSC2. The prevalence of Wolf-Hirschhorn syndrome is estimated to be 1 in 50,000 births. Wolf-Hirschhorn syndrome occurs in about twice as many females as males . 87% of cases represent de novo deletions, while in 13% of cases, one of the parents is a balanced translocation carrier. The deleted segment has a variable size. The major features of this disorder include a characteristic facial appearance, delayed growth and development. Typical craniofacial features include microcephaly, hight forehead, hypertelorism, widely spaced and prominent eyes, a 'Greek warrior helmet appearance' of the nose, triangular nose opened microretrognathy, cleft lip and palate. The fingers are long and fine, umbilical hernia may be evident.

WE PRESENT THE FOLLOWING CASES:

Case 1: MOM, 4 days, newborn from second pregnancy born after 40 gestation week. Weight at birth- 2000 g. After birth the following symptons were observed: delayed growth , hypotonia, heart defect and characteristic facial appearance: mycrocephaly with head circumference 30,5 sm.; hypertelorism; exophtalm, strabizmus; wide nose; microretrognathy; poorly modeled and incorrectly positioned ears shells; a 'Greek warrior helmet appearance' of the face. The patient also had umbilical hernia and long and fine fingers, syndactyly of the toes (fig 1). The newborn was referred by pediatric dysmorphologist of University Hospital St. Marina with Dg. Wolf-Hirshhorn syndrome for cytogenetic analysis. It detected a deletion of the distal part of the short arm of chromosome 4 - 46,XX,del(4)(p15.2), confirmed the working diagnosis (fig.2). This is a de novo chromosomal aberration, because the karyotype of the parents is normal. The child survived till two months of age.


fig. 1

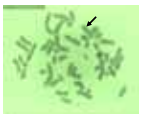


fig. 2

Case 2: Baby № 1996, 1 day, prematurely born in 35 gestation week after a second pregnancy, weight at birth-1410 gr. and head circumference 27 sm. The low weight, the mycrocephaly and the dysmorphologic features – hypertelorism, microretrognathy, poorly modeled ears shells, typical rectangular shape of the nose, seizures and heart failure, were confirmed by pediatric- genetic counselor of University Hospital St. Marina, as suspicious about specific chromosome disorder - Wolf-Hirshhorn syndrome (fig.3). A cytogenetic analysis was performed and it established a karyotype 46,XX,del(4)(p14). In this case, the deleted region has a larger size and includes almost the whole short arm of the fourth chromosome, on the basis of which the diagnosis was confirmed - Wolf-Hirschhorn syndrome (fig4). No cytogenetic analysis was performed of the parents. The child survived only a week, which may be related with the large size of the deleted region.

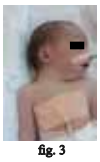


fig. 3


fig. 4

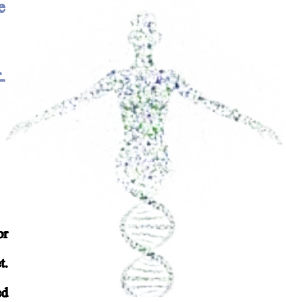
Case 3: KIIB, 1 month, a child from first pregnancy, premature birth due to rupture of the membranes. With severe birth asphyxia and critical medical state– respiratory and heart failure; abnormal neurological status with seizures; hypospadias. Head and face with congenital dysmorphologic features- mycrocephaly, hypertelorizm, short palpebral fissures, exophtalm, corneal leukoma of the left eye, cheilognathopalatoschisis, mandibular hypoplasia.(fig.5) Muscular hypotrophy of the limbs, pes ecvinovarus. To determine the cause of dysmorphologic features, the child was referred by pediatrician to our department, with Dg. MCA for cytogenetic analysis, which found a deletion of the short arm of a chromosome 4 - Wolf-Hirshhorn syndrome (karyotype: 46,XY,del(4)(15.1)) (fig.6). The child survived till three months of age.


fig.5


fig.6

CONCLUSION:

1. In these three cases, the deletion region of the short arm of the fourth chromosome is of varying size, but including the critical region 4p16.3, mapped candidate genes WHSC1 and WHSC2. The cytogenetic findings in the presented cases confirm that the WHS may be a result of the different size of the deletion of the short arm of chromosome 4.
2. The specific phenotype (facial appearance, delayed growth and development), which suggest the presence of a chromosomal disease - Wolf-Hirschhorn syndrome, confirmed by the cytogenetic analysis, can be suspected by specialists – dysmorphologist with genetics qualification.



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WHOLE-GENOME SEQUENCING IN NEWBORN SCREENING – MEDICAL PROFESSIONALS' ATTITUDES AND OPINIONS IN BULGARIA

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Introduction

Whole-genome sequencing (WGS) is viewed as a major vehicle for translating genetic and genomic advances into population health gains. The debate as to whether to include WGS in newborn screening (NBS) is currently taking place in many jurisdictions. There are considerable concerns among health care practitioners, including limited benefit of WGS for most healthy people in the general population, lack of expertise among non-genetic health care providers, potential negative implications for society and scarce economic resources.

Objective

The purpose of this study was to assess the attitudes and opinions of pediatricians and geneticists on the potential use of WGS for NBS in Bulgaria.

Material and methods

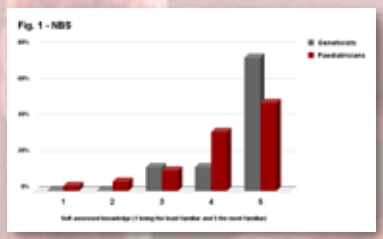
This study is based on the concept of non-selective WGS for all newborns and analysis of all genes. This definition of WGS was presented to all study participants and included in the subsequent discussion of the results.

Study participants included geneticists and pediatricians from Bulgaria. A total of 299 individuals were contacted by email to participate in the survey with an invitation letter describing the study. The study was conducted through an online survey in March–April, 2017 in Bulgaria.

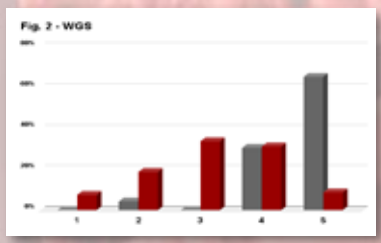
Results

Knowledge and awareness of NBS and WGS

In total, 103 out of 299 invited participants completed the survey, with an overall response rate of 34.4%. The groups of pediatricians (n=80) and geneticists (n=23) were found to be similar in terms of sex (p=0.543), age (p=0.707) and professional experience (p=0.385).



54.5% of the study sample indicated a maximum level of knowledge on NBS on a self-assessment scale, while only 21.4% reported highest level of awareness on WGS (Fig. 1). The difference in the case of WGS was statistically significant, with 15 out of 23 geneticists indicating highest level of knowledge in comparison to only 7 out of 80 pediatricians (p=0.000). The split in self-assessed knowledge of NBS and WGS strongly reflected on the question whether WGS could be implemented as an adjunct to NBS in Bulgaria (Fig. 2).



Pediatricians and geneticists largely agreed on their assessment of WGS's potential benefits. WGS was seen as especially helpful for early diagnosis and treatment (Fig. 3).

Nevertheless, a stable scoring pattern was observed with pediatricians giving higher ratings than geneticists. The same outcome was observed when respondents were asked to evaluate WGS's overall benefits in specific cases(Fig. 4).

Regulatory settings and organizational issues

Regulatory setting / organizational issue	Pediatricians, % (n)	Geneticists, % (n)
Legal mandate of WGS in NBS		
Mandatory	20.00% (16)	0.00% (0)
Optional, but highly recommended	35.00% (28)	26.09% (6)
Upon parental request	42.50% (34)	43.48% (10)
Other	2.50% (2)	30.43% (7)
Disclosure of WGS results		
All results	75.00% (60)	26.09% (6)
Upon decision by the physician	3.75% (3)	17.39% (4)
Upon decision by the family	17.50% (14)	34.78% (8)
Upon decision by the physician (further decision by the person after attaining legal age)	3.75% (3)	21.74% (5)
Additional research of WGS samples		
No additional research conducted	6.25% (5)	0.00% (0)
Consent required	71.25% (57)	65.22% (15)
Assumed consent	2.50% (2)	4.35% (1)
No consent required	20.00% (16)	30.43% (7)
Timing of WGS if apart from NBS		
At birth	70.00% (56)	47.83% (11)
At age of 0-6	13.75% (11)	4.35% (1)
After attaining legal age	0.00% (0)	13.04% (3)
In case of specific symptoms	16.25% (13)	34.78% (8)

Table 1. Regulatory settings and organizational issues

Discussion

The demonstrated lack of clear consensus on whether WGS should be incorporated into NBS illustrates that there is a need for discussion and collaboration among all stakeholders before any major changes are being implemented.

Ethical issues were the most commonly cited concerns by geneticists and pediatricians in our study. Although participants greatly believed WGS and precise medicine are the future, a considerable number expressed disquiet about a number of potential risks, including discrimination, psychological stress and invasion of privacy.

While there is a significant number of objections about non-selective WGS for all newborns, both geneticists and pediatricians supported expanding NBS with selective WGS for specific disorders, provided that broad societal consensus is reached and project funding is allocated.

Conclusion

While non-selective WGS for all newborns is not currently perceived as feasible, pediatricians and geneticists do believe that selective WGS could strengthen current NBS programs. Further research, including multi-stakeholder partnerships among pediatricians, geneticists and patients, should help guide the development of health policy and practice regarding this concept.

Universiteti i Tiranës
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IDENTIFICATION OF CYP2C9*2 ALLELIC VARIANT IN HEALTHY ALBANIAN POPULATION

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INTRODUCTION

Enzyme CYP2C9 is part of the cytochrome P450 (CYP450) enzyme group (1). This enzyme is mainly expressed in the endoplasmic reticulum of the liver cell (2). Enzyme CYP2C9 catalyzes the metabolism of a variety of endogenous lipophilic substances (steroids, fatty acids, retinoids, and vitamins) and exogenous (drugs), transforming them into hydrophilic and facilitating their elimination in urine. Among the drugs that are metabolized by CYP2C9 enzyme, are S-warfarin, phenytoin, diclofenac, mefenamic, phenotynoin, losartan, irbesartan, tolbutamidine, glipizidine and torsemide. It also oxidizes a variety of non-steroidal anti-inflammatory drugs such as ibuprofen and flurbiprofen (3). CYP2C9 is the second enzyme with the highest expression in the liver following the CYP3A4 (4) enzyme and results to be responsible for 15-20% metabolic clearance of all drugs that undergo the first stage metabolism (5). CYP2C9 is found in the long (q) arm of chromosome 10 at position 23.33., which has a length of approximately 84Kb and is very polymorphic (6) Fig.1

Fig. 1. Location of CYP2C9 gene on chromosome 10

More than 60 genetic variants have been identified (7).Three most common allelic variant are found in most of the studied populations: CYP2C9*1, CYP2C9*2, and CYP2C9*3. The most common allele is the wild type allele, CYP2C9*1. CYP2C9*2 allele differs from the wt allele as a result of the replacement of a single nucleotide, respectively C430>T in exon 3. As a result of these changes the Arginine amino acid is replaced by the Cysteine amino acid at position 144 of the enzyme structure (Arg144Cys). Conformational changes occurring in the active site of the enzyme become the cause of the change in the metabolic activity of the CYP2C9 enzyme. CYP2C9*2 allele encodes for a protein with reduced enzymatic activity. The inter individual and interethnic variations in the CYP2C9 polymorphisms are clinically significant especially in the patients on anticoagulation therapy with warfarin. This allelic variant reduces the metabolism of warfarin with 30-50% (8) Fig 2. The distribution of genotypes in the population differs from their ethnic composition. In Caucasian populations approximately 2/3 have a normal genotypes CYP2C9*1/*1 ; 1/3 carries either the *1/*2 or *1/*3 genotypes while less than 2.5% of Caucasian individuals carry the *2/*2, *2/*3 and *3/*3 have genotype which make them poor metabolizers of CYP2C9 substrates. Gender specific differences were not apparent in the distribution of polymorphic alleles in these populations (9). The frequency of CYP2C9*2 allele found in Caucasian population is higher (10-20%) than Asian ones (1-3%) and African (0-6%) (10).

Fig 2. A series cardiovascular conditions treated with warfarin

Aim of the study: There are no data for allelic variants of the CYP2C9 gene in Albanian population This study aims to determine the frequency of CYP2C9*2 allelic variant in Albanian population and the percentage of poor metabolizers, the category of individuals that metabolizes slowly the drugs metabolized by CYP2C9 enzyme. Based on our study on healthy population we intend to predict the percentage of patients who should re-evaluate drug dosages based on respective genotypes for CYP2C9 gene .

MATERIALS AND METHODS

Sample size: We have analyzed 100 healthy Albanian subjects from different regions of Albania, randomly selected. In our study we have male and female in equal proportion. The mean age of the subjects was 37±14.48 years old.

DNA extraction from blood: A blood sample (3mL) was drawn from each subject into K3 EDTA tube. DNA was extracted using DNA blood kit (Qiagen, Hilden, Germany) and Invitrogen (DNA extracting mini preparation kit) by following the manufacturer's protocol. The presence and quantity of DNA was confirmed by running DNA in 1% agarose gel. DNA samples were stored in -20℃ until analysis was performed.

Genotyping of CYP2C19*1 and CYP2C19*2 alleles: PCR-RFLP: DNA amplification was carried out by using a copy of primers which were selected from published literature, Aynacioglu et al (11) and confirmed by BLAST analysis. One specific reaction for CYP2C9*2 were conducted for each specimen in a final volume of 25µl. For the amplification of the DNA we have used CL1 5'-CAC TGG CTG AAA GAG CTA ACA GAG and CR1 5'- GTG ATA TGG AGT AGG GTC AC CAC primers at 94°C as initial denaturation for 10 min with 35 cycles of 94°C for 30 sec as a denaturation, annealing at 60°C for 10 sec polymerization at 72°C for 60 sec and final extension 10 min at 72°C. The fragment of interest has a length of 374bp.

RFLP analysis: The amplified DNA fragments were digested by AvaII restriction enzymes (New England Biolabs, Schwalbach, Germany). For the digestion of the DNA we incubated it with the enzyme AvaII in 37°C overnight.

Fig.3 Equipment used for identification of relevant genotypes, electrophoresis and UV-transiluminator

Identification of genotypes by gel electrophoresis of PCR products: The resulted RFLP products were analyzed in 2% agarose gel electrophoresis immersed in TBE buffer and visualized with ethidium bromide staining and ultraviolet illumination Figure 3 Digestion of CYP2C9*2 amplicon with AvaII resulted in products of 300bp and 74bp fragments (homozygous wild type), 374bp, 300bp and 74bp (heterozygous for *1 and *2) and a single undigested product of 374bp (homozygous for *2 allele) Figure 4.

*Fig.4 Identification of genotypes CYP2C9*1/*1, *1/*2, *2/*2*

Statistical analysis: The calculation of frequencies of CYP2C9*1 and CYP2C9*2 allele was done according to the standard formula, used in population genetics. Hardy-Weinberg equilibrium was calculated according to the online Encyclopedia for Genetic Epidemiology (OEGE) software. To compare the CYP2C9*2 allele frequency found in our study with those of other populations was used the Fisher Exact test statistic, p<0.05 values are considered not significant.

RESULTS AND DISCUSSION

1. Amplification of the DNA

The genomic DNA of sufficient quality and quantity extracted from 100 individuals was successfully amplified. In Fig. 5 is shown the gel electrophoresis with amplified products of 374 bp after PCR reaction for 13 individuals.

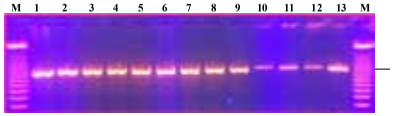

Fig. 5 Photo of gel electrophoresis of PCR products for 13 individuals. M is the marker, 50bp ladder.

2. Determination of CYP2C9 genotypes of CYP2C9 gene

In Fig.6 are presented the results of genotypes for 4 individuals. The PCR fragments are digested with restriction enzyme Ava II. The 374bp products are digested in two fragments of 300bp and 74bp, which indicates that all subjects are homozygous for the normal allele (CYP2C9 *1/*1).

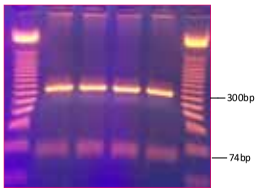

Fig. 6. Photo of gel electrophoresis for identification of CYP2C9 genotypes for 4 patients. M is the marker, 50bp ladder

3. Determination of genotype frequencies

The frequencies of CYP2C9*1/*1,*1/*2 and *2/*2 genotypes are presented in Fig 7.

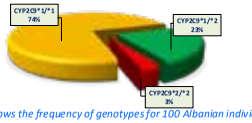

Fig.7. The graph shows the frequency of genotypes for 100 Albanian individuals.

As it is seen by the pie chart 23% of Albanian population are intermediate metabolizers (CYP2C9*1/*2) while 3% are poor metabolizers for these group of substrates. The frequencies found are in Hardy-Weinberg equilibrium ($\chi 2 = 0.528$).

4. Determination of allele frequencies

Results for CYP2C9*1 and CYP2C9*2 alleles are presented in Fig.7

*Fig.7: The graph shows the allelic frequency of CYP2C9*1 and CYP2C9*2 alleles in Albanian population*

As it is seen the CYP2C19*1 and CYP2C19*2 allele frequencies in Albanian population are 85.5% (CI 95%: 84.75-86.25) and 14.5% (CI 95%: 12.69-16.31) respectively.

5. Comparison of the frequencies between different populations

*Table1. Distribution of CYP2C9*2 allele among different ethnic groups*

Populations	n	CYP2C9*2 allele frequency (%)
Albania	100	14.5
Turkey	499	10.6 (NS)
Greece	283	12.9 (NS)
Bosnia and Herzegovina	151	15.0 (NS)
Italy	157	11.2 (NS)
Hungary	535	12.5(NS)
Croatia	200	16.5 (NS)
Rumania	332	11.3 (NS)
Macedonia	184	13.9(NS)
Slovenia	129	12.3 (NS)

*NS- statistically not significant

CONCLUSIONS

- The CYP2C9*1/*1 , CYP2C9*1/*2 and CYP2C9*2/*2 genotype frequencies in Albanian population are respectively 74%, 23%, 3%.
- About 1/4 of Albanian population are intermediate and poor metabolizers for drugs metabolized by CYP2C9 enzyme.
- The frequencies of allele CYP2C19*1 and CYP2C19*2 in Albanian population are respectively 85.5% and 14.5%.
- The distribution of CYP2C9 *1 and CYP2C9 *2 allelic variants are consistent with the frequency range reported by other Balkan and European populations.
- These data should be taken in consideration by physicians, to determine the right dosage of medications for patients based on their corresponding CYP2C9 genotype profile.

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The presence of premutation in the *FMR1* gene in patients with clinical picture of degenerative ataxia, tremor and Parkinsonism

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STATEMENT OF PURPOSE

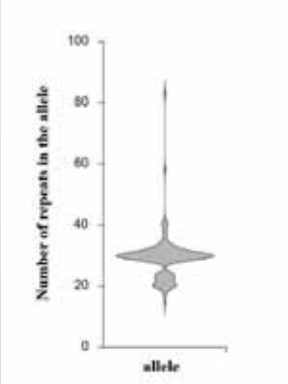
Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder found in some male and female carriers of the permutation expansion (55-200 CGG repeats) of the *fragile X mental retardation 1 (FMR1)* gene. It is considered to be related to mRNA toxicity which results in sequestration of proteins and mitochondrial disfunction. Principal features include intention tremor, cerebellar ataxia, Parkinsonism, brain atrophy, brain white matter disease, and cognitive decline. The aim of our study was to determine the frequency of premutation in the *FMR1* gene in patients with clinical signs of degenerative ataxia or unexplained tremor in combination with Parkinsonism and cognitive decline.

METHODOLOGY

The study included 100 patients, 67 men and 33 women, with clinical picture of degenerative ataxia, tremor and Parkinsonism, and negative molecular-genetic testing for autosomal-dominant spinocerebellar ataxias (SCA1, 2, 3, 6, 7, and 17) in whom the disease began after the age of 49. Molecular-genetic testing was performed by specific amplification of CGG repeats in the *FMR1* gene.

SUMMARY OF RESULTS

We detected the presence of premutation in the *FMR1* gene in 2% of patients with 83 and 58 CGG repeats, respectively. The presence of *FMR1* alleles with 41 CGG repeats was found in 2% of patients. The average number of CGG repeats in the allele was 29.2 ± 7.2 (27.9 - 30.5). The number of repeats in the allele had a bimodal distribution with the highest frequency of alleles with 29-30 and 23-24 CGG repeats.



Graph 1. The number of repeats in the *FMR1* gene shows bimodal distribution

CONCLUSION

Fragile X-associated tremor/ataxia syndrome should be considered in the differential diagnosis of patients with unexplained action tremor and ataxia, especially when there is a strong family history of movement disorders or autism spectrum disorders. Parkinsonism and gait ataxia may also be seen in individuals with 41 CGG expansions.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of education, science and technological development of Serbia, grant number 175090.

CASE REPORTS

PATIENT 1

An eighty-year-old male patient with hypothyroidism (on substitution therapy) and type 2 diabetes (on oral antidiabetics), started having mild tremor of the hands at the age of 63. This occurred while performing some acts, such as drinking coffee or shaving. Four years later, the tremor got worse and the tremor of the head appeared. Seven years from the onset of the symptoms gait instability and frequent falls started to occur, and soon after slowing down and speech impairment became evident. At the same time, there were changes in the mood (intolerance, irritability, indifference, sensitivity) and forgetfulness. In the eighth year of evolution of neurological disease, autonomic disorders began, first fecal incontinence, and two years later, urinary incontinence. The patient was diagnosed with Parkinsonism plus syndrome and probable multiple system atrophy (MSA).

Family history was informative for neurodevelopmental/psychiatric disorders in children.

Neurologic examination at the age of 63 revealed tremor of the head, mild dysarthria and hypophonia, intermittent statopostural tremor of the hands, and signs of hypokinesia. Mild ataxia was present in cerebellar upper and lower limb examination. Standing was impaired with tendency to fall backwards. Loss of control of sphincters was present.

On transcranial sonography of the brain parenchima we have found hyperechogenicity of the substantia nigra on the right side of 0.17 cm2, large hyperechogenic area in the dentate nucleus on the right of 0.66 cm2. Ventricular system was very dilated (III chamber 15mm).

A positive tilt test of pressure measurement in the lying and standing position after 1 and 3 minutes indicated the presence of orthostatic hypotension.

Using molecular-genetic testing, we established an *FMR1* allele of 83 CGG repeats (premutation).

Picture 1. Electropherogram of a male with 83 CGG repeats (premutation) in the *FMR1* gene

PATIENT 2

A seventy-two-year-old female patient with type 2 diabetes (on insulin therapy) came to the Neurological Clinic for the first time at the age of 62. Her first disturbances began at the age of 56 in the form of tremor of the hands and after several years, tremor of the head. Four years later, she started having gate instability, wide stance, loss of balance and a tendency to fall backwards. The problems gradually progressed. In addition to these motor disturbances, the patient complained of forgetfulness, and periods of confusion have also been noticed.

The patient has two children. Her uncle had a pronounced tremor on his hands and a "no" tremor of the head.

In the neurological finding, there was a tremor of the chin and occasionally a "no" tremor of the head. She had dysmetria and intentional tremor in the finger to nail test, ataxia in the heel to knee test, symmetrical postural tremor of the upper and lower extremities. She was ataxic and had a wide stance. The patient could not stand in Romberg's position and had the tendency to fall backwards. She had urgency of urination.

The finding of electromyneurography (EMNG) revealed sensory, axonal-demyelinating polyneuropathy, severe on the lower extremities, and moderate in the upper extremities. Brain NMR showed supratentorial changes of nonspecific morphology and distribution, as well as periventricular ischemic leukoencephalopathy and cortical reductive changes.

The patient has been diagnosed with essential tremor, polyneuropathy and small blood vessels disease of the brain.

Using molecular-genetic testing we established *FMR1* alleles of 32 and 58 CGG repeats (heterozygous premuatation carrier).

Picture 1. Electropherogram of a female with an allele of 32 (normal allele) and an allele of 58 CGG repeats (premutation) in the *FMR1* gene

From clinical to genetic diagnosis in an 11-year old boy with Familial Hypercholesterolemia: A Case Report

DEDICATED TO HUMANITY

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Introduction

Familial hypercholesterolemia (FH) is a genetic disorder that causes severe dyslipidemia manifested through symptoms of early generalized atherosclerosis. Symptom severity is much higher in homozygous patients than in heterozygous.

Case report

Anamnesis: An 11-year old boy, who at first had newly-appeared round, yellowish skin lesions on both knees and elbows.

Family history revealed early onset coronary artery disease and sudden cardiac death in first as well as in second degree relatives:

- ✓ The father of the boy had died at 30 due to a heart attack.
- ✓ The grandfather on the same side had suffered a sudden death at the age of 40.
- ✓ Laboratory tests of the mother revealed total cholesterol of 9.5 mmol/l. Her parents had non-fatal heart attacks at the age of 45 and 50 respectively.

Examination

Physical examination:

Height – 145 cm, weight – 46 kg, BMI – 18.62 kg/m²; BP – 115/70 mmHg; heart rate – 95 bpm. Arcus cornealis was present in both eyes of the patient.

Laboratory tests:

1) Blood analysis revealed extremely elevated total and LDL cholesterol levels (**18mmol/l and 15mmol/l respectively**) with normal triglycerides and HDL.
2) Further laboratory tests gave a ratio of **ApoB/ApoA1 – 2,9,** (Apo-B – 3.5 g/l, Apo-A-1.4 g/l).

Genetic analysis:

Genetic analysis revealed that the patient carries two different mutations of both alleles of the LDL-receptor (compound heterozygosity): c.1591A > G; p.(Lys507Glu) and c.2403_2406del, a condition that is equivalent in severity to the homozygous form of the disease.

Functional tests:

1) ***ECG*** *at rest was unremarkable.*
2) ***2D-Echocardiography*** *revealed posterior annular calcification of the mitral valve and thickened and sclerotic aortic valve without a pathological gradient (figure 1).*

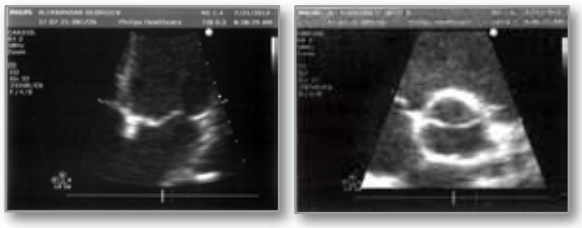

Figure 1: Mitral and aortic valve

3) ***US doppler*** *exam of the arteries was performed and showed no atherosclerotic lesions.*
4) ***Cardio-pulmonary test*** *was performed as well.*

Differential diagnosis

1) Dysbetahyperlipoproteinemia (type III hyperlipidemia)
2) Sitosterolemia (Phytosterolemia)

Treatment

Treatment began with **Atorvastatin 20 mg** – increased to 40 mg over 3 months – Total cholesterol levels reached *9.2* mmol/l, *LDL-cholesterol 8.7* mmol/l, ApoB/Apo-A1 – 2. After **Ezetimibe** was included – total cholesterol levels – 7.2 mmol/l, LDL-cholesterol 5.7 mmol/l, ApoB/Apo-A1 – 1.7. After turning 12 years of age, treatment with PCSK9 inhibiting **monoclonal antibodies** will be started – a new class of recently approved cholesterol-lowering drugs (figure 2).

Figure 2: Xanthoma before and after treatment

Conclusion

Hopefully, the addition of PCSK9 inhibitors will enable adequate lipid control. LDL apheresis is currently not available in Bulgaria.

The role of molecular genetic analysis in the diagnostics of congenital heart diseases

GENOME CENTER BULGARIA

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GENICA

INTRODUCTION

Heart disorders are the most common type of birth defects, accounting for more than 30% of all infant deaths. Such examples are: DiGeorge syndrome, Hypoplastic left heart syndrome, etc.

RESULTS

By MLPA analysis we established the genetic cause of congenital heart defect in five fetuses out of 36 tested (14%). In three of them a deletion in the genetic locus 22q11.21 responsible for DiGeorge syndrome was found (Figure 1). In the remaining two fetuses microduplication in 22q11.21 region (*SNAP29* gene) and the presence of additional genetic material in the 22q11-q13 region were identified respectively, which are associated with variable phenotype including congenital heart defects and other malformations (Figure 2 and 3). Based on the published data and our preliminary research we propose a diagnostic algorithm which detects the genetic reasons for congenital heart diseases (CHD) established prenatally during ultrasound examination (Figure 4). By NGS we clarified two CHD cases. The first case is a patient with dilated cardiomyopathy, heterozygous for p.Gln1916X in the *MYH7* gene (Figure 5). The second case is a patient with clinical diagnosis Long QT syndrome, heterozygous for p.Ala561Val in the *KCNH2* gene (Figure 6).

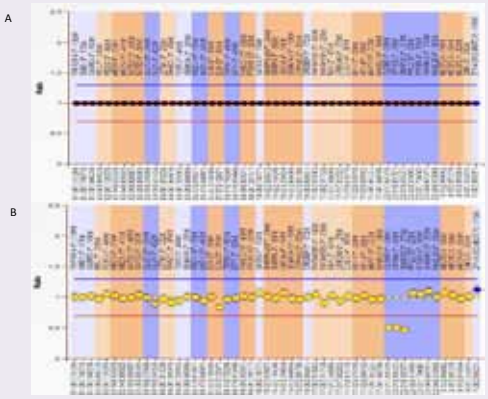


Figure 1: Deletion in the gene locus 22q11.21
A) Control sample; B) Patient

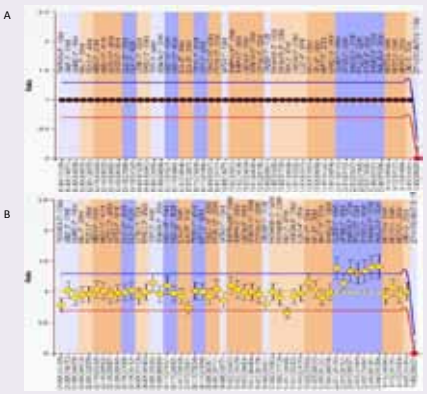


Figure 2: Microduplication in 22q11.21 region.
A) Control sample; B) Patient

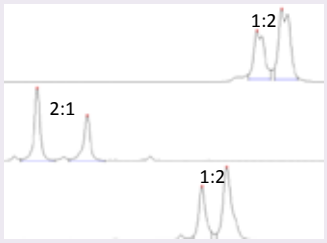


Figure 3: STR markers on chromosome 22 showing a peak ratio 2:1

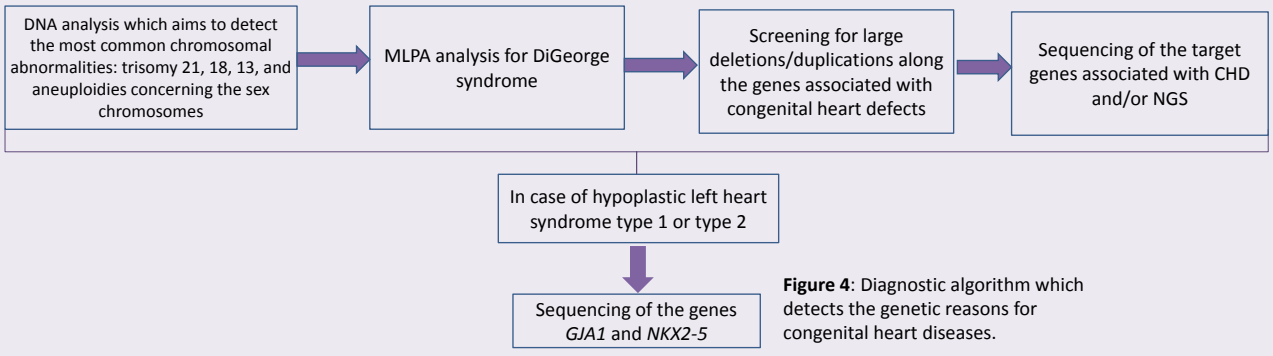


Figure 4: Diagnostic algorithm which detects the genetic reasons for congenital heart diseases.

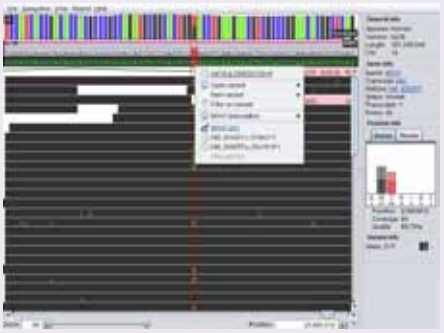


Figure 5: Heterozygous nonsense variant p.Gln1916X in the *MYH7* gene

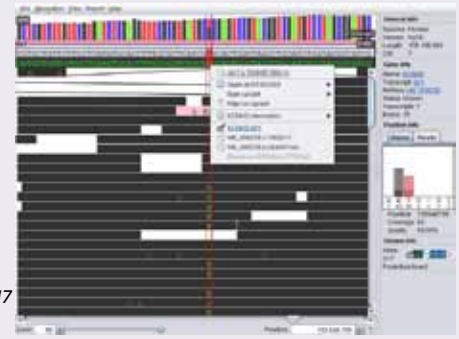


Figure 6: Heterozygous missense variant p.Ala561Val in the *KCNH2* gene

CONCLUSIONS

Verifying the genetic cause of the heart defect allows not only determining the accurate diagnosis, prevention and therapy after birth, but also the accurate genetic counseling and family planning.

The study was partially supported by Medical University Sofia, Grant number Д-61/2017.

VIII НАЦИОНАЛНА КОНФЕРЕНЦИЯ ЗА РЕДКИ БОЛЕСТИ
8-10 септември 2017 - гр. Пловдив

Атипична клиника при инфекциозна мононуклеоза

Е. Исмаил, Е. Якубова
Факултет "Медицина", Медицински университет - Варна

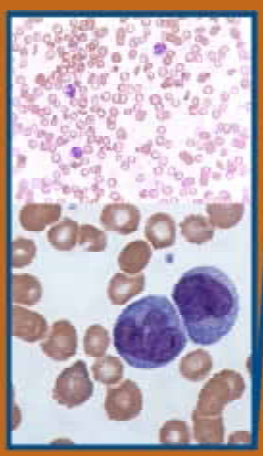
Обективно състояние

Анамнеза

Снета по данни на пациентката. Постъпва на 21.07. 2017 г. във Второ ВО, УМБАЛ „Св. Марина" с фебрилитет от 5 дни, придружен от втрисане. Температурата достига стойности до 39,9 ºС, без закономерност в часовете на фебрилност. Не се повлиява от антипиретични медикаменти. Изписана терапия с Augmentin от личния лекар, без ефект. Не съобщава за контакт с инфекциозно болни лица. Кърмачка (дете на 1г и 3м) живее в апартамент, няма домашни любимци.

Статус

Жена на видима възраст, отговаряща на календарната (31 г.), ало- и автоориентирана, фебрилна (38,5 С). Език - сух и обложен, кожа и видими лигавици - бледи, без обривни единици. Не се палпират увеличени лимфни възли. Аускултаторно не се долавя патологична находка в белите дробове. Няма съдечни шумове, RR 110/70 mmHg, P96, ритмичен. Корем - мек, неболезнен, не се установява хепатоспленомегалия. Крайници - без отоци.



Лабораторни находки

Кръв, биохимия – 21.07.2017

Лабораторните резултати в деня на постъпване показват левкопения, изразена моноцитоза, тромбоцитопения, понижени стойности на хематокрит и еритроцити и леко увеличени стойности на AcAT.

Hb - 120, Ery - 3.57, Hct – 0.323, Leu - 3.28 (Neu – 1.70; 51.9%), Mono# 0.86 (26.2%), Lym# 0.70 (21.3%), Tr - 117, AlAT – 47.1, AcAT – 54.2.

Кръв, биохимия – 26.07.2017

Повторните кръвни изследвания установяват значително повишени стойности на AlAT, AcAT, CrP. На кръвната натривка се установяват вироцити.

Hb - 115, Ery - 3.59, Hct – 0.343, Leu - 2.92 (Neu – 1.54; 52.8%), Mono# 0.44 (15.0%), Lym# 0.79 (27.2%), Tr - 189, AlAT – 105.0, AcAT – 65.0, CrP – 93.59

Лабораторните находките насочват към **вирусна инфекция**!

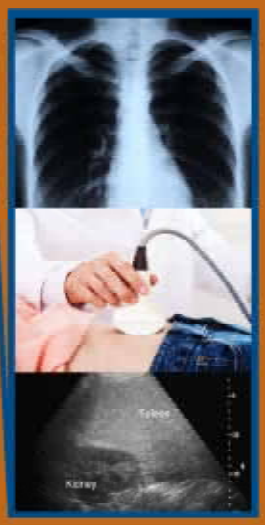
Образни изследвания

Рентгенография на гръдна клетка

Диафрагмени куполи – с нормално разположение, гладки и резки очертания. КДС – свободни двустранно. Белодробни полета – не се визуализират огнищни засенчвания и алвеоларни инфилтрати. Неразширени хилусни сенки. Горен медиастинум – неразширен. Нормален образ на видимите костни структури. Заключение: **нормално прозрачни белодробни полета**.

Ехография на коремни органи

Черен дроб – нормохомогенна структура, недилатирани съдове и интрахепатални жлъчни пътища. Жлъчен мехур – без конкременти. Панкреас – неувеличен, без кистични колекции. Ляв и десен бъбрек – нормални размери, запазен паренхим, без дренажни нарушения и конкременти над 4 мм. Слезка – 127/36 мм горногранично надтъжен размер, хомогенна структура. Пикочен мехур – без дефекти в изпълването. Липсва свободна течност в абдомена. Матка и яйчници – нормален УЗ образ. Заключение: **липсват патологични УЗ находки във видимите зони**.



Поставяне на диагноза

Поради особената клинична картина, диференциалната диагноза е широка. Участват много на брой специалисти, като състоянието на пациентката се обсъжда като треска с неизвестен произход. Изпраща се серум за изследване на кърлежопреносими заболявания, но резултатите отхвърлят тази хипотеза. Назначават се консулти с АГ и УНГ специалист. Извършват се микробиологични изследвания, както следва:

- Носен секрет: резидентна микрофлора;
- Гърлен секрет: резидентна микрофлора;
- Уринокултура: несигнификатна бактериурия;
- Хемокултура: посевките остават стерилни.

Назначени са широкоспектърни антибиотици, но фебрилитетът отново не се повлиява от лечението. След анализ на последните резултати от кръвната картина, се пристъпва към серологично изследване за вируса на Епщайн-Бар – **положителен**! Въпреки неспецифичната клиника, лабораторните данни позволяват да се постави диагноза – **инфекциозна мононуклеоза**. Пациентката бива приведена в инфекциозно отделение.

Какво прави случая атипичен ?

1) **Възрастта**: Инфекциозната мононуклеоза (ИМ) се среща предимно в юношеска възраст, а пациентката е на 31 години;
2) **Клиниката**: От триадата „треска, фарингит, лимфаденопатия" налице е само треската (с продължителност 15 дни);
3) **Лабораторните данни**: налице е левкопения (3,28-2,92), а не очакваната левкоцитоза. Отчита се и неутропения, но тя не е за сметка на лимфоцитите, а на общия брой левкоцити. Този на лимфоцитите не е увеличен, въпреки характерната за ИМ лимфоцитоза.

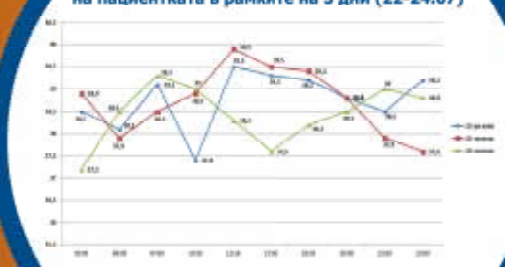
Друго заболяване ?

Установената левкопения насочва диагностичния процес към други възможни хипотези. Отхвърлят се аниктеричен EBV-свързан хепатит, поради отсъствието на астено-адинамичен синдром, както и CMV и HHV6, поради липсата им в серума.

Продължителният фебрилитет, достигащ до 39,9 С, неповлияващ се медикаментозно, както и неправилната температурна крива пораждат суспекция за развитие на септично състояние. Последното е отхвърлено след отрицателната хемокултура.

Температурна крива

Проследява измененията в температурата на пациентката в рамките на 3 дни (22-24.07)



Както преди, така и по време на престоя си в болницата, пациентката не достига афебрилност. Дневните флуктуации в температурата са между 2,1 и 2,3 градуса.

Дискусия и заключение

Поради неспецифичната клиника, пациентката е красноречив пример за нежно фебрилно състояние. Отхвърлени са били възпалителни, неопластични и автоимунни процеси.

Активното търсене на етиологичната причина чрез широк набор изследвания, добрата колаборация между различни специалисти (интернист, оториноларинголог, инфекционист, хематолог, микробиолог и др.), както и допускането на често срещани заболявания чрез необичайна клинична картина, са съществени за навременното поставяне на диагнозата и терапията. Подобен мултидисциплинарен подход е начин за предотвратяване на ненужно лечение с антибиотици и др. медикаменти, без поставена или погрешно поставена диагноза.

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4. **Andersson J, Ernberg I.** Management of Epstein-Barr virus infections. Am J Med. 1986 Aug 29; 81(2B):167-73.
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Genetic testing in coeliac disease – our experience in Bulgaria

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[2]Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria
[3]Laboratory of Medical Genetics, "St. Marina" Hospital, Varna, Bulgaria
[4]13 Zdrave str., Sofia

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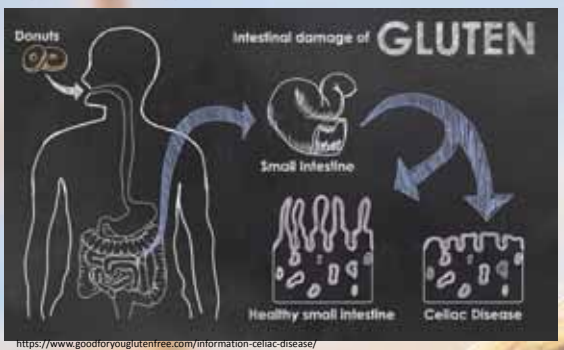


https://www.goodforyouglutenfree.com/information-celiac-disease/

INTRODUCTION

Coeliac disease (CD) represents a chronic inflammatory disease affecting the small intestine mucosa. CD develops because of intolerance to ingested gluten in wheat, barley and rye. The prevalence of coeliac disease is estimated to 1% in Europe, there are no available data precisely for the Bulgarian population.

GENETICS OF COELIAC DISEASE

It is widely accepted that CD has a strong genetic component. About 90-95% of CD patients carry the DQA1*05-DQB1*02:01 haplotype serologically denoted as **DQ2.5**. In almost all of the remaining cases DQA1*03 along with DQB1*03:02 allele are found, known as **DQ8**, or DQA1*05 or DQB1*02 alone. The risk HLA alleles are carried by about 25-40% of the general population. However, less than 0.5% of CD patients lack them justifying the **high negative predictive value** of genetic testing

METHODS

A simplified SSP-PCR (Sequence specific PCR) version has been applied by our team. HLA genotyping allows clinical risk assessment for coeliac disease in case of ambiguous histological and serologic results or of an already initiated gluten-free diet. The current method stands out also for its low cost allowing its wide use even in the sense of screening programs for high-risk groups.

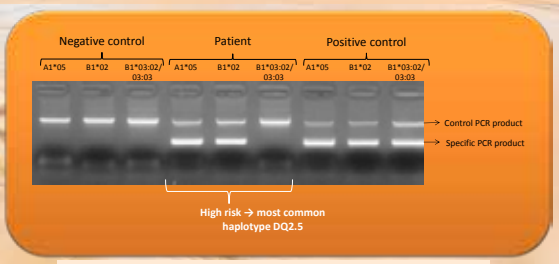


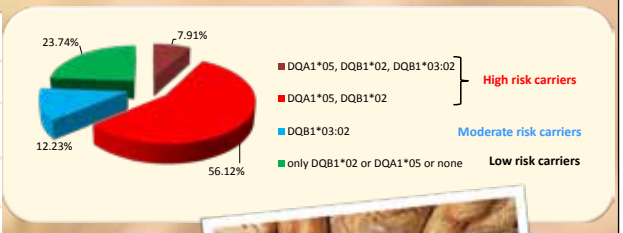
Figure 1. SSP-PCR HLA genotyping.

RESULTS

We performed SSP-PCR HLA genotyping of 139 individuals in total presenting with coeliac related complaints. In total 89 of them (64%) were determined as high risk carriers: 11 (8%) carriers of all the risk HLA variants meaning that they are concomitant carriers of DQ2.5 and DQ8; 78 (56%) carrying the DQ2.5 haplotype (positive for DQA1*05 and DQB1*02). As moderate risk patients were assigned 17 individuals because of presence of the DQB1*03:02 allele (DQ8) either alone or in combination with DQA1*05 or DQB1*02 alleles. From all the tested individuals 33 (24%) were assigned to the low risk group being without any of the tested CD predisposing alleles or carrying only the DQB1*02 or the DQA1*05.

Table 1. and Figure 2. Results from HLA genotyping of patients with coeliac related complaints.

	High risk carriers		Moderate risk carriers	Low risk
DQ group	concomitant DQ2.5 and DQ8	DQ2.5 haplotype	DQ8	-
Positive alleles	DQA1*05, DQB1*02, DQB1*03:02	DQA1*05, DQB1*02	DQB1*03:02	only DQB1*02 or DQA1*05 or none
Number of patients	11	78	17	33
Percent	7.91%	56.12%	12.23%	23.74%
Number of patients	89			
Percent	64.03%			
Total	139			



The authors declare that they have no conflict of interest.

Genome Center Bulgaria

VIII НАЦИОНАЛНА КОНФЕРЕНЦИЯ ЗА РЕДКИ БОЛЕСТИ

Пловдив, 8-10 септември 2017 г.

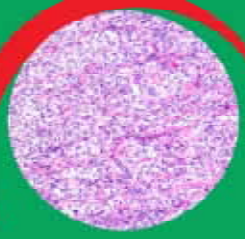
Пациент със светлоклетъчен сарком на горния крайник със сърдечни метастази – клиничен случай

Каназирев, Б.1; Димова, М.1, Златева, В.1, Трифонова, М.1, Бончев, П.1; Исмаил, Е.2, Якубова, Е.2
1УМБАЛ „Св. Марина" – гр. Варна
2Факултет „Медицина", Медицински университет – гр. Варна

Анамнеза

Касае се за 41-годишен мъж, който постъпва в онкологичната клиника на УМБАЛ „Св. Марина" на 22.12.16 г. по повод химиотерапия при злокачествено новообразуване Z51.1, КП № 240 – светлоклетъчен сарком на горен крайник rpT1b cN cM1 (pul), МКБ10: C49.1. Състояние след ампутация на дясна предмишница, белодробна метастаза, пирамидектомия вляво, 6 цикъла от първа линия ХТ . Прогресия до rpT1b cN cM1 (pul, ple, oth). Втори цикъл от трета линия ХТ.

Хистология



Морфологичен резултат: капсулирана туморна формация от разрастнали в гнезда атипични клетки с овални или хиперхромни ядра с централни проминиращи нуклеоли, еозинофилна на места вакуолизирана цитоплазма, единични многоядрени и „физалитни" клетки. Липсват кръвоизливи и некрози, митози < 4MF/10 HPF; инфилтрация в капсулата от туморните клетки.

ИХХ: S100(+), HMB45(+), Desmin(-), CD34(-), Ki67=20%

Химиотерапия

Въпреки неубедителните литературни данни за успех на химиотерапията при СКС, са проведени общо 6 курса на лечение. 4 цикъла от първа линия ХТ - Епирубицин 60 mg/m2, Ифосфамид 1500 mg/m2. Следват 4 цикъла от трета линия ХТ с Гемцитабин 1400 мг, Доцетаксел 150 мг, Филграстим 300 мг.

Терапията в нито един етап не потиска развитието на СКС.

Дискусия

Най-честите неоплазии, метастазиращи в сърцето, могат да инфилтрират различни части от него, с различна честота, в зависимост от първичния тумор – 9% от всички тумори и 14% от метастатичните. Диференциална диагноза с малигнен тумор на периферните нерви (Меркел-клетъчен тумор), синовиален сарком, дълбоко разположен епителоиден сарком, фибросарком, меланотичен шваном, малигнен меланом и др.

Заключение, източници

Въпреки че първичните сърдечни тумори са изключително редки, метастазите в сърцето се срещат по-често. Ехокардиографията е добър способ за откриване на сърдечно ангажиране след първични или метастатични тумори.

1. Медицинската документация на пациента;
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Хирургични интервенции

2008 г. Екстирпация на туморна формация в основата на китката вдясно. Липсва хистологичен резултат в документацията.

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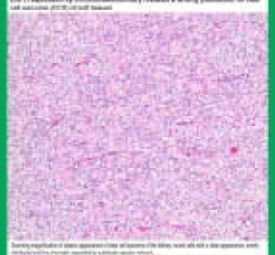
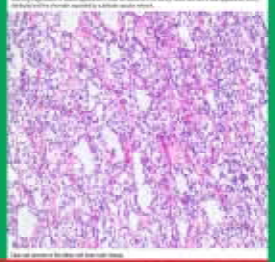
Август, 2015 г. Ампутация на нивo прокимална трета на предмишница – рецидив от СКС, доказан имунохистохимично.

Септември, 2015 г. Хоспитализация в клиника по гръдна хирургия. Пирамидектомия вляво.

Ноември, 2016 г. Ексцизия на туморна формация от език – метастаза от СКС.



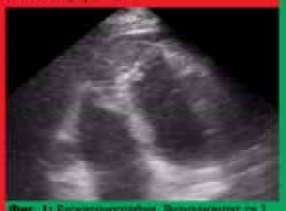


Метастатичен ход

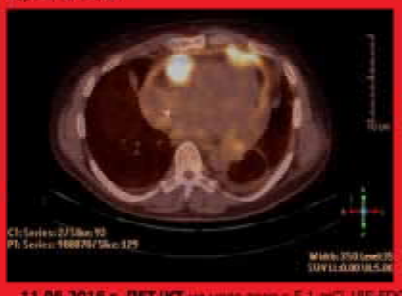
31.03.2015 г. Целотелесно ПЕТ/КТ изследване на 90/170 мин. с ясно изявена активност на 18F-FDG 10.0 mCi. В левия бял дроб – единична нодуларна лезия в контакт с долнолобарния бронх и проксимално в паренхима на 9-ти сегмент, с размери 14 мм и SUVmax 3.0. Увеличената фиксация е суспектна, но неубедителна за малигнена формация. За проследяване.

17.02.2016 г. ПЕТ/КТ на цяло тяло с 8,8 mCi 18F-FDG и време на фиксация 70 мин. Заключение: две метаболитно активни находки – в областта на някои тъкани медиално от остеохондрален преход на 8-мо ребро вдясно и по базата на дясната камера. Независимо от крайно необичайната локализация, и двете находки са суспектни за метастази, особено лезията в областта на реброто, която е ясно структурирана като некротъканна формация.

25.02.2016 г. ЯМР на гръдна клетка, аксиални и коронарни Т2 образи: Локално изпъкване по контура на дясна камера – овална зона с размери около 23/27 мм, изоинтенсивна на миокарда, неотграничима от него, вероятно интрамиокардна. Ниска информативност, с препоръка за ехоКГ.



Фиг. 1: Ехокардиография. Визуализират се 2 формации, суспектни за метастази – вдясно на апекса и в миокарда на лява камера нефрина.

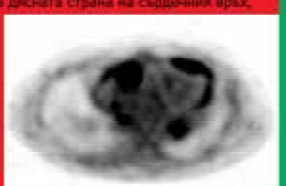


Фиг. 2: На ПЕТ/КТ с FDG се виждат 2 метастази: на апекса на сърцето вляво, на преход между дясно предсърдие и дясна камера, а също и в миокарда на тази предсърдие, инвазиращо и перикарда.

11.06.2016 г. ПЕТ/КТ на цяло тяло с 5,1 mCi 18F-FDG с време на фиксация 78 мин. В областта на основата на дясна камера латерално вдясно се оконтурира изоденсна на миокарда зона с висока метаболитна активност до SUV 9,6 и приблизителни размери 36/25 мм. Находката е нараснала в сравнение с предходното скенириране почти двойно (от 21/11 мм). Открива се втора, ясно разграничима лезия по перикарда, контактна на дясната страна на сърдечния връх, 16 мм, с висока активност до SUV 3,5.

27.10.2016 г. ПЕТ/КТ. Находките от предходните изследвания са провизорно леко увеличени. Ниско в долен лоб на левия бял дроб се скенира плеврално базирана лезия, контактна на латералната плевра, 14 мм – периферна белодробна метастаза.

10.11.2016 г. ПЕТ/КТ с цел рестадиране – данни за прогресия на заболяването. Пациентът е насочен за провеждане на 4 цикъла от трета линия ХТ.



VIII НАЦИОНАЛНА КОНФЕРЕНЦИЯ ЗА РЕДКИ БОЛЕСТИ

8-10 септември 2017 г. - Пловдив

Диагнстични прийоми и терапевтични практики при BCKD дефицит (болест на урина като кленов сироп)

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Факултет "Медицина", Медицински университет - Варна

Същност, генетични аспекти и разпространение

Болестта на урината като кленов сироп (MSUD; МКБ E71.0) е хомозиготно рецесивно заболяване, което се дължи на ензимен дефект в BCKA-комплекса. Последният е свързан с вътрешната митохондриална мембрана и притежава 3 каталитични компонента (E1, E2 и E3) и 2 регулаторни ензима (киназа и фосфатаза). Осигурява декарбоксилирането на алфа-кетокиселините на разклонените АК левцин, изолевцин и валин. Дефекти в E1 – тип A и тип B, E2 и E3 водят до MSUD. Биалелните гени, патологичен еквивалент при MSUD са:
- BCKDHA, кодиращ гена за алфа-субединицата на BCKA декарбоксилазата (E1) (MSUD тип 1A);
- BCKDHB, кодиращ гена за бета-субединицата на BCKA декарбоксилазата (E1) (MSUD тип 1B);
- DBT, кодиращ гена за дихидролипоил трансацилаза (E2) субединицата (MSUD тип 2);
- DLD, кодиращ гена на липоамид дехидрогеназа (E3) субединицата (MSUD тип 3).

Заболеваемостта от MSUD в световен мащаб е около 1:180-185 000, докато в някои изолирани популации тя е далеч по-висока:
- 1:176 живородени при менонитите (религиозна общност) в Пенсилвания, САЩ. Описана е точкова мутация (замяна на тимидинова с аденинова база) в E1-алфа субедицата на гена за BCKA декарбоксилазата;
- Хомозиготна 1-bp делеция (117delC) в BCKDHA гена при португалски цигани. Носителството на тази делеция достига честота от 1,4%.

Сбирка на менонити от Старим Орден - Пенсилвания, САЩ

Клинично обособени форми и прояви

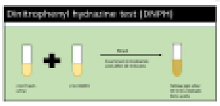
Тип MSUD	Начало (възраст)	Клинични находки	Биохимични белези	BCKAD активност (%)
Класически	неонатална	Мирис на кленов сироп на церумена, лошо хранене, раздразнителност, летаргия, опистотонус, фокална дистония, стереотипни движения, замъглено съзнание, кома, централна дихателна недостатъчност	Увеличено ниво на разклонени АК в плазмата, увеличен плазмен алоизолевцин, увеличени кетокиселини в урината, положителен DNPH-тест, кетонурия	0-2%
Междинен	вариира	Мирис на кленов сироп на церумена, нарушен растеж и хранене, раздразнителност, забавяне в развитието, енцефалопатия	Подобни на класическия фенотип; по-слабо изразени	3-30%
Интермитентен	вариира	Нормален ранен растеж и развитие, епизодични декомпенсации с тенденция към влошаване	Нормални нива на разклонени АК при отсъствие на симптоми / подобни на класическия фенотип при болест	5-20%
Тиамин-чувствителен	Вариира	Подобен на междинния фенотип	Подобрение в левциновия толеранс с приложение на тиамин	2-40%

Всички новородени с класически MSUD се представят в неонаталния период. За останалите форми от значение са няколко фактора, в т.ч. протеинов и калориен прием, темп на растеж, брой и тежест на инфекциозни заболявания и др.

Както при междинната, така и при интермитентната форма на MSUD, тежките неврологични и биохимични прояви могат да наподобят класическия фенотип. Физиологичният стрес е достатъчен да преодолее остатъчната BCKAD активност или тази активност е намалена от преходни промени във фосфорилиращото състояние на ензимния комплекс.

Необходимо е биохимичните признаци да се интерпретират в контекста на левциновия толеранс и преобладаващите клинични обстоятелства. Левциновата толерантност (в mg/kg/ден) се определя като прием на левцин в стационарно състояние, който позволява нормален растеж и поддържа плазмената концентрация на левцин в нормалния диапазон.

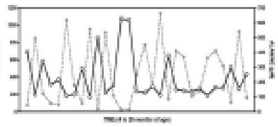
Насоки за установяването на MSUD



Диагностични прийоми

Биохимични тестове:
- Количествен анализ на плазмените аминокиселини: повишена плазмена концентрация на левцин и изолевцин (>5 µmol/L);
- Профилиране на АК на базата на тандемна масспектрометрия (MS/MS) – скрининг програми между 24-тия и 48-тия час след раждане;
- Екскреция на алфа-хидроксикиселини и алфа-кетокиселини с разклонена верига в урината (BCKA): газова хроматография - мас спектрометрия, динитрофенилхидразинов тест (DNPH).

Молекулярно-генетични тестове:
- BCKDHA (19q13.2), BCKDHB (6q14.1) : ДНК секвениране, таргетен анализ за патогенни варианти, анализ за делеции/дупликации;
- DBT (1p21.2): ДНК секвениране, анализ на делеции/дупликации.

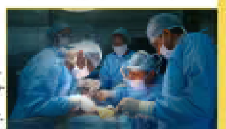


!! **Диференциална диагноза:** метаболитни нарушения с прояви на неонатална енцефалопатия - хиперкетозни синдроми, дефекти в урейния цикъл, глицинова енцефалопатия, пропионова/метилмалонова ацидемия (редки).

Плазмените АК стойности на дете между 4 и 26 месеца с класически MSUD показват силна реципрочна връзка между левцин (сини ромбчета) и аланин (бели кръгове).

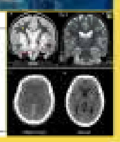
Терапевтични практики

Терапевтичният подход при MSUD се изразява в 2 направления – дългосрочен контрол на заболяването и корекция на епизодите на метаболитна декомпенсация. Последната се повлиява от нетно натрупване на протеини, както и третиране на преципитационния фактор (инфекция, дехидратация, болка, треска и др.). Ето защо, основна причина за хоспитализация на деца с вече установен MSUD са вирусни и бактериални инфекции, като водещи са гастроентеритите и респираторните.

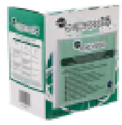


Ортотопичната чернодробна трансплантация е една от алтернативите при лечение на MSUD. Повлиява отлично кризите на метаболитна декомпенсация, като ги преустановява напълно. Неврологични прояви също не се установяват, но вече настъпилите не се повлияват.

Цели на болничното лечение	Основни терапевтични подходи
Нормализиране на плазмената Leu концентрация под 750 µmol/l за 24 h; Натриева концентрация 138-145 mEq/l с минимални флуктуации, избягване на промени в осмоларитета, по-големи от 5 mosm/l на ден; отделяне урина - 2-4 ml/kg/h с осмоларитет 300-400 mosm/l; избягване на хипотонични течности; предвиждане и предотвратяване на вторични електролитни нарушения, свързани с глюкозна и инсулинова терапия (хипокалиемия, хипофосфатемия).	1. Установяване и лечение на преципитиращото състояние (инфекция/възпаление/травма); 2. Назначаване на антиеметик (Ondansetron 0.15 mg/kg/dose); 3. Калориен прием, надвишаващ поне 25% нормата, с дял на мазнините 40-50%; внимание за хиперглекемия; 4. АК суплементиране по време на криза: изолевцин и валин (20-120 mg/kg/ден), глутамин и аланин (250-400 mg/kg/ден), тирозин (100-400 mg/kg/ден) – според декомпенсирания статус.



Диета



Диетичната терапия продължава цял живот! Достъпни са няколко формули и храни без АК с разклонена верига или с намалени нива на такива. Продуктите са използват както от новородени, така и от младежи и възрастни, като напр. **MSUD Express**. Приемът на левцин се изчислява на индивидуално, след измерване на плазмените аминокиселини с разклонена верига. Това трябва да става редовно, на подходящи интервали за първите 6-12 месеца от живота. В допълнение към диетичната терапия, се прилага тиамин (10-20 mg /ден) в продължение на 4 седмици, за определяне реакцията към тиамин.

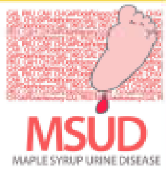
Дискусия

Честотата на заболяването, отнесена към раждаемостта в България, предполага възникването на няколко случая за десетилетие. По тази причина липсва клиничен опит в разпознаването, а настъпилите неврологични смущения лесно се асоциират с друга, по-широко застъпена патология. Това превръща болестта на урината като кленов сироп в диагностичен проблем.

Заключение

Въпреки ниската честота, с която се среща, BCKD-дефицитът следва да бъде подозиран и изследван при наличие на основната симптоматика.

Източници



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7. **Chuang DT, Chuang JL, Wynn RM.** Lessons from genetic disorders of branched-chain amino acid metabolism. J Nutr. 2006;136:243S–9S;
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BECKWITH-WIEDEMANN SYNDROME IN FOUR UNRELATED CASES – FROM THE GENETIC COUNSELING IN MBAL "ST. MARINA" VARNA

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INTRODUCTION

Beckwith-Wiedemann syndrome (BWS)(OMIM#130650) is a congenital overgrowth syndrome that occurs in aproximately one in 13,700 births with about equal incidence in boys and girls. The condition is caused by dysregulation of the expression of imprinted genes in the 11p15.5 chromosomal region.
The syndrome presents with typical manifestations such as macrosomy at birth, macroglossia, defects of the anterior abdominal wall, an increased risk of developing certain childhood tumors, hypoglycemia in the newborn period due to hyperinsulinism and unusual ear creases or pits. Children with BWS may also have hemihyperplasia. Genetic background is variable – abnormal methylation in region KvDMR1 (gene KCNQ1OT) and gene H19DMR of chromosome 11p15.5 evaluated by MLPA analysis. Indirect DNA analysis with polymorphic markers D11S1984, D11S922, TH, D11S4088 and D11S1346 identify UPD11p15.5.
Cytogenetically detectable abnormalities involving chromosome 11p15 are found in 1% or fewer of affected individuals.

CASE 1

A boy from a first, uneventful pregnancy, 1 year old with the following symptoms:
Fetal Macrosomia – weight 4420,height 51cm at birth,
Macroglossia,
Facial hemangioma,
Omphalocele, Diastasis recti (2mm),
Hepatomegaly,
Dermoid cyst in the left temporal lobe,
Ear lobes – Anterior linear ear lobe creases,Posterior helical pits.
Advanced bone age
Thorax-widely spaced nipples,pectus excavatum

Diagnosis
1. Conventional cytogenetic analysis: normal male karyotype 46,XY,21ps+
2. DNA Analysis - Abnormal methylation (demethylation) at region KvDMR1 (gene KCNQ1OT) of chromosome 11p15.5

CASE 2

A 2-months-old boy from a first pregnancy presented with:
Fetal Macrosomia – weight 4050,height 51cm at birth
Macroglossia,
Hemihyperplasia,
Hemihypertrophy (height - measurement taken on the left side of the body 60cm, on the right side:57.5 cm,
Head circumference 42.5
Undescended testis on the right side,

Facial asymmetry,overgrown of the skull on the left side, ear lobes – anterior earlobe creases,
Hepatomegaly,splenomegaly,
Diastasis recti
Umbilical hernia

Diagnosis
1. Conventional cytogenetic analysis: normal male karyotype 46,XY
2. DNA Analysis:
   a. Abnormal methylation (hypomethylation) at region KvDMR1 (gene KCNQ1OT) of chromosome 11p15.5.
   b. Paternal uniparental disomy 11p15.5 with focus on phenotype – genotype correlation

CASE 3

A two-months old girl from a first uneventful pregnancy, with the following symptoms:
Fetal macrosomia – weight 4050 g,height 53 cm at birth
moderate birth asphyxia
Hypotonia
Neonatal hypoglycemia
Omphalocele
Macroglossia
Caput succedaneum

Diagnosis
1. Conventional cytogenetic analysis: normal female karyotype 46,XX
1. DNA Analysis:Abnormal methylation (demethylation) at region KvDMR1 (gene KCNQ1OT) of chromosome 11p15.5

CASE 4

A three-months-old girl a second uneventful pregnancy with the following symptoms:

Fetal Macrosomia – weight 4000 g,height 54 cm at birth
Macroglossia
Facial dysmorphism
Hypertrophy of right upper and lower limbs,
Anterior linear ear lobe crease on the left ear
Omphalocele

Diagnosis
1. Conventional cytogenetic analysis: normal female karyotype 46,XX
2. DNA Analysis:Abnormal methylation (hypermethylation) of H19DMR.

DIAGNOSIS

Conventional cytogenetic analysis was performed in all cases to exclude a regular chromosomal disorder. DNA Analysis - Methylation test in region KvDMR1 (gene KCNQ1OT) and gene H19DMR of chromosome 11p15.5 and MS-MLPA analysis confirmed clinical diagnosis in all patients, showing remarkable variability of the DNA findings. This could be a good start for interpreting genotype-phenotype correlation in future research cohorts.

References:
Лукова, М., А. Тодорова, Т. Тодоров, Т. Кадийска, Р. Тинчева, Д. Авджиева, Е. Симеонов, Л. Ангелова, В. Митев. Синдром на Beckwith-Wiedemann и синдром на Silver-Russell в България. Молекулярно-генетична диагностика
https://www.ncbi.nlm.nih.gov/books/NBK1394/
https://ghr.nlm.nih.gov/condition/beckwith-wiedemann-syndrome
https://rarediseases.org/rare-diseases/beckwith-wiedemann-syndrome/

THE CASE OF BRIC-sindrom

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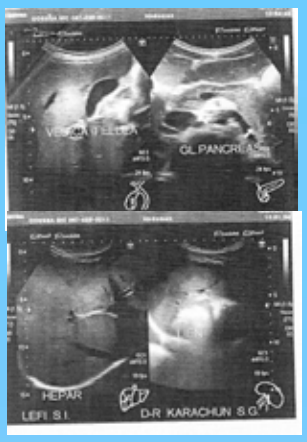
INTRODUCTION Benign Recurrent Intrahepatic Cholestasis (BRIC) is a rare hereditary disease. It is characterized by periodic episodes of cholestasis, itching, jaundice, an increase in the level of transaminases, alkaline phosphatase, with a relatively normal level of GGT. An important criterion is the absence of progressive liver damage. Type of inheritance is autosomal recessive. At present, the mutation leading to the development of the disease is known, ATP8B1

OBJECTIVE to study the increase in the activity of transaminases, which can be of a relatively benign nature

METHODS among the 3000 patients with an increase in transaminases examined in the center, BRIC syndrome is registered for the first time



RESULTS AND DISCUSSION in metabolic disorders, the liver is involved in the process in 95% of patients, so often we face with a high level of transaminases. After correction of the underlying disease, hepatic parameters approach the reference values. We give an example where the treatment did not lead to the expected results.

THE PATIENT S., 15 years old, complained of general weakness, fatigue, periodically - itchy skin, jaundice of the skin – has addressed to our center.

From anamnesis it is known that from the age of 6 the child was actively engaged in sports. She grew and developed according to age norms, has transferred: ARD, chicken pox. At 12 years with a planned medical examination for the first time the biochemical parameters were monitored, a 10-fold increase in the level of transaminases, GGT, alkaline phosphatase, bilirubin levels were slightly increased, and moderate hepatomegaly was noted. Child did not have any complaints.

Taking into account the biochemical picture, in the first place, a search was made for markers of viral hepatitis - the result is negative. Bacterial, TORCH-infection, were also excluded. Due to the fact that the child was intensely engaged in sports, toxic liver damage was suspected, but this diagnosis was not confirmed

Markers of viral hepatitis A, E, B and C were repeatedly examined - the result is negative.

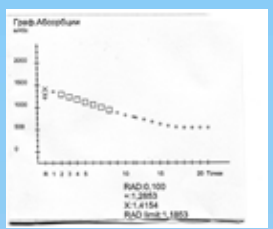
DIAGNOSED acute hepatitis of non-etic etiology. The girl was prescribed hepatoprotective therapy, ursofalk. Against the background of taking medications, the level of transaminases did not stabilize.

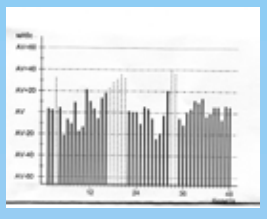
With the growth of the child began to arise uncaused periods of jaundice, itching. During the transient period there was a high level of transaminases, alkaline phosphatase, total bilirubin, but GGT indices increased slightly. The proband was suspected of hepatitis of autoimmune etiology. A comprehensive examination was carried out, this diagnosis was not confirmed. Differential diagnostics included Konovalov-Wilson syndrome: level of copper in blood and urine, ceruloplasmin, ophthalmological examination for the detection of Kaiser-Flasher ring – no changes were detected.

The study of activity of acidic lipase was investigated to exclude Wolman's disease, this diagnosis was excluded. For refining diagnosis biopsy of the liver was performed - cirrhosis, fibrosis excluded. The family turned to our center

Biochemical indicators:

Indicators	2016	2017 April	2017 June
ACT	290.01 ↑	182 ↑	279.73↑
ALT	680.23 ↑	552 ↑	660.82 ↑
GGT	48 ↑	30↑	33 ↑
Alkaline phosphatase	170 ↑	152 ↑	
Total bilirubin	25.2 ↑	20.0 (normal)	15.48 (normal)
Direct bilirubin	6.1↑	6.0 ↑	6.0↑





Gas chromatography of urine: changes in metabolites
-the decrease in metabolites of the Krebs cycle, -reduction of neurotransmitters, - depletion of glutathione
With ultrasound examination of the abdominal cavity organs, a moderate increase in echolarsity of the liver was revealed.

CONCLUSIONS Having analyzing this clinical case (wave-like course, absence of complaints during the inter-attack period, absence of changes on the part of the liver with a significant increase in transaminase activity, absence of markers of viral hepatitis), we came to the conclusion that the patient has the BRIC syndrome. To search for a molecular target, a mutation in the ATP8B1 gene is searched.

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METABOLISM OF VITAMIN D SYSTEM IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

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INTRODUCTION Today autism spectrum disorders (ASD) is considered as an epidemic – in the last 5 years, the frequency of pathology has increased from 1: 166 to 1:68 children (Center for Disease Control and Prevention USA (CDC), 2014).

Now there is no the single etiopathogenetical mechanism of ASD, but there are some theories of its (genetic and non-genetic). The metabolism of vitamin D plays the important role. The system of vitamin D regulates the metabolism of neurotransmitters (such as serotonin), involves in detoxification and performs epigenetic effects (controls about 3% of the human genome).

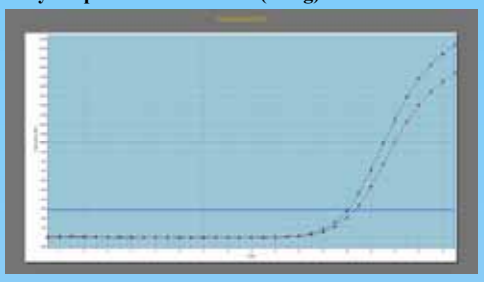
VDR gene has several allelic variants, the most clinically are Bsm I, Fok I and Taq I. VDR gene is localized on the long arm of chromosome 12 (12q13.11).The above data show the relevance of studying the peculiarities of metabolism of vitamin D in children with ASD for the elaboration pathogenetic treatment.

PURPOSE To study of the distribution of polymorphism Bsm I of gene VDR, their association with metabolism of vitamin D and state of folate-methionine cycle in children with ASD.

Polymorphism	*ASD (n=74)*	*Helthy (n=46)*	*Population frequency, Ukraine (O.Grechanina, R.Matalon, 2008) n=1938*
VDRBsm I BB (normal)	21 (28.38%)	19 (41.31%)	
VDRBsm I Bb (hetero)	40 (54.05%)	26 (56.52%)	
VDR Bsm Ibb(pathological)	13 (17.57%)	1 (2.17%)	
MTHFR 677 C/C (normal)	32 (43.24%)	25 (54.35%)	48.0%
MTHFR 677 C/T (hetero)	35 (47.30%)	17 (36.95%)	43.3%
MTHFR 677 T/T (pathological)	7 (9.46%)	4 (8.70%)	8.7%
MTRR 66 A/A (normal)	20 (27.02%)	10 (21.74%)	21.2%
MTRR 66 A/G (hetero)	30 (40.54%)	23 (50.00%)	41.8%
MTRR 66 G/G (pathological)	24 (32.44%)	13 (28.26%)	37.0%
MTR 2756 A/A (normal)	35 (47.30%)	31 (67.39%)	
MTR 2756 A/G (hetero)	32 (43,24%)	12 (26,09%)	
MTR 2756 G/G (pathological)	7 (9.46%)	3 (6.52%)	

MATERIAL AND METHODS The study will use classical genetic methods (somatic genetic study with syndromic analysis, clinical and genealogical analysis) and modern technology (molecular genetic methods, high performed liquid chromatography, mass spectrometry/gas chromatography).

Polymorphism MTHFR 677 (hmzg)



Bsm I VDR gene (htzg)

RESULTS We examined 130 children: 74 with ASD and 45neurotypical. The polymorphism Bsml of the VDR gene was studied: bb (pathological homozygote) - 13 (17.57%) and 1 (2.17%), respectively, Bb - 40 (54.05%) and 26 (56.52%), BB - 21 (28.38%) and 19 (41.31%). 87.84% of children with ASD showed a decrease of 25-OH-D level in the blood, whereas in the control group in only 6.52% cases. Polymorphic variants of the genes mutilation cycle were studied: an increase in the frequency of MTHFR 677 C/T polymorphisms (47.30% and 36.95%, respectively), MTRR 66 G/G (32.44% and 28.26), MTR 2756 A/G (43.24% and 26.09%), MTR 2756 G/G (9.46% and 6.52%, respectively).98.65% of children with ASD showed a decrease of homocysteine level in the blood, whereas in the control group in only 45.62% cases

CONCLUSIONS 1. The specific weight of vitamin D deficiency in children with ASD occupies one of the leading places 2. Diagnosis and correction of vitamin D deficiency (with correction of folate-methionine cycle) in children with ASD should be part of the examination and treatment algorithms.

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THE CASE OF A COMBINATION OF FOLATE TRANSPORTER, METHYLENETETRAHYDROFOLATE REDUCTASE AND PYRIDOXINE DEFICIENCY IN CHILD WITH EPILEPSY AND PSYCHOMOTOR AND SPEECH RETARDATION

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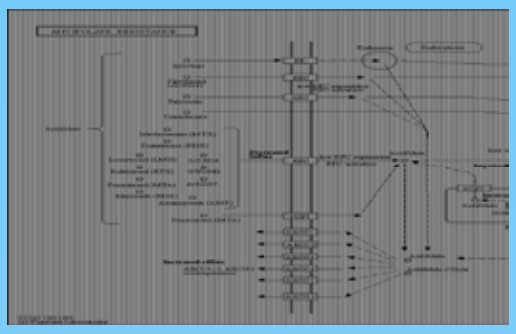
INTRODUCTION The study of the folate-methionine cycle KhISMGC-CR(O)D (together with the University of Houston, USA, R. Matalon) has been engaged since 2007 (O. Grechanina, Y. Grechanina). Over 11,000 patients with suspicion of metabolic disorder were examined. The accumulated experience makes it possible to demonstrate extremely rare combinations of metabolic disorders.

2 year (2016)

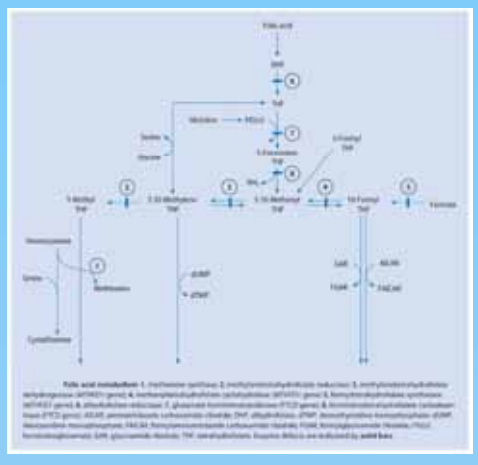
3 year (2017)

MATERIAL AND METHODS The study will use classical genetic methods (somatic genetic study with syndromic analysis, clinical and genealogical analysis) and modern technology (molecular genetic methods, high performed liquid chromatography).

PURPOSE To study the clinical manifestations of a combination of folate deficiency, the deficiency of methylenetetrahydrofolate reductase and pyridoxine in the case of a clinical case.



DESCRIPTION Boy D., 2 years, complaints: delay in psychomotor and speech development, epilepsy, episodes of apnea after an attack. In the phenotype: the marbling of the skin, pronounced subcutaneous venous network, submicrocephaly, deformity of the thorax. At the examination: increased methionine, homocysteine; reduced pyridoxine, serine in the blood.



When carrying out the DNA analysis of the gene RFC-1 (folate transporter) - genotype 27Arg/Arg (pathological homozygote); of the polymorphisms MTHFR 677 T\T (pathological homozygote), MTRR 66 A\G, MTR 2756 G\G (pathological homozygote). Treatment: hypomethionine diet, vitamin B6, 5-MTHF, betaine. On the background of therapy, the child has a significant positive dynamics - improved motor skills, seizures are stopped.

CONCLUSIONS The case demonstrates a complex and rare combination of metabolic disorders in a child with a delay in psychomotor and speech development. Early diagnosis and individually selected therapy made it possible to achieve relief of epicas and improve development.

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THE CASE OF A COMBINATION OF WILSON-KONOVALOV DISEASE AND HEMOCHROMATOSIS, CAUSED BY HETEROZYGOUS CARRIAGE OF THE MUTATIONS C282Y AND H63D OF THE GENE OF HEMOCHROMATOSIS

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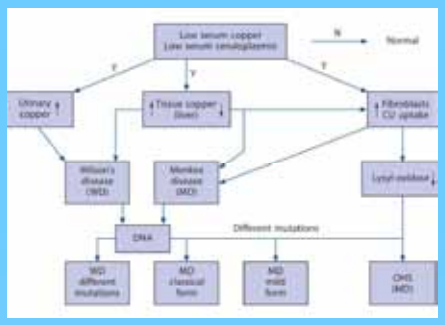
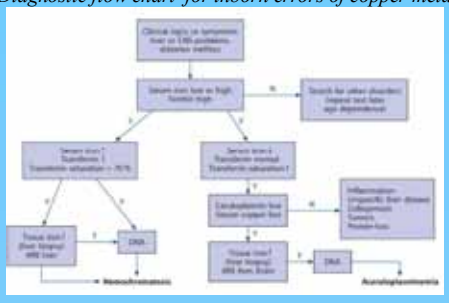
INTRODUCTION: Today, we are increasingly observing the phenomenon of synthropy, when one patient has a conglomerate of diseases, each of which contributes to the realization of the clinical picture of the pathology, which often complicates its diagnosis.

AIM: To study the features of the course of Wilson-Konovalov disease (WKD) in combination with hemochromatosis for the development of individual tactics of management.
MATERIALS AND METHODS: Among 11000 first-time patients in 2016, one case of WKD was diagnosed. Both classical and modern methods were used.

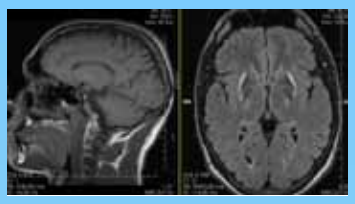
RESULTS: Patient A., 25 years old.
Complaints: muscle tension, stiffness in the body, speech impairment, salivation, choking, difficulty of movement, inability to walk without support, frequent falls, anxiety, disturbance of sleep.In 2015, after the stress, there was a feeling of chronic fatigue. After 6 months, the syndrome of portal hypertension and dyscirculatory encephalopathy were diagnosed. The disease is progressing. MRI of the brain – the accumulation of metal in the basal nuclei.In uriny – high copper excretion. The patient was hospitalized in a neurological clinic. Established the diagnosis: Wilson-Konovalov's disease. Chronic hepatitis in the transition to cirrhosis. Started the therapy (Kurantil, Unitiol). The treatment without effect The general condition worsened, a tremor appeared, muscle tension, stiffness, began to experience difficulty in walking The general condition worsened, a tremor appeared, muscle tension, stiffness, began to experience difficulty in walking.



Diagnostic flow chart for inborn errors of copper metabolism.



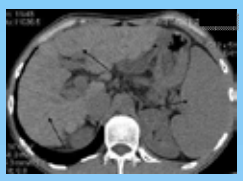
Diagnostic flow chart for inborn errors of iron metabolism.



MRI of the brain - accumulation of metal in the basal nuclei

The Kaiser-Fleischer Ring

In connection with the progression of the disease with a rapid growth of clinical symptoms, the ineffectiveness of the therapy, the patient turned to the Center to clarify the diagnosis and choose further tactics of treatment. Correction of therapy (Zincteral) was performed.
In the phenotype: hypomia, pronounced hypokinesia, speech modulation disorder, salivation, stiffness, lack of synergy in walking, manifestations of microangiopathy.



CT-signs of liver cirrhosis, portal hypertension and splenomegaly

SYMPTOMS	BEFORE THE TREATMENT	AFTER THE TREATMENT
Stiffnessinthebody	+++	+
Increased muscletone by plastictype	+++	++
Violation of speech modulation	++	+
Salivation	++	-
Dysfunction of swallowing	+	-
Hypomia	++	+
Hypokinesia	++	+
Difficulty of walking	+++	+
Frequentfalls	+	-
Inability to walk withou tsupport	+	-
Self-walking without tsupport	-	+
Absence of synergy in traffic	++	-
Unstable tremor of the head, fingers of the hand	+	-
Intensional tremor in the execution of the finger-nose test	+	+
Hepatosplenomegaly	++	+
The Kaiser-Fleischer Ring	+	+
Anxiety	+	-
Disturbance of sleep	+	-
Increased copper excretion in daily urine)	+	+
Increase inserumiron	+	-
Increase of blood lactate	+	-
Disturbance of microelements metabolism	++	+

In the neurological status expressed manifestations of subcortical, akinetic-rigid syndromes. In uriny – high copper excretion. In the blood – increase level of iron, AST, ALT, lactate, methionine, decrease level of cystine. Ultrasound examination – signs of portal hypertension, hepatosplenomegaly, pancreatopathy.
Molecular diagnostics – carrier of mutations C282Y and H63D of the gene of hereditary hemochromatosis; polymorphisms of folate-methionine cycle MTHFR 677 C/T, MTRR 66 A/G, MTR 2756 A/G.
DIAGNOSE: Combined metabolic metabolic disorder: Wilson-Konovalov's disease and hemochromatosis caused by heterozygous carriage of mutations C282Y and H63D of the hereditary hemochromatosis gene. Violation of the metabolic of sulfur-containing amino acids. Secondary mitochondrial dysfunction. Polymorphism of genes: MTHFR 677CT (risk allele), MTRR 66AG (risk allele), MTR 2756AG (risk allele)
Taking into account the revealed metabolic disturbances, an individual tactic of tretment aimed at their correction was developed.

CONCLUSIONS: The presence of synthropy-associated metabolic disturbance, in our opinion, underlies the rapid progression of the process, the trigger of which has been a long-term stress. Correction of the metabolic disorders stopped the progression of the pathology, reduced neurologic manifestations, improved the quality of life of the patient.

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GENETIC POLYMORPHISM DATA ON 15 AUTOSOMAL STR MARKERS IN ALBANIANS FROM ALBANIA

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AIM OF THE STUDY:

Albania is part of Balkan region, having a homogenous population of about 3.000.000 ethnic Albanians, representing about 97% of Albanian population living in Albania (Census 2011). To our knowledge, there are not published data on autosomal STR loci of Albanians living in Albania, useful for calculations of forensic parameters.

MATERIALS AND METHODS

This study included 140 unrelated adult Albanians from various regions of Albania, a part of the persons presented in our centre to perform DNA identification tests. DNA from blood and buccal swabs was isolated by various protocols.
PCR amplification was carried out on GeneAmp PCR System 9700, using the AmpFISTR Identifiler PCR amplification kit (Applied Biosystems, USA), according to the manufacturer's instructions.
The amplified products were separated by capillary electrophoresis using ABI PRISM 310 Genetic Analyser. Data analysis and allele detection were carried out using data collection software, Genescan3.1 and Genotyper software v3.7.
Allele frequencies and other statistical parameters commonly used in forensic and paternity tests were calculated with PowerStats software v1.2.

RESULTS

Figure 1. DNA profile using 15 STR AmpFISTR Identifiler Plus markers.

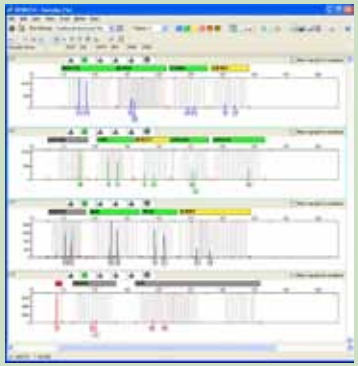

Table 1. Allele frequencies and related statistical data for 140 individuals of Albanian population

The allele frequencies of the 15 STR loci and forensic parameters are presented in Table 1. No statistically significant deviation (P< 0.05) from the H-W equilibrium was found for any of the analyzed loci. The combined power of discrimination and the combined power of exclusion for all 15 loci were 0.99999999999996 and 0.9999997325712, respectively.

Table 2. Population differentiation exact test probability values resulting from comparison of Albanians with geographically close populations

Locus	Kosovo	Serbia	Montenegro	Bosnia&Herzego.	Croatia	Albanians of Italy
D8S1179	0.23765±0.0119	0.06345±0.0090	0.38429±0.0098	**0.01490±0.0024**	0.11349±0.0076	0.83709±0.0097
D21S11	0.13226±0.0154	**0.00007±0.0001***	**0.00018±0.0001***	**0.04839±0.0095**	**0.00023±0.0002***	0.31589±0.0233
D7S820	0.07845±0.0097	**0.00375±0.0019**	0.63307±0.0185	0.06562±0.0032	**0.02613±0.0078**	0.18468±0.0162
CSF1PO	0.45970±0.0159	0.62790±0.0166	0.10975±0.0100	**0.02957±0.0040**	0.41929±0.0173	0.77025±0.0110
D3S1358	0.20063±0.0093	0.54845±0.0191	0.52950±0.0186	0.72086±0.0132	0.40694±0.0177	0.12397±0.0160
THO1	0.15912±0.0084	**0.00176±0.0006***	0.57445±0.0135	**0.00192±0.0010***	**0.00556±0.0017**	**0.01953±0.0023**
D13S317	0.34835±0.0235	0.55176±0.0136	0.42354±0.0155	0.46218±0.0158	0.94625±0.0053	0.29177±0.0130
D16S539	0.89440±0.0036	0.38316±0.0160	0.40578±0.0155	0.72588±0.0136	0.76393±0.0130	0.70242±0.0093
D2S1338	0.10036±0.0077	0.17648±0.0122	**0.04948±0.0067**	**0.01934±0.0047**	0.09138±0.0067	--
D19S433	**0.00868±0.0018**	0.48039±0.0221	**0.00000±0.0000***	0.34384±0.0146	0.55124±0.0196	--
vWA	**0.00884±0.0017**	0.28043±0.0167	**0.00037±0.0002***	**0.04575±0.0114**	**0.02039±0.0037**	0.85356±0.0076
TPOX	0.17696±0.0094	0.05912±0.0071	0.13854±0.0129	0.44660±0.0121	0.31660±0.0201	0.11345±0.0117
D18S51	**0.00747±0.0021**	0.57897±0.0182	0.14240±0.0195	0.52314±0.0186	0.39247±0.0251	0.18966±0.0164
D5S818	0.75235±0.0210	0.58429±0.0205	0.75982±0.0166	0.55779±0.0151	0.82542±0.0162	0.67934±0.0162
FGA	0.41828±0.0206	0.06703±0.0088	0.06690±0.0126	0.17030±0.0184	0.11411±0.0193	0.27776±0.0191

P values ± SE (an estimation of the error on the P value according to Arlequin software)
Significant values P<0.005 marked in bold
* Significant values after applying Bonferroni correction (P<0.0033)

In order to compare the Albanian population with other populations, the exact population differentiation test was performed using Arlequine software v3.5. The comparison of the allele frequency distributions in our sample with other populations are presented in Table 2. Significant P-values are marked in bold.

DISCUSSIONS

We compare the results obtained from our sample with other populations in the region using exact test. The exact population differentiation test performed using Arlequine software v3.5 revealed statistically significant differences between our sample of Albanians and all compared populations.

The lowest differences were found with Kosovo (D19S433, vWA, D18S51) and the highest differences were found with Montenegro and Bosnia& Hercegovina (D21S11, THO1, vWA, D8S1179, CSF1PO, D2S1338). The most variable loci in Albanian population were found to be THO1, D21S11 and vWA, loci that differed in 4 from 6 compared sample populations.

We consider our data valuable for an Albanian Reference Database, suitable for forensic application and for population genetic studies. To our knowledge, these data are the first published data on autosomal STR loci of Albanians living in Albania. We hope data presented in our study could be useful for calculations of forensic parameters involving Albanians in Europe and in other continents.

The lowest differences are with Kosovo (D19, vWA, D18), and North Greece (D3, THO1), and the biggest differences are identified with Italy (Calabria) (7 loci) and Austria (6 loci).

After applying Bonferroni correction, statistically significant differences remained at one locus when compared to the following populations: the North Greek population at THO1 locus (from 2 loci D3, THO1) and the Croatia population at D21S11 locus (from 4 loci D21, D7, THO1, vWA). The Serbian sample from Vojvodina province differed from our sample at two loci: D21S11 and THO1, as well as the Italian sample from Calabria at D18S51 and D21S11 loci. There were significant differences between our sample and Montenegro population at three loci: D21S11, vWA, D19S433, as well as with Austrian population at THO1, D18S51 and FGA loci.

We also compared the sample of our study, with a sample of Albanians from Kosovo (4) and with a sample of Albanian emigrants from Albania, living in Torino Region, Italy, studied by Forensic Institute of Torino (14). The lowest differences are with Albanian emigrants, living in Torino Region, only the locus THO1, and with Kosovo sample we found differences in three loci (D19, vWA, D18). The Italian sample of Albanians was analyzed using PowePlex16 kit of Promega, that not included 2 variable loci, D2S1338 and D19S433. After applying Bonferoni's correction, statistically no significant differences remained with Kosovo sample and Albanian emigrants living in Italy.

The most variable loci in Albanian population were identified THO1 and D21S11, that differed in 8 from11 considered populations, after was vWA locus (6/11), D18S51 (5/11) and FGA (5/11) loci. From comparison of our sample with other populations we confirm the solid and unique data of Albanians living in Albania, and considered our data valuable for an Albanian reference Database suitable for forensic application and population genetic studies. These are the first data published on autosomal STR loci of Albanians living in Albania.

We hope our data to be useful for calculations of forensic parameters involving Albanians in Europe and in other continents.

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PRELIMINARY RESULTS OF GENETIC VARIANT CYP1A2 1*F, INVOLVED IN CAFFEINE INTOLERANCE, IN ALBANIAN POPULATION

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INTRODUCTION

CYP1A2 is a human enzyme involved in the metabolism of 5-10% of drugs of large use, as clozapine, imipramine, caffeine, fluvoxamine, paracetamol, phenacetin, theophylline, tacrine, etc. More than 15 allelic variants of gene CYP1A2 were identified, which determined different activity of CYP1A2 enzyme. Polymorphism of A nucleotide in position 163, called variant 1*F (-163C> A), represented alellic variant with higher frequency in human populations. Genetic variants of CYP1A2 gene were identified, variant F is considered a fast metabolizer and variant C is considered a low metabolizer. Personal identification of genetic variants is important in the treatment of patients by dose specific drugs. In particular, genetic variant CYP1A2 1*F is part of DNA test for caffeine intolerance, considered as a preventive test for the risk reduction of myocardial infarct. Each ethnic population presented a particular pattern of CYP1A2 genetic variants and their identification in Albanian population could be of genetic and clinical interest.

Figure 1. CYP1A2 gene, chromosomal position, other genes around and the genomic structure.

AIM OF THE STUDY

Up to know we have not Albanian population data about the frequency of CYP1A2 1*F allele in Albania. Our aim was the analysis of 100 healthy Albanians for the identification of CYP1A2 1*F allele frequency. The setting up of the protocol and the analysis of 50 individuals was part of a Master experimental thesis in Molecular Biology.

MATERIALS AND METHODS

MATERIALS: 50 healthy individuals from Albanian population randomly selected from subjects analyzed in Biochemical Lab of University Hospital of Obstetrics and Gyneacology "Mbreteresha Geraldine", in Tirana were analysed for CYP1A2 polymorphism. 2 ml blood samples with EDTA were collected and DNA was extracted.

METHODS

DNA extraction. DNA was extracted from total blood according standard protocols using Qiagen DNA blood extraction kit. DNA samples were conserved in +4°C, and used for DNA analyses immediately, otherwise are conserved in -20oC. The concentration of DNA was about 20 ng/µl.

PCR-RFLP protocol used in determination of CYP1A2 *1A and *1F alleles

Figure 1. Gene structure and primers P1 and P4 r used for amplification of intron 1 of CYP1A2 gene, and thermocyler used for PCR.

Primers used: CYP1A2*1F (5' – CCCAGAAGTGGAAACTGAGA – 3') and CYP1A2 *1R (5' – GGGTTGAGATGGAGACATTC – 3'). Primers were purchased from Applied Biosystem. Primers amplified intron 1 of CYP1A2 gene and the PCR product was a fragment of 243 bp in size.

PCR reactions were performed in 25 µl volume, containing 6 µl BufferGold Mix (MgCl2 1.5 mM, dNTPs 200uM for each nucleotide), 1 µl for each primer (10 pmol/µl), 0.5 µl TaqGold polymerase, 11.27 µl H2O and 5µl DNA (~100 ng). PCR program was as described: 10 min at 95°C, 35 cycles of 30s at 94°C, 30s at 60°C, 30s at 72°C, and the last extension for 10 minutes at 72oC.

The control of PCR products was performed by gel electrophoresis in 2% agarose. A fragment of 243 bp should be visible by gel electrophoresis in all samples amplified.

RFLP (Digestion of PCR fragment by Restriction Enzyme Apa1)

Allele CYP1A2 *1F (-163C>A in intron 1) is identified by digestion with ApaI. A new Apa1 site was created by mutation (-163 C>A). Restriction reaction was performed in 25 µl final volume, containing 10 µl DNA from PCR reaction, 2.5 µl Buffer A, 0.5 µl Apa1(10 units/ul) and 12 µl H2O. DNA samples were incubated in a block heater at 37°C, overnight.

Identification of Apa1 digestion pattern of PCR products by gel electrophoresis. The presence of 243bp fragment after digestion by Apa1 means that the subject have a wild type genotype in homozygotes. The presence of only two fragments, 119bp and 124bp, means that a new Apa1 site was created by mutation and the genotype is homozygote for the mutation CYP1A2 *1F. The presence of three fragments 243bp, 119bp and 124bp, means that the subject have a heterozygote genotype CYP1A2 *1A/CYP1A2 *1F. The possible digestion patterns of PCR-Apa1 protocol is shown schematically in Fig 2.

Frequency of genotypes and alleles CYP1A2 *1A and *1F

Calculation of genotypes frequency and allele frequencies of CYP1A2 *1A and *1F was performed according to known formulas of population genetics.

*Figure 2. Schematic presentation of three digestion patterns of CYP1A2 genotypes *1A/*1F and electrophoresis equipments used in our laboratory.*

RESULTS

Determination of genotypes and alleles *1A and *1F frequencies in the sample of 50 individuals from healthy Albanian population.

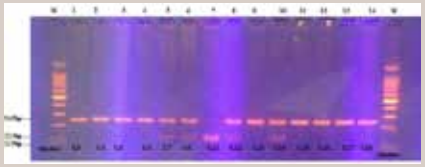

Figure 3. Photo of gel electrophoresis PCR products digested by Apa1 restriction enzyme.

Frequency of genotypes and alleles. Homozygote genotypes CYP1A2 *1A/*1A were found at frequency of 64.2%; Heterozygote genotypes CYP1A2*1A/*1F were found at frequency of 32.0 %; Homozygote genotypes CYP1A2 *1F/*1F were found at frequency of 3.8 %.

The frequency of allele CYP1A2 *1A (wild type) was found to be about 80% and the frequency of allele CYP1A2 *1F was found to be about 20%. Calculating standard deviation we found that with CI (Interval of Confidence) 95% the frequency of CYP1A2 *1F allele could be from 12% to 28% in Albanian population. Although the sample size is small we have now preliminary data in Albanian population, that can permit a comparison with European and regional populations.

DISCUSSIONS

We have some data from literature about European and regional populations. In Serbia were analyzed genetic variants of CYP1A2 gene in 114 healthy subjects and a frequency of 22% was found for *1F allele. Distribution of genetic poymorphisms of CYP1A2 in Turkish population was analyzed in 110 healthy individuals by PCR-RFLP protocol and a frequency of CYP1A2 *1F of 27% was found. In Italian population 95 healthy individuals were analyzed and the frequency of CYP1A2 *1F was found to be 33%. As mentioned above the European populations have an allele CYP1A2 *1F frequency about 25%. In our sample of 50 subjects the frequency of allele CYP1A2 *1A (wild type) was about 80% and the frequency of allele CYP1A2 *1F was about 20%. The frequency of genotypes intolerant to caffeine is about 1/3 of Albanian population. We are working to complete the sample size of about 100 subjects.

Ethnic groups	Sample size	−163C>A (*1F)
British	114	0.333
German	236	0.32
Italian	95	0.33
Swedish	194	0.286
Turkish	110	0.27
Serbs	114	0.22
Albanians	50	**0.20**

*Tab. 1. Frequencies of genetic variant *1F of CYP1A2*

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IDENTIFICATION OF HLA B57:01 ALLELE, CONFERRING HYPERSENSIBILITY REACTION TO ABACAVIR, IN HLAB57 INDIVIDUALS OF ALBANIAN POPULATION BY AS-PCR

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INTRODUCTION

In clinical practice, using pharmacogenetics has risen in importance since the technologies for genomic variation searches for different responses to drugs became widespread, easier, more rapid, and affordable for a specialized laboratory. Other applications are being developed to detect genetic molecular markers associated with resistance or hypersensitivity/adverse reactions to antiretroviral drug treatment in Human Immunodeficiency Virus (HIV) infected patients. HIV treatment is known to be limited by adverse drug reactions and the development of resistance. One significant example is the strong association of the Abacavir hypersensitivity reaction with HLA-B*5701 in HIV-positive patients. Abacavir, a common drug for treating HIV infected patients, is an efficient nucleoside reverse-transcriptase inhibitor with a beneficial long-term toxicity profile, often used with other antiretroviral agents. However, Abacavir can yield adverse effects such as immunologically-driven hypersensitivity in 5 to 8% of HIV-positive subjects during the first six weeks of use.

The hypersensitivity reaction to Abacavir has been reportedly associated with the presence of the major histocompatibility complex class I allele HLA-B*57:01 and this association has been confirmed in several replication studies in different ethnic groups of HIV-positive patients.

It is important to remark that HLA-B*57:01 frequency is different among populations. The Caucasian group has a HLA-B*57:01 prevalence of 5–8%.

One model of the hypersensitivity reaction to Abacavir is shown in Figure 1.

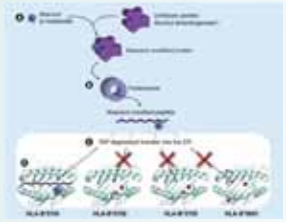

Figure 1.(A) *Abacavir or a metabolite haptenates an unknown cellular protein, possibly alcohol dehydrogenase, which is involved in abacavir metabolism.* **(B)** *This protein then undergoes proteosome-mediated degradation to generate a peptide pool, including an abacavir-modified peptide.* **(C)** *This peptide is transferred into the ER via the TAP and loaded onto the MHC in a tapasin-dependent manner.* **(D)** *Loading is only facilitated by the specific landscape of HLA-B*5701; differences in the shape and electrostatic landscape of the binding grooves of other alleles prevent the loading and binding of this peptide.*

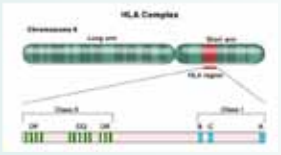

Figure 2. HLA region of Chromosome 6

The **human leukocyte antigen (HLA)** system or complex is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. These cell-surface proteins are responsible for the regulation of the immune system in humans. The HLA gene complex resides on a 3 Mbp stretch within chromosome 6p21 (Figure 2). HLAs corresponding to MHC class I (**A, B,** and **C**) present peptides from inside the cell. Foreign antigens presented by MHC class I attract killer T-cells (also called CD8+ or cytotoxic T-cells) that destroy cells.

Regarding the frequency of HLA B * 57 genotype in the Balkan Peninsula, the data represented in Table 1 shown frequencies varies from 0,7% to 4,5%. The highest frequency was found in Bulgarian (4,5%) and Greece (4,22%) populations. The lowest frequencies were found in Albania (1.3%) followed by Romania population with a frequency of 0.7%.

Balkan Countries	Allelic frequencies B57
Bosnia and Hercegovina	0.0320
Bulgaria	0.0450
Croacia	0.0251
Greece	0.0422
Kosovo	0.0290
Republic of Macedonia	0.0160
Rumania	0.0070
Serbia	0.0340
Albania	0.0130

*Table 1. Presentation of the frequency of HLA B * 57 on the Balkan Peninsula.*

AIMS OF THE STUDY

Applying a method based on the DNA analysis for identifying carrier individuals with HLA B * 57: 01. In these case we applied PCR alel-specific methods in healthy individuals to identify HLA B * 57 genotype from peripheral blood.

MATERIALS AND METHODS

A sample of 13 Albanian individuals were selected for the application of PCR-SSP and nested PCR methods to determine HLA B*57 and HLA B*57:01 alleles. These individuals were previously identified as HLA B * 57 from about 500 healthy Albanian subjects. They were analyzed by the PCR-SSP method from Immunological Laboratory of the University Hospital "Mother Teresa". 5 ml of blood was collected in EDTA tubes.

DNA extraction from blood using Invitrogen method

DNA was extracted using the Invitrogen method and samples were stored at 4 ºC. The concentration of DNA was about 20 ng/µl (Figure 3).

Figure 3. Extraction of DNA from the blood sample

HLA B57 and HLAB57:01 Identification

PCR-SSP Primers used for HLA B57 Identification
HLA B*57F (5' – GTCTCACATCATCCAGGT – 3')
HLA B*57R1 (5' – CGTCTCCTTCCCGTTCTC – 3')
PCR-SSP of exon 3 in HLA B * 57 genotype provide a 262 bp fragment (Fig.4)

SSP PCR Reaction
The PCR-SSP reaction was performed in a 25 µl final volume, containing 6 µl Buffer Gold Mix (MgCl2 1.5 mM, 200 µM dNTPs for each nucleotide), 1 µl HLA B 57F primer and 1 µl primer HLA B * 57R with 10 pmol / µl concentration, 0.25 µlTaqGold polymerase, 11,80 µl H2O and 5 µl AND (100 µg).
PCR is developed with a program as follows: initial duration 10 min to 95 ºC followed by 32 cycles from 30s to 94 ºC, 30s to 60 ºC, 30s to 70 ºC, and a cycle of 10 minutes at 72 ºC .

Nested-PCR Primers used for HLA B:57*01 allele identification
HLA B*57F (5' – GTCTCACATCATCCAGGT – 3')
HLA B*57:01R2 (5' – ATCCTTGCCGTCGTAGGCGG – 3')
Nested-PCR for HLA B * 57: 01 provide a 94 bp fragment (Figure 4).

Nested PCR Reaction
The nested PCR reaction was performed at a final volume solution of 25 µl.
PCR is developed with a program as follows: initial duration of 10 min to 95ºC followed by 30 cycles of 30s to 94ºC, 1 min at 60 ºC, 1min at 72 ºC, and a cycle of 10 minutes at 72ºC .

Figure 4. Schematic representation of primers and fragments amplification in exon 3.

RESULTS AND DISCUSSIONS

HLA B57 Genotyping identification

PCR-SSP amplification of 13 samples were analyzed by agarose gel electrophoresis and visualized at UV TRANSILUMINATOR to check the presence and the size of expected fragments. 5 µl of PCR products were mixed with 5 µl of H2O and with 2 µl of colorant-charger for loading in agarose gel. In 7 of the 13 samples, B1-B4 and B6-B8, we have the presence of the 262 bp fragment, and in 6 samples, B5,B9-B13, we don't found (providing) the expected fragment of 262 bp (Figure 9).

Figure 9. Control of PCR-SSP products on gel electrophoresis for 13 samples.

HLA B57:01 allele identification

The PCR-SSP-amplified DNA for the 7 samples providing 262 bp fragment and one sample not providing the fragment, were used for the second amplification, Nested – PCR, of HLA B*57:01 allele. 5µl DNA from the first PCR product was added.

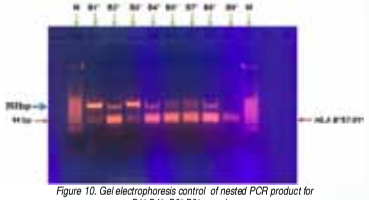

Figure 10. Gel electrophoresis control of nested PCR product for B1-B4*; B6*-B9* samples.*

All amplified samples provided the 94 bp fragment (Figure 10). Almost in all the samples was identified the 262 bp fragment, which represented the product of the first PCR. The presence of 94 bp fragment revealed that the samples contain HLA B*57:01 allele. The B9 * sample, which in the first amplification did not present the fragment 262 bp, resulted to have the 94 bp fragment. In this case, the HLA B * 57 amplification was performed in a insufficient level not revealed by gel electrophoresis.

Direct PCR amplification

We decided to test a direct DNA amplification of the DNA samples, which consist on amplification using the nested PCR program with DNA not amplified previously. The amplification of the 94 bp fragment, would show that HLA B*57: 01 allele could be directly identified without previously detecting HLA B*57. A direct amplification for the 13 samples was performed, using HLA B * 57: 01 primers. In 13 samples it was identified the 94 bp fragment.(Figure 11).

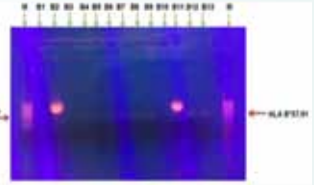

Figure 11. Control on the gel electrophoresis ofdirect amplification for 13 samples

Flooreshence of DNA bands was low, because the amount of the charged DNA was insufficient,and the fragment 94 bp was better identified, when we charged 10 µl of the product.

CONCLUSIONS

- In this study it was possible to apply the PCR-SSP and nested PCR method for the identification of HLA B * 57: 01 allele starting from human DNA.
- The presence of allele HLA B * 57: 01 was found in 100% of the analyzed HLA B * 57 genotypes.
- The methods used in this study are more specific and at a lower cost compared to the methods used by the Immunology Lab.
- This protocol is applicable to HIV-infected patients for the identification of allele HLA B * 57: 01 in order to prevent hypersensitivity to Abacavir, based on the Scheme 1.

Scheme 1. The dilution-direct' nested PCR workflow system

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