# Rare Diseases and Orphan Drugs

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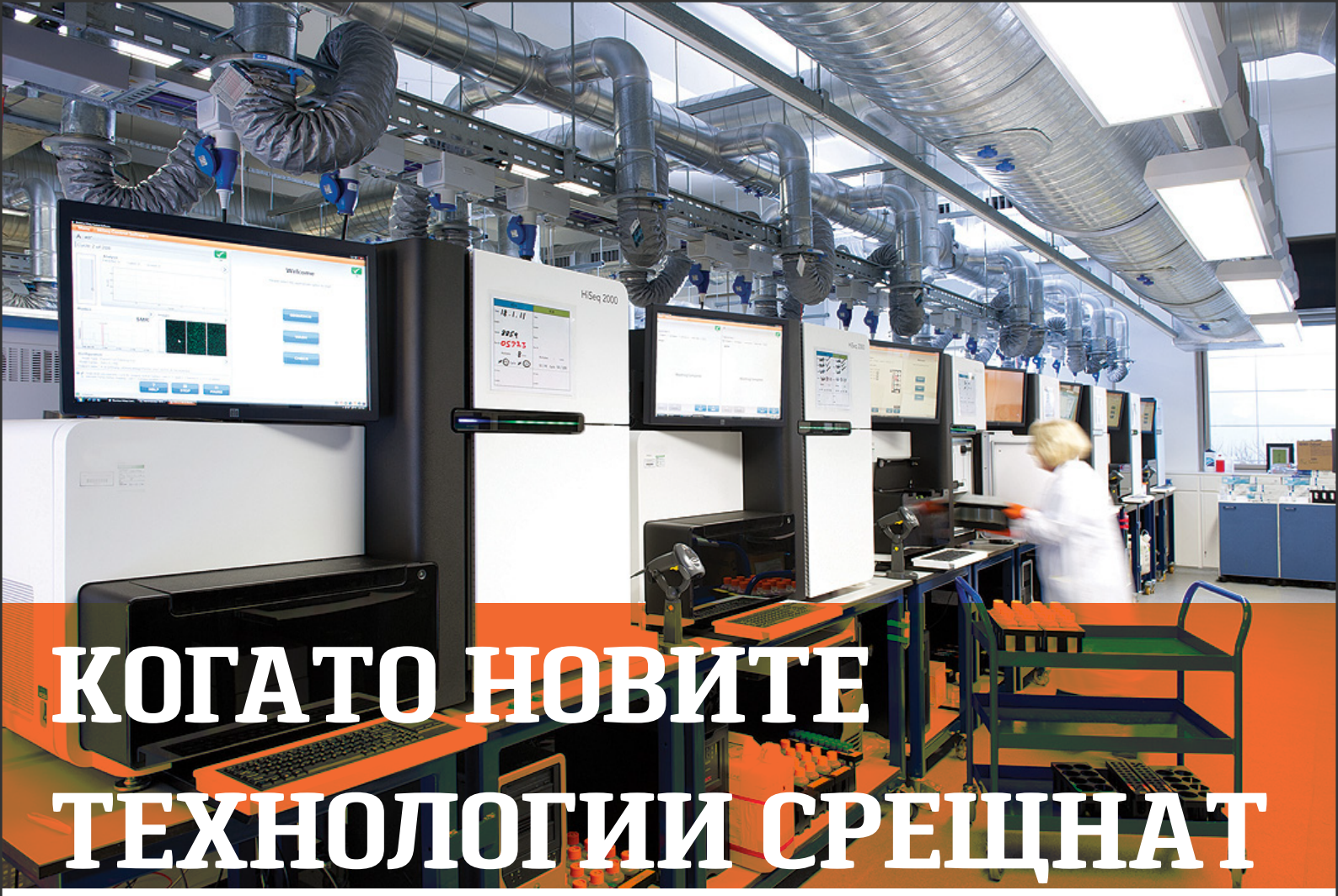
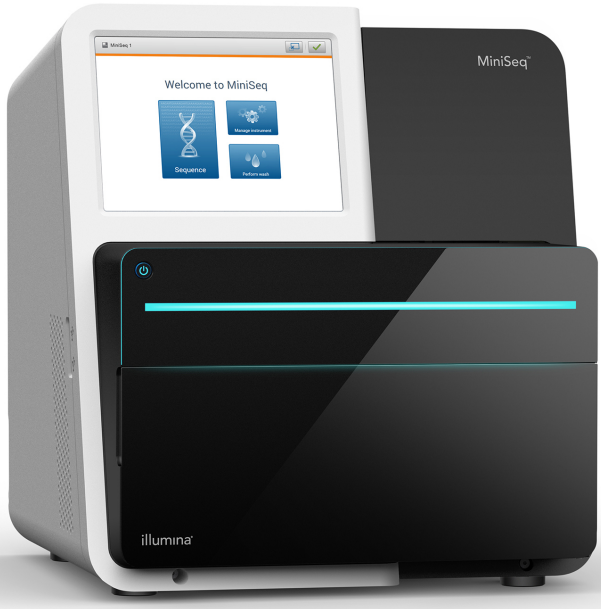


# КОГАТО НОВИТЕ ТЕХНОЛОГИИ СРЕЩНАТ ГОЛЕМИТЕ ИДЕИ

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.

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

**8-10 September 2017**

**Grand Hotel Plovdiv, Bulgaria**

## 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**

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**Комисия по здравеопазване към 44-то Народно събрание**

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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**

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***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***

# PLENARY LECTURES / ПЛЕНАРНИ ДОКЛАДИ

## PL-01 **DEVELOPMENT OF GENOMIC MEDICINE IN BULGARIA**

*Toncheva D*

Department of Medical Genetics, Medical University of Sofia, Medical Faculty

After finishing the first version of the Human Genome Project, the tremendous progress in technologies paved the way of the new postgenomic era in medicine. The subsequent large-scale projects deepened the knowledge about the variants in the human (respectively the tumor) genome and created a premise for the development of individualized (personalized) medicine.

Bulgaria participates actively in the ongoing genomic revolution in medicine. We are the 5th country in Europe which has acquired a next-generation sequencer for genomic analyses and we took part in several big international consortia. Due to our participations, Bulgaria exists on the European and global maps of genetic variants. The genomic technologies were used widely and successfully in reproductive medicine, pediatrics, common diseases and oncology for molecular post-natal diagnostics, for invasive and non-invasive prenatal diagnostics, pre-implantational genetic screening and diagnosis, genetic tumor profiling and targeted therapy.

The molecular profile of each tumor is different depending on the stage and development of the malignant process. Genetic tumor signatures are used for genetic classification, to diagnose prognostic markers and to manage the personalized treatment of tumor patients. The genomic analyses of Bulgarian patients discovered a large number of new pathogenic mutations, revealed new molecular pathways, leading to diseases and new variants for genetic predisposition to severe common disorders.

The information encoded in the human genome is related not only to pathology but also is connected with human longevity.

## PL-02 **GENOMIC MEDICINE IN HEALTH CARE SYSTEMS: THE TIME FOR CHANGE**

*Peterlin B*

Clinical Institute of Medical Genetics, University Medical Center Ljubljana

New genomic technologies, especially next generation sequencing have a significant impact on the recognition of etiology of hundreds of human diseases. Recognition of etiology is however of paramount importance for timely diagnosis, treatment, and prevention. Moreover, identification of people at an increased risk for developing diseases or transmitting them to their offspring is a key step in futuristic medical concepts like personalized and preventive medicine. Identification of highly penetrant genetic variants might be therefore considered as an important public health issue and relevant goal of public health genomics.

Health systems are usually resistant to rapid integration of novel technologies due to several barriers including lack of clinical guidelines and pathways as well as professional standards for genomic applications, limited access to expertise, limited evidence of benefit/value and finally lack of reimbursement.

Consequently, the potential of new technologies remains currently relatively poorly exploited in the health systems worldwide, but especially so in economically deprivileged countries.

We suggest that timely implementation of new innovative genomic technologies in the framework of new generation health services might significantly improve the efficacy and quality of health systems with favorable economical effects.

The Slovene model of translation of genomic technologies into public health system will be presented.

## PL-03 **INTERPRETING NEXT GENERATION SEQUENCING AND ARRAY DATA OF PATIENTS WITH RARE CONDITIONS**

*Yararbas K, Alanay Y, Ozbek U*

Acibadem Mehmet Ali Aydinlar University School of Medicine Department of Medical Genetics

The routine use of next-generation sequencing (NGS)- particularly whole exome sequencing and array karyotyping in human disease made the geneticist identify many genetic variants per individual. Especially for rare diseases, the genetic background is also full of

"orphan" genes and chromosomal loci. So interpretation of the data became as important as finding them. First dilemma is whether to start with exome sequencing or array karytyping or both, and when to decide to choose only one of them. Array karyotyping is considered as first tier test in intellectual disability, autism spectrum disorders and developmental delay. The array data is usually distinct form sequencing data, and to be evaluated and confirmed with different methodology, whereas results of NGS is usually confirmed again by sequencing -that is Sanger-. NGS findings usually fall outside of protein-coding regions, and by the advance of whole genome sequencing technology, these type of data will emerge. So the next problem will be to identify whether they are involved in regulation of gene functioning which will need completely different methodologies.

So it is important to decide whom to test, what to test, and what to to with the data obtained. We will share the Acibadem Group experience and suggest an algorithm for interpreting the array and exome data obtained from a patient tested for such a condition.

## PL-04 **NEXT GENERATION CLINICAL GENETICS: GENOTYPE FIRST OR PHENOTYPE FIRST APPROACH**

*Writzl K, Maver A, Peterlin B*

Clinical institute of Medical Genetics, University Medical Centre Ljubljana, 3, Šlajmerjeva Street, Ljubljana 1000, Slovenia

A major challenge in rare and genetically heterogeneous disorders is to find an etiological diagnosis. Traditionally, the detailed characterization of a patient's phenotypes was followed by genotyping to discover the responsible pathogenic variant. Next-generation sequencing (NGS) has revolutionized the diagnosis of rare disorders by allowing interrogation of all genes in a single assay and thereby opening the door to phenotyping directed by results from genomic testing. What is the role of clinical geneticists in the pre-NGS-test and post-NGS test phenotyping? The results of NGS analysis will be presented, illustrated by interesting cases.

## PL-05 **PRECISION AND PERSONALIZED MEDICINE – A PROMISE OR AN IMPERATIVE**

*Koeva-Balabanova J*

Chair of the Board of BAPPM

Overview of the development of precision and personalized medicine – global, regional and national trends, recent developments, current issues and barriers. Snapshot of the stage of development and implementation of precision and personalized medicine in Bulgaria, as well as stepping stones for problems solving and future directions for further developments are proposed.

## PL-06 **RASOPATHIES: CLINICAL AND MOLECULAR CORRELATIONS**

*Neri G*

Institute of Genomic Medicine, Catholic University School of Medicine, Rome, Italy and Greenwood Genetic Center, Greenwood (SC), USA

The term RASopathy, recently coined, denotes a family of multiple congenital anomalies/intellectual disability syndromes that share an overlapping phenotype, whose main characteristics are developmental delay with short stature, peculiar face, ectodermal involvement, congenital heart defects. Members of this family are the Noonan syndrome and its variants, the Mazzanti syndrome, the cardiofaciocutaneous (CFC) syndrome, the Costello syndrome and, somewhat distinct, neurofibromatosis type 1 (NF1) and the Legius syndrome. All of these conditions are inherited as autosomal dominant traits, even though the most severe ones, like CFC and Costello syndrome are of sporadic occurrence, due to new mutations. The underlying reason that explains the phenotypic similarities consists in the causal role of genes encoding proteins that operate within the same RAS-ERK signaling pathway, that regulates cell proliferation, differentiation and programmed death. The list of these genes is long and yet to be completed, including, among others, PTPN11, RAF1, SOS1, SHOC2, BRAF, MEK1/2, HRAS. Interestingly, the same syndrome can be caused by mutation of different genes (e.g. PTPN11, RAF1, SOS1 for Noonan), while, on the other hand, different syndromes can be caused by mutation of the same gene (e.g. BRAF for Noonan and CFC). The pathogenic mutations are normally gain-of-function mutations, upregulating the RAS-ERK pathway. Notably, somatic mutations of these genes are found in many types of cancer, such as melanoma and bladder carcinoma. However, these mutations are different from those causing the RASopathies, probably having a stronger effect, that results in embryonic lethality.

Nonetheless, in Noonan syndrome, and more so in Costello syndrome, there is increased risk of developing some forms of childhood cancer, such as neuroblastoma and rhabdomyosarcoma, calling for the adoption of a surveillance protocol. The existence of drugs inhibiting the RAS-ERK pathway, already experimented and used in specific types of cancer, indicates that the pathway is targetable, lending hope to the eventual discovery of a pharmacological treatment of RASopathies.

## PL-07 **CHERUBISM – A RARE GENETIC DISORDER**

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**Background:** Cherubism is a rare genetic disorder involving the mandible and occasionally maxilla in early childhood. SH3BP2 gene is the only one in which mutations are currently known to cause 80% of isolated cherubism.

**Objectives:** Our aims were to find out the genetic cause of mandibular deformity and to avoid misdiagnosis and inappropriate treatment risks.

**Methods:** Clinical, radiographic, biopsy and histological examinations were performed on two-female unrelated patients with bilateral asymmetric enlargement of the mandible. A thorough family history was obtained as well. The patients and their family members were genetically tested for mutations in SH3BP2.

**Results:** Clinical features, such as, facial appearance with swollen cheeks, round and asymmetrically full lower face, were suggestive for cherubism in both cases. Radiological examination revealed bilateral multilocular radiolucent areas within the bone. Histological examination reported the replacement of the normal bony structure with fibrous tissue. There was no family history of prominent facial swelling or similar disorders. The disorder seems to be occurred sporadically within the both families. Direct sequencing of SH3BP2 gene identified an A-to-G transition, resulting in an arg415-to-gln (R415Q) substitution in one patient. The second patient presented no SH3BP2 gene mutation.

**Conclusion:** Failure to identify a SH3BP2 mutation in affected individuals suggests possible genetic heterogeneity.

## PL-08 **RATIONAL DESIGN OF MIMOTOPE LIBRARY FOR IGM REPERTOIRE STUDIES**

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A reasonably small set of peptide probes for global antibody analysis was rationally designed as a human IgM mimotope library. Phage display representing the diversity of random 7-mer peptides was selected on IgM repertoire pool from 10000 donors then adsorbed on monoclonal IgM myeloma. Phage selected in a single round were amplified, their DNA extracted and the region coding the random library was sequenced by NGS. 224087 different 7-mers were selected out of 2x106 quality sequences and 790 clusters of sequences were defined using the Gibb's Cluster 2 algorithm. Because of their relatively small number, probably these profiles characterize structural context themes rather than individual specificities. The optimized library was defined as 594 mimotopes representing the highest scoring sequences of the most significant clusters. Compared to 9 other peptide libraries (random, depleted of canonical sequences, individual clusters, etc) the optimized library yielded the best high dynamic signal against 10 sera. The library was further tested with sera from 38 brain tumor patients of 6 different diagnoses. No single IgM reactivity correlated strictly with diagnosis but recursive elimination with "leave one out" validation identified sets of 40-100 mimotopes from each group with consensus of 6-24 mimotopes for each diagnosis that separated the tested patients from the rest of the cases and allowed for the construction of SVM predictors. The typical sizes of these sets indicate that the library has the potential to distinguish over 1040 different physiological and pathological states.

## PL-9 **APPLICATION OF TARGETED NGS IN DIAGNOSTIC WORK-UP OF PATIENTS WITH RARE DISEASES**

*Vazharova R[1, 2, 4], Balabanski L[2], Andonova S[3], Ivanova M[3], Sinigerska I[3], Bichev S[3], Ivanov S[2], Atanasoska M[2], Savov A[3, 5], Toncheva D[2, 5]*

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[5] Medical University Sofia

**Background:** Clarifying the genetic basis of rare Mendelian diseases is difficult and many patients remain undiagnosed despite extensive clinical, laboratory and imaging studies. The conventional diagnostic process used by most clinical / medical genetics specialists begins with the recognition of a specific phenotypic pattern which guides a step by step series of laboratory studies of candidate-genes. Although NGS of the exome / genome has the potential to change the diagnostic paradigm in medical genetics, it is still not clear which patients would benefit from such a test and at what stage of their clinical follow-up.

**Materials and methods:** The study includes a series of 43 consecutive cases referred for genetic testing because of a suspected diagnosis of monogenic disease. Targeted NGS was performed on the MiSeq system using the TruSight One gene panel (Illumina, San Diego, CA, USA). The search for the disease-causing mutations focused on variants with a quality score ≥20 and coverage ≥20×, located outside of segmental duplications and simple repeats.

**Results:** A specific genetic alteration associated with the leading clinical manifestations was found in 48% of all patients studied (21 out of 43 cases), this share being less among patients with an "unknown" diagnosis – 38% (10 of 26 cases). In four patients with a definite pathological finding and a genotype suggesting a phenotypic manifestation of the disease, the genetic diagnosis differs from the clinically suspected. In two patients with "unknown" clinical diagnosis, certain pathological gene variants were identified that only partially explained the phenotype manifestations and in one patient there was found the presence of pathological variants in genes associated with three different monogenic diseases and a genotype suggesting the phenotypic manifestation of all three conditions.

**Conclusion:** Applying targeted next generation sequencing using a 4813 gene panel has the potential to detect a genetic defect associated with the phenotype in about 48% of patients with rare monogenic diseases.

## PL-10 **PRE-IMPLANTATIONAL GENETIC TESTING FOR MONOGENIC AND CHROMOSOMAL DISORDERS: THE EXPERIENCE IN BULGARIA**

*Hadjidekova S[1, 2], Staneva R[1, 2], Andonova S[3], Savov A[3], Bitchev S[3], Yaneva S[1], Pancheva M[2], Serafimova M[2], Toncheva D[1], Stamenov G[2]*

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The treatment is the major goal in the control of genetic diseases, unfortunately, most inherited conditions cannot be cured. Although current breakthrough in genome editing technologies, it is still not a realistic option for managing the genetic disorders and the prevention remains the main approach. Over the years, the primary method of prevention in families with genetic disease was the prenatal diagnosis and termination of pregnancy for medical reasons. Nowadays pre-implantational genetic testing (PGT) provides a new opportunity for disease prevention, thus avoids the trauma of interruption of a desired pregnancy and the possible medical complications. PGT is a method for detection of genetic anomalies in IVF embryos before the transfer in the uterus. In most cases the genetic status determination is performed at the blastocyst stage, after biopsy of trophectoderm cells, followed by whole genome amplification and application of various platforms for genetic analysis. The world's first successful PGT in humans was made in 1990 for cystic fibrosis (CF). Today PGT is possible for all chromosome disorders and a large number of monogenic diseases (beta-thalassemia, sickle cell disease, spinal muscular atrophy, myotonic dystrophy, Huntington's chorea, Alzheimer's Charcot-Marie-Tooth, dystrophy and many others).

We report our experience in PGT for six monogenic diseases (myotonic dystrophy, Huntington's disease, fragile X syndrome, epidermolysis bullosa, beta-thalassemia, hemophilia A) and for chromosomal testing in families with reciprocal and complex chromosomal translocations. Different genetic methods were applied: Sanger sequencing, real time PCR with FRET (Fluorescence resonance energy transfer), DNA fragment analysis by triplet repeat primed PCR and array CGH analysis.

Due to the tremendous progress in the assisted reproduction and the technical development in genomics technologies PGT has the potential to become a powerful tool for transmission control of the genetic diseases and has changed the landscape for prevention of the inherited conditions.

## PL-11 **PHENOTYPE AND GENOTYPE HETEROGENEITY OF THALASSEMIA INTERMEDIA**

*Plaseska-Karanfilska D*

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The term thalassemia intermedia (TI) describes patients who have clinical manifestations that are too severe to be termed minor yet too mild to be termed major. The hallmark of TI disease process includes ineffective erythropoiesis, chronic haemolytic anaemia and iron overload. TI encompasses a wide clinical spectrum; mildly affected patients are completely asymptomatic until adult life, experiencing only mild anemia, while patients with more severe TI generally present between the ages of 2 and 6 years and often require transfusion therapy.

In addition to the phenotypic diversity TI is extremely heterogeneous at the genetic level, thus making the molecular diagnosis very chalanging. The genetic basis for phenotypic diversity of beta TI is best explained in terms of primary (broad diversity of beta globin gene mutations), secondary (modifying the degree of globin chain imbalance), and tertiary genetic modifiers (having effect on the complications of the disease). Commonly, alpha thalassemia intermedia presents as HbH disease due to deletions removing three out of the four alpha globin genes. Non-deletional alpha TI is less common, but has a more severe clinical phenotype.

Both beta and alpha TI have been diagnosed among Macedonian patients. The most common genetic determinants of beta TI are inheritance of silent or mild beta globin mutation, coinheritance of genetic determinants of high fetal hemoglobin as well as coinheritance of alpha globin gene defects. Alpha TI is caused both by deletions and non-deletional defects. We have recently identified two unrelated TI patients with Romani ethnic origin who were homozygous for a rare Hb variant, Hb Agrinio (HBA2: c.89T>C, p. 29 Leu>Pro). This may imply that Hb Agrinio is a founder mutation among Romani Balkan population.

Since TI patients may experience serious clinical manifestation later in life, timely diagnosis is very imortant for proper management of TI.

## PL-12 **NON-TRANSFUSION-DEPENDENT THALASSAEMIA**

*Kaleva V*

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Department of Paediatrics, Medical University of Varna

Non-transfusion-dependent thalassemias (NTDT) is a term used to label patients who do not require lifelong regular transfusions for survival, although they may require occasional or even frequent transfusions in certain clinical settings or at later in their life. They have a genetic defect or combination of defects that, compared to transfusion-dependent thalassemia, affect less hemoglobin synthesis and encompasses three clinically distinct forms: β-thalassemia intermedia, HbE/β-thalassemia and α-thalassemia intermedia (HbH disease). The carrier frequency of NTDT is high (up to 80% in some parts of the world) but the prevalence of symptomatic patients varies with geography and is estimated to be from 1 in 100,000 to 1 in 100. NTDT has a variable presentation that may include mild to severe anaemia, enlarged spleen and/or liver, skeletal deformities, growth retardation, elevated serum ferritin and iron overload. The contributing factors to disease progression are ineffective erythropoiesis and increased haemolysis, which lead to chronic anaemia. Due to the constantly activated erythropoiesis, extramedullary haematopoiesis is often observed. Diagnosis of NTDT is largely clinical but can be confirmed by Hb electrophoresis and genetic sequencing. Management of NTDT is based on managing symptoms, and includes blood transfusions, hydroxyurea treatment, iron chelation and sometimes splenectomy. Prognosis for well managed patients is good, with most patients living a normal life. It is particularly important to identify and diagnose patients early, thereby preventing complications.

## PL-13 **COLORECTAL CARCINOMA – FROM GENETIC MARKERS, RESPONSE PREDICTORS FOR THE TREATMENT TO PERSONALIZED THERAPY AND NEW GENETIC CLASSIFICATION**

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**Purpose:** The personalized oncology concentrates on the ability to segregate the subgroups of patients with a specific genetic profile, to estimate the progression of the condition as well as, the genetically based sensitivity to treatment, metabolism and toxicity. Optimal therapeutic treatment can be identified based on the attained individual molecular subtype, correlating prognosis and drug sensitivity of the patients.

In cases of colorectal carcinoma (CRC), the established chemotherapy treatment regiments with or without a combination of target molecules, not always cause the expected therapeutic response. This can be due to the individual genetic profile of the patient. Studying said genetic profile can improve the prognosis of the progression of the condition as well as the personalized treatment approach.

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;
- CSM3, (metabolic, 13%), epithelial, metabolic dysregulation, multiple KRAS mutations;
- CSM4, (mesenchymal, 23%), microsatellite instability, insufficient mismatch repair protein (MRR), stromal invasion and angiogenesis;
- CSM5, 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.

Attempt at differentiating the response of treatment of patients depending on the location of the primary tumor (left/right colon) is a possible secondary objective of the project.

**Methodology:** The project includes studying a pilot group of 40 patients, diagnosed with CRC in different clinical stage (I-IV). Patients are divided into two groups, depending on their RAS status:

- 20 patients with RAS mutated type, under the treatment of chemotherapy course in combination with a target agent – Bevacizumab or Aflibercept, depending on the line of the treatment;
- 20 patients with RAS wild type, under the treatment of chemotherapy course in combination with a target agent – Panitumumab or Cetuximab.
- All patients:

o Genetic markers are tested (KRAS, BRAF, MSS or MSI);
o Tracing the general condition by testing tumor markers CA19-9, CEA and ECG analysis;
o Tracing the condition of the treatment with image diagnostics – computer tomography, with the application of intravenous and per-oral contrast;
o Preparation of estimation index, based on the molecular subtype of the patients and tracing its objectivity;
o A precise personalized therapeutic regime, based on the analyzed genetic markers, defined subtypes, the prognosis of the disease and the objectiveness of the treatment's results.

**Results:** The project's results will be presented and defined after processing and analysis of the collected data.

## PL-14 **NEUROMUSCULAR DISORDERS IN ROMA (GYPSIES)**

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Historical and current migrations have made the Gypsy citizens of every European country. The Roma (Gypsies) are a transnational minority of Indian/Pakistan origin with an overall population size estimated between 10 and 15 million (Liegeois, 1994). The arrival of the Gypsies in Byzantine Empire 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. The formation of the present-day Romani populations of European countries is the compound product of the early migrations from the Balkans into Western Europe, completed by the 15 century,

and three superimposed migration waves: the first during the end of the 19th century, after the abolition of Gypsy slavery in Romania (Hancock, 1987; Fraser, 1992); the second out of Yugoslavia, during 1960s and 1970s; and the third during the last decade following the political and economic changes in Eastern Europe.

In Bulgaria the Roma community is also non-homogeneous. It is divided into two main groups: settled Roma (Yerlii) and Walachian Roma who have come to Bulgaria after the abolition of slavery in the Walachian Principality in 1868. These two main groups are further divided into smaller subgroups and meta-groups based on occupation, dialect, religious affiliation, culture, customs, and traditions. 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).

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.

During 20 years "door-to-door" epidemiological study of NMD in Gypsies was conducted in Bulgaria, covering of 2500 residential areas and around 97% of the Gypsy population. The survey identified 842 NMD patients with CMS, HMSN –Lom, CCFDN, LGMD2C, GNE myopathy and SMA being most prominent.

Genetic studies have reported considerable differences in the frequencies of the mutations in the different Romani populations, resp. in the prevalence of the neuromuscular disorders in the different Roma groups, which should be considered in planning and organising prevention activities.

A community-based carrier testing program was implemented in Bulgaria. The program incorporated an educational component, screening for several common founder mutations, genetic counselling and prenatal testing of pregnancies at risk. It was accompanied by wider-ranging activities, such as training and employing of 215 Roma health mediators, training of 22 Roma medical doctors and 84 Roma nurses and midwives for work with affected families, promoting equal access of Roma patients to health and social services.

## PL-15 **ENGINEERING INTEGRATED DIGITAL CIRCUITS WITH ALLOSTERIC RIBOZYMES FOR SCALING UP MOLECULAR COMPUTATION AND DIAGNOSTICS OF RARE DISEASES**

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Here we describe molecular implementations of integrated digital circuits, including a three-input AND logic gate, a two-input multiplexer, and 1-to-2 decoder using allosteric ribozymes. Furthermore, we demonstrate a multiplexer−decoder circuit. The ribozymes are designed to seek-and-destroy specific RNAs with a certain length by a fully computerized procedure. The algorithm can accurately predict one base substitution that alters the ribozyme's logic function. The ability to sense the length of RNA molecules enables single ribozymes to be used as platforms for multiple interactions. These ribozymes can work as integrated circuits with the functionality of up to five logic gates. The ribozyme design is universal since the allosteric and substrate domains can be altered to sense different RNAs. In addition, the ribozymes can specifically cleave RNA molecules with triplet-repeat expansions observed in genetic disorders such as oculopharyngeal muscular dystrophy. Therefore, the designer ribozymes can be employed for scaling up computing and diagnostic networks in the fields of molecular computing and diagnostics and RNA synthetic biology.

## PL-16 **GENOME STRUCTURE OF MODERN BULGARIANS**

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The previous analysis of the mitochondrial DNA and Y-chromosome diversity of modern Bulgarians positioned them among other European populations. We have continued with the characterization of the genome structure of Bulgarians by participating in large-scale analyses of single nucleotide and copy number variants in studies performed from a European, Eurasian and global perspective.

Initially we took part in a survey of more than 270 000 single nucleotide polymorphisms in 3112 Europeans, including Bulgarians. This study depicted the European genetic map in which the genetic diversity has northwest to southeast gradient and the populations are positioned according to their approximate geographic origin.

Afterwards, we were involved in a population-based genome-wide association analysis of five population samples: Irish (n=1142), Scottish (n=656), Swedish (n=620), Bulgarian (n=1129) and Portuguese (n=347). The obtained results confirmed the genetic relationship between European populations and their geographic distribution and detected the largest genetic differences in genes for pigmentation and immunity.

We were also part of an international group examining the selective signatures of duplication and deletion copy number variants in 236 individuals from 125 distinct human world-wide populations. The obtained results showed that duplications are more likely to be stratified between human populations than deletions.

In another study, in which we have participated, the dataset of more than 450 000 single nucleotide variants in 63 West Eurasian populations, including Bulgarians, was used to define and date admixture events in the formation of the Western Eurasian genetic landscape. The established genomic composition of Bulgarians in this study reflects admixture events dating to the Migration Period and shows minimal Mongolian admixture.

In conclusion the Bulgarian genomic structure fits within the continuum of genetic variants in Europe and its analysis in broader contexts will further contribute to the formation of substantial basis for assessing the clinical significance of genetic variants.

## PL-17 **MEDICAL LAW CHALLENGES IN GENETIC TESTING**

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European Institute for Healthcare Law, Healthcare Management and Public Health

**Goal:** To introduce the medico-legal aspects, innovations and challenges related to genetic testing (?). To introduce practitioners in the field of genetic testing and the analysis and discovery of hereditary diseases to the existing legal framework and relevant changes, as well as to inform them on potential legal risks related to their professional activities.

**Materials and approach:** This work takes into consideration the existing norms – national laws and specific regulations, as well as the existing body of case law (court practice).

**Methodology:** Comparative legal analysis of the main alternatives provided by the Law, which are obligatory for the practice of genetical testing.

**Results and findings:** To introduce practitioners to the legally defined obligations and possible liabilities. Underline the potential risks for both practitioners and patients in genetic testing. To introduce practitioners to the process of informed consent (consultation) in genetic testing. To present the changes in legislation related to the administration of sensitive, private and health data of patients related to genetic testing.

The comparative legal analysis provides the possibility of bringing into focus the majority of flaws and omissions in current regulation when it comes to genetic testing. The paper explores the possibilities of improvement and possible alternative solutions that would achieve a legal framework that better safeguards the interests of patients and also provides a safer working environment for medical practitioners.

## PL-18 **ORPHAN DRUGS IN SERBIA: EVALUATION OF MARKET AUTHORIZATION, PRICING, REIMBURSEMENT AND EXPENDITURE**

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Orphan drugs (ODs) are medicinal products intended for treatment, prevention or diagnosis of life-threatening or chronically debilitating diseases affecting small patient populations. Policies specific to orphan drugs don't exist in Serbia. Furthermore, Serbia, being a country outside of the European Union (EU), cannot directly benefit from the EU orphan drug legislation. ODs should obtain market authorization from the Medicines and Medical Devices Agency of Serbia (ALIMS) 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 also allowed for ODs. They are exempted of the market authorization fees as well. Special policy regulates import of unauthorized medicines and there is a list of 255 rare diseases for which import may be authorized. There are no differences in pricing and reimbursement decision making

procedures regarding ODs. The Government sets the pricing criteria and maximum wholesale prices for market authorized prescription drugs on a national level. External reference pricing is used and Slovenia, Croatia and Italy are reference countries. Reimbursement decisions are made by the National Health Insurance Fund taking into account general (pharmacotherapeutic and pharmacoeconomic justification, financial plan) and special criteria (special contracts, priority order) for inclusion of medicines in the reimbursement list. Reference countries for setting reimbursement prices are Slovenia, Croatia and Italy or if drug is not priced there, Romania, Lithuania, Slovakia, Bulgaria, Hungary and Latvia. Twenty-three ODs were authorized, 20 were priced and only 7 were reimbursed in Serbia in June 2017, representing 24%, 21% and 7%, respectively, of 95 drugs with active orphan designation and market authorization in the EU in this period. Expenditure on ODs increased from EUR 1.5 million in 2006 to EUR 3.7 million in 2014, representing 0.30% and 0.46% of the total pharmaceutical expenditure, respectively (based on analysis of ALIMS's annual reports). There is a need for further improvement in accessibility of orphan drugs.

Keywords: orphan drugs, market authorization, pricing, reimbursement, expenditure

## PL-19 **MONITORING OF MOLECULAR RESPONSE DURING THE THERAPY WITH NILOTINIB AS FIRST-LINE TYROSINE KINASE INHIBITOR IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA**

*Balatzenko G[1], Bogdanov L[2], Goranov S[3], Goranova-Marinova V[3], Grudeva-Popova J[4], Ivanova S[1], Ignatova K[1], Mihailov G[1], Spassov B[1], Stojanova J[1], Tumbeva D[4], Hadjiev E[5], Hristova I[3], Hrischev V[1], Tsvetkov N[2], Tsvetkova G[5], Guenova M[1]*

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**Background:** Clinical trials demonstrated efficiency of the tyrosine kinase inhibitor Nilotinib as a first-line therapy in chronic myeloid leukaemia (CML). The depth of molecular response (MR) is an important parameter for therapeutic efficiency assessment. Real world evidence in line with these observations are of practical significance for the outcome prediction or TKI discontinuation inclusion.

**Aim:** To evaluate the molecular response to Nilotinib as a first-line therapy in chronic phase CML patients(pts).

**Materials and methods:** A total of 162 adult CML pts, treated with Nilotinib 300 mg b.d., were included in this study (mean follow-up 35±21 months(mo)). Initial RT-PCR testing revealed typical p210 BCR-ABL1 transcripts in 160 cases and p190 in 2 others. Molecular monitoring in p210BCR-ABL1 pts was performed by Cepheid\'s automated PCR-based platform. The results were reported as a BCR-ABL1/ABL1 (B/A) ratio.

**Results:** An optimal MR (OMR) was found in 85.1%(80/94) at 3rd(B/A≤10%), 84%(84/100) at 6th(B/A<1%), and 82.4%(89/108) at 12th(B/A≤0.1%) mo. Nineteen (11.9%) pts were resistant to therapy, and 2 others lost a previous MR. OMR rates at 6th and 12th mo were higher in pts with leukocytosis <250x109/l compared to those with >250x109/l: 87.5%(35/40) vs 57.9%(11/19)(p=0.018) and 92.1%(35/38) vs 57.9%(11/19)(p=0.004). After ≥15mo follow-up, major MR was detected in 88.5%(116/131), and deep MR in 67.9%(89/131), including undetectable BCR-ABL1 in 33 of them. In total, 8 pts (5%) died, in 4 of them death was caused by another malignancy. The cumulative 5-years overall survival (OS) for the whole cohort was 91.0% and was associated with the achievement of major MR after the 12th mo – 97.7% vs 75% without major MR (p=0.000). OS was better in women (97%) compared to men (75%) (p=0.013). OMR rate was significantly higher in pts with b3a2 transctips (45.3-91.3%) compared to b2a2(+) (25.8-77.8%) pts in all tested time points. B3a2 were also associated with a higher deep MR rate at 12th mo or later: 61.4%(35/57) vs 34.7%(17/49)(p=0.005) and 72.8%(59/81) vs 54.7%(41/75)(p=0.02). No MR was registered in p190(+) pts.

**Conclusions:** The results of our study confirmed the reported high efficiency of Nilotinib as a first-line therapy, with a better MR in patients with b3a2 transcripts.

# ORAL PRESENTATIONS / УСТНИ ПРЕЗЕНТАЦИИ

## OP-1 **DYSREGULATED PATHWAYS IN AUTISM SPECTRUM DISORDER**

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Autism Spectrum Disorder (ASD) combines a group of complex neurodevelopmental disorders that presents in the early childhood. Individuals with ASD are characterized by having deficits in social interaction, impaired communication and a range of stereotyped and repetitive behaviors. There are hundreds of different genes associated with ASD, but each of the mutations found account for more than a small subset of ASD cases. Because of this genetic heterogeneity of ASD, the information on gene function provided by transcriptional data is essential to further elucidate its genesis. The aim of this study is to identify common pathways and mechanisms in the pathogenesis of ASD.

To identify the biological pathways and profiles associated with ASD, an integrative functional analysis of the genetic changes has been applied. The sets of differentially expressed genes from 16 transcriptome expression studies (one of which ours) after 2011 and the targets of differentially expressed mi-RNA from two our studies were used.

The pathways with the most overlapping differentially expressed genes were evaluated and a system (network) of biological pathways was generated. We found that most of the differentially expressed genes are involved in major kinase and /or signaling pathways.

The integrative functional analysis of genetic changes and the network of biological pathways contributes to our understanding not only of individual genes but also of the function of the gene product, the network of reactions and their interactions.

## OP-2 **GENETICALLY PROVEN CASES OF WOLMAN DISEASE IN BULGARIA AND MUTATION SCREENING OF TWO PRESUMABLE ENDEMIC REGIONS**

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Wolman disease is a rare autosomal recessive disorder. It belongs to the group of lysosomal diseases. Wolman disease is caused by mutations in the LIPA gene, localized on chromosome 10 (10q23.31). The LIPA gene encodes an enzyme called lysosomal acid lipase (LAL), which plays a key role in hydrolysis of the cholesteryl esters and triglycerides into free cholesterol and free fatty acids. Two unrelated patients aged 3 and 5 months were referred for genetic testing of the LIPA gene due to the clinical symptoms compatible with Wolman disease. DNA sequencing revealed two different mutations. The first mutation is a previously described disease-causing missense mutation, c.260G>T; p.Gly87Val in exon 4 of the LIPA gene. The second mutation is a splice-site change, c.822+1G>A in exon 7 of the gene. Both mutations substitute the purine base G with T (transversion) in the first variant and with A (transition) in the second one. Considering the high genetic heterogeneity of the Bulgarian population, it is extremely atypical to detect homozygous mutations in rare recessive conditions. Selective screening for these mutations was performed in two presumable endemic regions in Bulgaria. Endogamous marriages are very unusual for the Bulgarian population, supposing a high carrier frequency in this subpopulation. Altogether, 100 newborns were screened for p.Gly87Val mutation and the detected carrier frequency was about 1% (1/100), while in the group of 100 newborns screened for the c.822+1G>A mutation the detected carrier frequency was 2% (2/100). 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 counselling during family planning.

Keywords: Wolman disease, LIPA gene, Lysosomal acid lipase, mutations, selective screening

## OP-3 **QUALITY OF LIFE IN ACROMEGALY**

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Acromegaly is a rare chronic disorder caused by pituitary adenoma in almost all cases. Along with physical changes, patients with acromegaly show some psychosocial alterations and decreased self-perception of health. We have evaluated quality of life in patients with acromegaly using the disease-specific Acromegaly Quality of life (AcroQoL) questionnaire in respect to disease activity, treatment modalities and other factors. We studied 212 patients with acromegaly in a cross-sectional manner over a 6-year period in a single tertiary center. Out of them, seventy patients with active disease at baseline were followed-up prospectively, 45 of whom were in remission at re-evaluation. There was no significant difference in AcroQoL scores between controlled and uncontrolled patients in the cross-sectional study group. The mean change of scores from baseline was higher in controlled vs. uncontrolled patients, however without reaching significance. In the cross-sectional group the lack of disease control was a negative predictor of the appearance subscale (B=-7.39; p=0.041) while IGF-1 index had negative impact on the same scale in the uncontrolled patients (B=-4.9; p=0.044). Radiotherapy, age and female gender negatively affected various scales. Personal relations subscale was inversely related to duration of remission in controlled patients (B=-1.59, p=0.014) and use of somatostatin analog (SSA) in uncontrolled patients (B=-18.2, p=0.04). In uncontrolled patients from the prospective group, however, SSA was positively associated with the change from baseline of the appearance subscale (B=11.19, p=0.041). Achievement of remission was an independent predictor of improvement of the total scale – OR 4.09 (95% CI 1.17-14.2; p=0.026). In conclusion, disease control was predictive for better appearance score and improvement of the global score. Other factors with significant influence on various scales of AcroQoL were age, female gender, radiotherapy, duration of remission and application of SSA.

## OP-4 **ACROMEGALY – PSYCHOLOGICAL CONSEQUENCES FOR THE PATIENT**

*Grigorova N*

Bulgarian Huntigton Association

Acromegaly is a rare condition which often leads to permanent change in the physical appearance of patients and is associated with adverse psychological outcomes. During the initial diagnosis and/or primary brain surgery, patients can experience severe psychological trauma and posttraumatic stress disorder. Some of the reactions are related to the unexpected diagnosis of the chronic disease and unwanted brain surgery. After being diagnosed, patients very often exhibit a condition, which is symptomatically similar to grief and loss, while they are trying to cope with this life-altering disease. More than that they may feel that they are losing themselves, both literally and figuratively, because of the physical changes attributable to the disease and the knowledge of the lifelong diagnosis. Many of the patients do not manage to cope in adaptive ways with the diagnosis and its consequences on their life, which ultimately leads to psychological issues like depressive and anxiety symptoms, a negative impact on social interactions, like loss of partners, job and social surroundings. The author presents her work with patients with acromegaly in the period from June 2015 to June 2016. Various psychological phenomena and patients' mental suffering and inquiries are described. Of great importance is the fact that patients with maladaptive ways of coping with the disease are experiencing severe mental discomfort and stress, which is causing therapy managing problems and reduces quality of life.

## OP-5 **POMPE DISEASE – TREATABLE MYOPATHY**

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Pompe disease is a rare autosomal recessive metabolic disorder, whereby mutations in the GAA gene lead to partial or total absence of the lysosomal enzyme acid α-glucosidase. The disease presents as a spectrum of phenotypes, ranging from a rapidly fatal phenotype in infants to slower progressive phenotypes in older children and adults. Many adults with Pompe disease are diagnosed late in life, when they are already in an advanced stage of the disease. Enzyme replacement therapy (ERT) seems to have a beneficial effect on survival that is related to its positive effect on motor and pulmonary functions. In this respect timely diagnosis and treatment are crucial.

In April 2012, a screening program called 'Prevalence study of Pompe disease' was initiated in Bulgaria. Its main aim was to determine the prevalence of Pompe disease among patients with progressive limb-girdle muscle weakness with or without respiratory insufficiency, and with or without elevated creatine kinase levels. The study was based on two main stand points: retrospective study of patients with undiagnosed myopathies from the registries of Bulgarian National Genetic Laboratory and Clinic of Neurology, University Hospital Alexandrovska, Sofia; and prospective study of a cohort of patients who are visiting university hospitals and electromyography centers in Bulgaria. Twenty four centers, spread throughout Bulgaria, working in close collaboration, were included. The targeted screening encompassed 370 patients who underwent evaluation of the activity of GAA on DBS.

In Bulgaria, there were not any patients diagnosed with Pompe disease until the beginning of 2012. For the last 5 years, from the 370 tested individuals, 6 turned out to have decreased activity of GAA on DBS and were subsequently genetically verified as having Pompe disease. They had non-classical forms of the disorder. Their diagnosis was established 3–10 years after the clinical onset. Limb-girdle muscle weakness with prominent involvement of lower limbs and axial muscles was present in all of the affected.

The rarity of the disorder, variable clinical presentation, and overlap of signs and symptoms with other neuromuscular disorders often results in delays in diagnosis and treatment for many patients. Targeted screening of unclassified patients with LGMD by DBS can be a valuable tool for identification of Pompe patients.

## OP-06 **PRE-IMPLANTATION GENETIC DIAGNOSIS IN A FAMILY WITH WAARDENBURG SYNDROME**

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**Introduction:** Waardenburg syndrome (WS) is a rare genetic disorder, which is predominantly inherited in an autosomal dominant pattern. There are four main types of WS, which are distinguished by their physical characteristics and genetic cause. Symptoms vary between the different types and between patients from the same type. Mutations in multiple genes – EDN3, EDNRB, MITF, PAX3, SNAI2, SOX10, TYR, WS2B and WS2C, cause the various forms of WS.

**Materials and Methods:** DNA from two siblings with a clinical diagnosis of Waardenburg syndrome was extracted. Library preparation was done using a TruSight One panel (Illumina) and sequencing was performed on a MiSeq platform. After the causative mutation was identified, the family of one of the patients decided to undergo PGD. Four embryos were biopsied and tested for carrier status of the mutation through targeted NGS.

**Results:** Both siblings were found to be carriers of a heterozygous non-sense mutation in PAX3: Chr2:g.223160283T>A, NM_181459.3:c.415A>T, NP_852124.1:p.Lys139Ter. Performing PGD we established that two of the four embryos examined were with normal genotype. The third one was a carrier of the mutation and the result of the last one was not informative due to potential allele dropout. According to the established genotypes, two of the embryos were suitable for transfer. One was transferred, but pregnancy was unfortunately not achieved in this cycle. A transfer of the second embryo is forthcoming.

**Conclusion:** We successfully performed pre-implantation genetic diagnosis in a family where the father was a carrier of a pathogenic PAX3 mutation. PGD is an alternative to prenatal diagnosis in the first or second trimester of pregnancy for families at risk of a monogenic disorder. Its advantage is that it offers couples the chance of having an unaffected child without facing the unpleasant experience of termination of pregnancy or having a child with an inherited genetic condition.

## OP-07 **APPLICATION OF NEXT-GENERATION SEQUENCING FOR BALANCED TRANLOCATIONS IN PREIMPLANTATION EMBRYOS**

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**Statement of purpose:** Carriers of a balanced translocation are phenotypically normal, as there is no loss of genetic material. The pathological effect occurs when they segregate as unbalanced in the conceptus. This may be a cause of infertility, miscarriages and malformations in the offspring. We report 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.

**Methodology:** In the presented case we report an infertile couple with recurrent implantation failures (RIF). Karyotyping by G-banded was performed using standard cytogenetic technique. After analysis, a partner carrier of balanced translocation was detected. The woman in the couple was reported as a carrier of translocation with karyotype 46, XX, t(2;15)(q2;qter) After genetic counseling, PGS was used according to the patients' will. For detection of chromosomal imbalances a laser-assisted biopsy was performed on trophectoderm (day 5) followed by whole genome amplification (WGA) and Next- Generation Sequencing (NGS) was performed according to manufacturer's instructions. In the case for analyzing chromosomal and sub chromosomal structural imbalances Veriseq assay (Illumina Inc., USA) was performed.

**Summary of results:** Nine embryos were biopsied and analyzed, two of them were with euploid karyotype. The rest were with different chromosome anomalies as 48, XXX( +8; -11; +15; +X) , 48, XX(+1; +3; -4; +14), 44, XX (+8; -14; -16; -17). In this embryo potential aberrations in the affected chromosome 2 and 15 were detected. One of the embryos was chaotic and one was with a gain of chromosome 2 and a loss of chromosome 15. Two of the embryos were euploid but with potential subchromosomal aberrations of chromosomes 2 and 15.

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.

## OP-08 **STEM CELL TRANSPLANTATION – PRESENT AND FUTURE**

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Allogeneic and autologous hematopoietic stem cell transplantations (HSCT) have been performed routinely for more than 30 years now and with the vast experience gathered, the procedures have been mostly perfected. Allogenic hematopoietic stem cell transplantations are part of the treatment of malignant diseases whereas autologous hematopoietic stem cell transplantations accompany and enable the main treatment of mainly oncohematological diseases (Hodgin`s disease, non-Hodgin lymphoma, multiple myeloma, acute myeloid leukemia etc.). In spite of all the enhancements of the stem cell transplantation procedures, autologous HSCT is not part of per se pathogenetic treatment and thus its efficiency is limited. Allogenic HSCT treatment is pathogenetic (graft versus leukemia and graft versus tumor effect), however it cannot be separated from the adverse graft versus host effect.

With the development of targeted treatment approaches for malignant diseases, there is real possibility for complete control over the disease. Currently there are several approaches, which can be combined, eliciting and sustaining a tumor specific immune response (immunotherapy) and targeted manipulation of cancer driving signaling pathways, including in cancer stem cells. When these approaches reach sufficient level of maturity, hematopoietic stem cell transplantation may become obsolete.

We comment on our two year` experience on autologous hematopoietic stem cell transplantations at the Centre of translational medicine and cell therapy at the University hospital "Saint Marina" Varna.

## OP-09 **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*

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**Introduction:** The Thracian and Proto–Bulgarian population, who existed in different periods from the past, cause huge research interest. Therefore, we have examined Thracian and Proto-Bulgarian ancient mtDNA for the first time. The Thracian samples are dated from III Millenium B.C. and Proto-Bulgarian from VIII – X Century. They are taken from different necropolis in the Bulgrian lands.

**Material and methods:** In order to clarify the genetic diversity of the Thracians and Proto-Bulgarians, 43 samples of 7 separate cemeteries, were analyzed. Two main fundamental methods are used for mitochondrial DNA analysis - the classical method and NGS platform of Illumina.

**Results:** The results of the ancient HVSI haplotype and whole mitochondrial genomes from Thracian and Proto-Bulgarian bone/teeth materials are unique. From the obtained results of Thracian samples the percentages of macro-haplogroups are: H – 41.6%, N and JT – 12.5 % each; K, U, HV and D – 8.3% each. From the obtained results of Proto- Bulgarian samples the percentages of macro-haplogroups are: H – 47%, T – 15.8%, J and U4 – 10.5% each, H5, HV and U3 – 5% each. The established haplogroups are predominantly Eurasian. They determine the possible position and role of Thracians and Proto-Bulgarians in the formation of the modern Bulgarian gene pool and their genetic relationship with other West Eurasian populations.

**Conclusion:** Based on Principal Component Analysis a genetic distance of Thracian, Proto-Bulgarians and contemporary Bulgarians was observed. 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, Slovaks. A large genetic distance exists between Proto – Bulgarians and populations from Volga-Ural region, Tatars and Turks. For the first time the presence of polymorphisms associated with high or low risk of development of different diseases was proved in Thracians and Proto-Bulgarians.

## OP-10 **MOLECULAR DEFECTS DETERMINED AMONG HEMOPHILIA PATIENTS IN REPUBLIC OF MACEDONIA**

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Hemophilia A and B are common X-linked bleeding disorders caused by deficiency or dysfunction of coagulant factors VIII and IX, respectively. The knowledge of the causative gene defect has become an important tool in hemophilia care with respect to prediction of the patients' clinical course and safe genetic counseling of relatives.

The aim of this study was to determine the molecular defects underlying hemophilia A and hemophilia B patients in Macedonia. Seventy unrelated hemophilia A patients and 25 hemophilia B patients were referred to RCGEB for molecular diagnosis. Long range PCR was applied for detection of inversions in intron 1 and 22 in factor VIII gene, while PCR followed by direct sequencing was performed for mutation analysis. The molecular defect was determined in 54 hemophilia A patients, while molecular defect was elucidated in all hemophilia B patients.

In hemophilia A patients the most frequent molecular defect was intron-22 inversion, determined among 25 (46.3%) patients, followed by intron-1 inversion found in 5 severely affected patients or 9.3%. Among hemophilia A patients 24 private nucleotide substitutions or small deletions were identified. Two nucleotide substitutions were novel: c.1735G>A; p.Asp579Asn in exon 11 and c.6823T>G, p.Tyr2275Asp(2256) in exon 25 of the factor VIII gene, both determined in Macedonian hemophilia A patients with mild form of disease.

Among hemophilia B patients 10 different nucleotide substitutions were determined. Nucleotide substitutions c.339T>G,p. Asn113Lys(67) and nt.30150c.835G>A;p.Ala279Thr(2 33) were common variants with frequency of 28% and 20%, respectively, determined among Albanian mild to moderate hemophilia B patients. As a novel events, one small deletion/insertion in exon 2, c.230_231delTT insA, p.Val77Aspfs*27 was determined in a severely affected hemophilia B patient of Macedonian origin, while one nucleotide substitution c.536G>C, p.Gly179Ala in exon 6 of factor IX gene, was determined in a severely affected hemophilia B patient of Roma origin.

## OP-11 **MOLECULAR PROFILING OF HEREDITARY BREAST AND OVARIAN CANCER IN BULGARIA**

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Aiming to determine the mutation spectrum and prevalence of BRCA1/2 pathogenic mutations in Bulgarian Hereditary Breast and Ovarian Cancer (HBOC) families we screened 400 breast/ovarian cancer patients. BRCA genes were sequenced with either Sanger sequencing or NGS using validated AmpliSeq™ BRCA1/2 Community Panel on PGM platform, followed by MLPA analysis.

The overall mutation prevalence observed was 21,25%. Altogether 31 different mutations were detected, including 7 recurrent (4 in BRCA1 and 3 in BRCA2) and 24 singletons (10 in BRCA1 and 14 in BRCA2). The recurrent mutations accounted for 71,76% of all detected mutations. They were dominated by the founder mutations in BRCA1 (c.5263_5264insC and c.181T>G), followed by BRCA2 (c.5848_5851delGTTA and c.9098_9099insA). The other recurrent mutations include a new variant in BRCA2 (c.9908delA) and two in BRCA1 (c.2019delA and c.3700_3704delGTAAA). BRCA mutations explain only 21,25% of the inherited predisposition to breast and ovarian cancer in the analysed cohort and additional studies are needed to unveil the other genes, contributing to increased risk in HBOC families. With the current technological developments, the diagnostic application of NGS using a panel of cancer related genes became feasible. In a pilot study we selected 70 BRCA1/2 negative patients and NGS sequencing was performed on MiSeq (Illumina), using TruSight Cancer Panel. Pathogenic mutations in 11,94% of the screened families were found in PALB2, CHEK2, NBN, MUTYH, MLH1, and RET, including missense and frameshifts. Probably pathogenic mutations in 7,46% of the patients were detected in ATM, MLH1, MSH6, BRIP1, and BLM.

This study provides the first comprehensive estimation of the frequencies and type of mutations in BRCA1/2 and other genes contributing to HBOC in Bulgarian patients. Based on it optimal cost-effective strategy for the routine diagnostic testing is offered allowing genetic counseling, risk assessment and risk management in HBOC families.

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## OP-12 **DIFFERENTIAL EXPRESSION OF 12 MICRORNAS IN BREAST CANCER AND THEIR POTENTIAL USE AS MARKERS FOR DIFFERENT CLINICOPATHOLOGIC FEATURES**

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MicroRNAs differential expression is widely studied in breast cancer (BC). Aberrant expression of certain miRNAs has been found to be associated with different cancer types, stages, grades or receptor statuses. These findings suggest that miRNAs could be used as biomarkers for cancer diagnosis.

In this study we investigated the differential expression and significance of 12 miRNAs in BC patients and their potential use as markers for different clinicopathologic features.

The 12 studied microRNAs (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) were selected based on literature data as well as initial microarray analysis of 6 pairs of breast tissues (normal and malignant). A total of 130 pairs of BC tissues were included in the study. The tumors were grouped based on their grade, node and receptor status. Total RNA was extracted using RNeasyMini kit from Qiagen. TaqMan microRNA assay were used for qPCR analysis. Statistical analysis was performed using R Bioconductor software for the microarray, DataAssist software v.3.0 for qPCR, and SPSS v.19.0 for ROC curves construction.

QPCR analysis revealed that miR-21, miR-155, miR-200a, miR-205, miR-142-3p and miR-139-5p were differentially expressed in malignant in comparison to normal breast tissue. MiR-142-3p, miR-146a, miR-200a, miR-21 and miR-210 showed progressive

upregulation of the expression with the higher grade. ROC curve analysis of 7 miRNAs (miR-21, miR-139-5p, miR-142-3p, miR-146a, miR-205, miR-210 and miR-320c) showed that miR-210 can discriminate G3 grade from other tumor grades. MiR-210, miR-21 and miR-320c were significantly upregulated in triple negative tumors. Additionally, miR-125b was found to be downregulated in HER2+ tumors.

Our results showed that miRNAs could be useful for distinguishing tumor/normal BC samples, tumor grade and different receptor statuses.

## OP-13 **STUDY OF THE SIGNALING PATHWAYS OF T- AND B-CELL ACTIVATION IN COMMON VARIABLE IMMUNODEFICIENCY – SIGHT TO THE PATHOGENESIS OF DISEASE**

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Common variable immunodeficiency (CVID) includes a heterogeneous group of disorders characterized by hypogammaglobulinaemia, severe, recurrent bacterial infections, tendency to autoimmune manifestations and oncological diseases. It is the most common humoral immune deficiency requiring substitution therapy. The exact pathogenetic mechanisms leading to the development of the disease are poorly studied. It is assumed that the most essential in pathogenesis is defective B-cell differentiation and function, but defects in T-cell function are also permissible. The aim of this study is to examine the intracellular JAK/STAT and mitogen activated protein kinase (MAPK) signal pathways in lymphocytes of CVID patients.

**Matherials and methods:** Blood samples of 10 patients diagnosed with common variable immune deficiency and 10 clinically healthy unrelated individuals were investigated. By performing multicolor flowcytometric analysis we evaluated post-stimulation intracellular expression of STAT3, STAT5, STAT6, Erk1/2 and p38MAPK.

**Results:** The analysis of cell populations in patients in comparison with healthy controls showed increased spontaneous Erk activation in CD19+ B-lymphocytes and CD4+ T-cells. IL-2 / STAT5 induced activation and increased STAT5 activation index in CD4 + cells are increased in combination with reduced spontaneous STAT5 activation of CD8 + lymphocytes, but increased STAT5 IL-2-dependent activation index. IL-6-dependent STAT3 activation in CD19+ B-lymphocytes and STAT 3 activation index are decreased. CD8+ T-lymphocytes are with decreased IL-4 induced STAT6 activation and reduced STAT6 activation index.

**Discussion:** The obtained data indicate that in patients with CVID are observed disturbances in the intracellular signaling activation pathways leading to impaired function of T- cells. The impairments we found could explain the cytokine Th1 / Th2 disbalance in disease, and also alterations observed in CVID helper and cytotoxic T cells as well as B cells count, phenotype and function. Such a research approach in a larger group of patients in combination with genetic analysis of defective signaling pathways would have not only scientific but also clinical and therapeutic potential.
This work was partially granted by Medical University Sofia, Grant#56 project №339/15.01.2015

## OP-14 **ATAXIA TELANGIECTASIA: CLINICAL CASE**

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Ataxia telangiectasia (AT) is a rare, neurodegenerative multi-system disease characterized by cerebellar ataxia, variable immune deficiency, ocular telangiectasia, progressive respiratory failure and an increased risk of malignancies. AT is part of fragile chromosome syndromes and is caused by mutation in the ATA gene on chromosome 11q22.3.

**Clinical case:** A 23-year-old woman with AT, with difficulty moving, tremor of the body and speech difficulties, enters the clinic due to frequent respiratory tract infections in the last two years. The first symptoms of the disease occurred at 3 years of age (conjunctival telangiectasia) followed by ataxia and cytogenetically diagnosed AT at the age of 5 years. After that when she was 6 years old immunological studies were carried out, because of suffering frequent infections (sinusitis and bronchitis), by which the imbalance in cell populations and changes in serum immunoglobulin levels were established. In this connection, immunotherapy with IVIG was performed with a good clinical response for 12 months. Immunological studies have shown a significant decrease in serum IgA levels <0.02 g/l, decreased IgG2 1.65 g/l and elevated IgM 2.88 g/l. T and B cell deficiency has been proven to be non-progressive. Imbalance in the percentage distribution of naive and memory CD4+ T cells was established. Also, normal expression of CD69 from total T lymphocytes after stimulation with PHA and significantly elevated NK cells were found. Markers for early detection of neoplasms and diabetes mellitus were examined. Treatment with Immunovenin intact at a dose of 250 mg/kg was performed.

**Conclusion:** Patients with AT require a specialized care, tailored to the individual needs of the subject. This can only be achieved in Rare Disease Centers like this in the University Hospital Alexandrovska for PID, where threatment and follow-up is offered not only to the patients, but also to their family members. We believe that such a comprehensive approach gives a chance for improvement and allows a better quality of life for these patients

## OP-15 **TTR FAP IN BALKAN COUNTRIES**

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TTR amyloidosis was first identified in Northern Portugal, where it was found to be associated with a Val30Met mutation of the TTR gene. Portugal, Brazil, Japan, and Sweden are considered endemic regions. More than 120 different mutations were identified in the whole world.

In the last ten years there is a big advance in identification of the disease in Balkan countries. The disease was found in Bulgaria, Turkey, Romania, Macedonia, Kosovo.

In Bulgaria 112 TTR-FAP patients and 94 asymptomatic carriers from 80 affected families were identified with the following mutations: Glu89Gln – 63 families; Val30Met – 8 families; Ser77Phe – 6 families, Ser52Pro – 1 family and Gly47Glu – 2 Roma (Gypsy) families.

In Turkey 28 TTR FAP patients were diagnosed – 19 in Istanbul and 9 – in Ankara. Val30Met – 11 patients. The following mutations were found: Glu89Gln – 5 patients; Gly47Glu – 4 patients, Gly53Glu – 3 patients; Thr49Ser – 2 patients; Glu54Lys – 2 patients and Glu54Gly – 1 patient.

In Romania four TTR-FAP cases were diagnosed. All of them have the same mutation - Glu54Gln.

In Macedonia and Kosovo several TTR FAP families were identified with Glu89Gln mutation.

In conclusion there exist significant genetic and clinical heterogeneity of TTR FAP. Val30Met mutation was found in the most of the countries of Central and Eastern Europe and it causes TTR FAP with late onset. Glu89Gln is a specific regional Balkan-Mediterranean mutation, identified in Turkey, Bulgaria, Macedonia, Kosovo, Italy (Sicily). Gly47Glu is second regional Balkan-Mediterranean mutation, identified in Italy, Greece, Bulgaria. In Bulgaria the mutation was identified in Roma (Gypsies).

## OP-16 **GENETIC SCREENING FOR TRANSTHYRETIN (TTR) AMYLOIDOSIS IN BULGARIA. GENETIC PROFILE OF TTR-FAP: GLU89GLN FOUNDER EFFECT.**

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Transthyretin (TTR) amyloidosis is an autosomal dominant systemic disorder characterized by the formation of amyloid fibrils in the extracellular space. 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. TTR-FAP is characterized by a progressive, axonal sensory autonomic and motor peripheral neuropathy. TTR-FAC is a classic form of an infiltrative cardiomyopathy. It is progressive lethal disease caused by an infiltrative restrictive cardiomyopathy.

In our population the genetic profile of TTR-FAP is dramatically different in comparison to the rest of the countries. We have clinically well described and genetically screened endemic region in the South-West part of the country with 75 affected families involving 203 patients mainly (76%) with Glu89Gln mutation. We also screened more than 100 random anonymous newborn DBS samples which originated from the endemic region, but now mutation carriers were detected outsides the effected families.

This finding was the first milestone in our research in genetics of TTR-FAP and we suspected founder effect of the mutation. We reconstructed the most likely haplotype of the Glu89Gln carriers by genotyping 7 highly informative STR markers localized closely to the TTR gene. Our preliminary results from the first 10 families (more than 70 patients) clearly confirmed the founder effect of the mutation. We also start preliminary determination of the mutation age based on these haplotype results.

It is interesting that our Bulgarian endemic region is remarkably well geographically defined in spite of the fact that our country is a crossroad between Europe and Asia and frequent population migrations during centuries. Our screening program during the last few years showed very few occasional spreading of the mutation across the country.

## OP-17 **GENETIC PROFILE OF TTR-FAP IN BULGARIA: PARENT-OF-ORIGIN DIFFERENCE IN PENETRANCE**

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Transthyretin (TTR) amyloidosis is an autosomal dominant systemic disorder caused by mutations in the TTR gene. It may present as familial amyloid polyneuropathy (TTR-FAP), or cardiomyopathy (TTR-FAC). The most common TTR mutation worldwide is Val30Met. Another TTR mutation common in Sicilia, Italy is Glu89Gln. Different patterns of clinical presentation have been described for both mutations and the genotype-phenotype correlation remains incompletely defined. In Bulgaria the genetic profile of TTR-FAP is dramatically different. We have clinically well described and genetically screened 75 affected families (203 patients). The disease was mainly caused by the common mutation Glu89Gln (76%). Some authors report markedly different penetrance according to the gender of the transmitting parent. Based on our previous studies we have some preliminary expectations for different penetrance in some Bulgarian families. In Glu89Gln mother-son pair we found more than 5 years difference in the age at onset. We also found anticipation in father-son inheritance: a twin with neurological onset at the age of 36, the second brother not affected at this age and their father with carpal tunnel syndrome operation at the age of 46 and cardiac manifestation at 65. The aim of the present study was to better understand the difference in the disease penetrance by evaluating the ratio of mutant versus wild type transcripts. We sequenced the TTR RT-PCR product and compared the mutant versus wild type transcript in families with markedly different age at onset. The RNA was extracted from plasma and urine. The obtained results showed different expression levels of mutant versus wild type transcripts, which coincides with the disease onset and phenotypic manifestation in a single family. Such an experiment to assess mutant versus wild type TTR transcripts has not been performed so far. The anticipation need to be considered in genetic counselling and in follow-up of mutation carriers.

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## OP-18 **GENETIC HETEROGENEITY OF CARDIO VASCULAR DISEASES ASSOCIATED WITH PATHOLOGY OF GREAT VESSELS**

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**Purpose:** Serious advances have been made in identification of genetic basis of cardiovascular diseases (CVD). However clarification of the genetic causality is complicated by the genetic heterogeneity of the group. The aim of the study was identification of genetic variants obtained through next-generation sequencing (NGS) associated with phenotype of cardio vascular diseases with pathology of great vessels.

**Methodology:** DNA samples from patients with pathology of great vessels were analyzed by targeted next generation sequencing of 174 genes included in TruSight Cardio gene panel (Illumina). Detected variants were validated by Sanger sequencing.

**Results:** Double heterozygosity for novel ELN:c.890-1G>A and known SCN5A: p.Gly9Val was detected in a patient with SVAS and pulmonary valve stenosis. Heterozygous variants in gene ACTA2 were detected in two patients: p.Lys52Glu in a patient with aneurysm of abdominal aorta and p.Arg258Cys, in a patient with aortic dissection type III. Three patients 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 associated with the syndrome. The first one with mitral valve prolapse and aortic dilation was a heterozygote for two variants of unknown clinical significance in genes TTN, p.Thr8843Met and ELN, p. Gly518Ser. The second, with scoliosis, bicuspid aortic valve and mitral valve prolapse had no mutations in any of the genes in the panel. In the third, a known heterozygous pathogenic variant in gene CBS, p.Ile278Thr was detected. Analysis of patient with family history and clinical diagnosis of Marfan's syndrome revealed heterozygous, probably pathogenic variant in gene FBN1:p.Cys982Arg.

**Conclusion:** Our results are in accordance to previous findings of high heterogeneity of genetic background of cardio vascular diseases with pathology of great vessels.

## OP-19 **GENETICS AND CARDIO-VASCULAR DISEASES**

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Cardiovascular diseases (CVD) are one of the world's leading causes of morbidity and mortality. Its prevalence will continue to rise over the next few decades. Until now, public prevention strategies have relied predominantly on managing environmental factors that contribute to CVD, such as obesity, smoking and lack of exercise. The recent and rapid development of molecular genetics in CVD has created a new understanding of their pathogenesis and natural history, and also new possibilities for the diagnosis of these genetic disorders through genetic testing. The genetic variants predisposing to CVD spread from rare and deleterious mutations responsible for Mendelian diseases, such as cardiomyopathies, familial hypercholesterolemia, to common polymorphisms that modulate the predisposition to complex diseases with a weak effect at individual level.

Cardiomyopathies are a heterogeneous group of heart muscle diseases associated with mechanical and/or electrical dysfunction that predispose patients to sudden cardiac death. Over the last 20 years, the association of specific genes involved with cardiomyopathies has emerged. Hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, left ventricular noncompaction cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy are all now recognized to have a genetic component

Familial hypercholesterolemia (FH) is an autosomal dominant disorder that causes severe elevations in total cholesterol and low-density lipoprotein cholesterol. FH affects approximately 1 in 500 people (10 million world-wide) and the elevated serum cholesterol concentrations lead to a more than 50% risk of fatal or non-fatal coronary heart disease by age 50 years in men and at least 30% in women aged 60 years.

The heritability of coronary artery disease (CAD) has been estimated between 40% and 60%, on the basis of family and twin studies. However it has a complex genetic trait with multiple genetic and environmental components contributing to the observed phenotype.

## OP-20 **RECOGNITION OF SYNDROMIC FORMS OF DISORDERS OF SEXUAL DIFFERENTIATION**

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**Purpose:** Disorders of sexual development (DSD) encompass etiologically heterogeneous group of patients including disorders of chromosomal or genetic sex, gonadal dysgenesis of disturbed phenotypic sex development due to the inappropriate hormonal action and

synthesis. The phenotypic spectrum of external genitalia, gonads and development of Wolfian and Mulerian duct derivatives varies in all patients. Rarely DSD, when either male or female sex phenotype is present is associated with a wide variety of malformations of other systems.

**Results:** We present seven cases with syndromic DSD. All patients have ambiguous genitalia with different Prader staging. In one case the phenotypic sex determination was not possible due to the extensive anomaly of the frontal abdominal wall. Five of them had XY, and two XX karyotype. Developmental delay was present in six of them. A spectrum of other malformations includes skeletal, intestinal, renal, cardial system, accompanied with evident dysmorphism in all. The combination the specific anomalies together with chromosomal and molecular findings in patients pointed to the specific syndrome in all.

**Discussion:** There are more than 70 syndromes associated with sexual ambiguity - either male or female phenotype separated in the separate group of DSD. 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. Clinical recognition directs the diagnostic procedure on the right path.

## OP-21 **GENETIC BACKGROUND OF STEROID-RESISTANT NEPHROTIC SYNDROME IN BULGARIA**

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Nephrotic syndrome is the most common pathology of the kidney glomeruli in children. The majority of the cases are successfully treated with corticoids, but some patients fail to respond to this therapy and progress towards a steroid-resistant form (SRNS). SRNS is a clinically and genetically heterogeneous group of diseases characterized by massive proteinuria, albuminuria and oedema. It is caused by changes in the glomerular ultra-filter, the slit diaphragm, which result in an increased renal filter permeability.

Several genes have been implicated in the pathogenesis of SRNS. Most commonly affected are NPHS1, NPHS2 ACTN4 and CD2AP - involved in constructing and maintaining the glomerular filter, as well as WT1 - a transcription factor with role in the formation of the genitourinary tract in the foetus and the cell subtype differentiation maintenance in adults. Genetic testing in SRNS facilitates both the choice of treatment and genetic counselling. In addition, identifying the causative variants allows us to better understand the molecular causes of the disease.

The goal of the present study was to understand the genetic background of SRNS in Bulgaria. Initially, our attention was focused on NPHS2 and WT1, the two genes most commonly affected in non-Finnish patients. We recruited and screened for mutations 28 patients from 24 families by Sanger sequencing. The genetic cause of the disease was determined in five families, where two novel heterozygous WT1 mutations (p. S395Y and p. Cys428Ser) and two homozygous or compound heterozygous previously described NPHS2 variants (p.Gly140Aspfs * 40; p.Leu169Pro) were identified. A unique case of maternal WT1 mutation mosaicism was documented. Based on recent publications confirming the involvement of NPHS1 in cases of non-Finish background, we are currently performing a screening for mutations in this gene in the remaining patients.

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## OP-22 **MOLECULAR BASIS OF DEVELOPMENTAL DISORDERS: A VIEW THROUGH THE KIDNEY FILTER**

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The fate of a cell is determined by complex interplay of transcription factors, epigenetic modifiers, signalling molecules, enzymes and structural molecules. Any change in these interactions may affect the development of the organism, causing disease. Since both common and tissue specific factors determine the differentiation of cells and architecture of tissues, some mutations affect single organs or physiological processes, while others lead to more complex phenotypes.

Congenital anomalies of the kidney and urinary tract (CAKUT) are among the most common developmental disorders affecting 3-6 in

1 000 births. They are a major cause of end-stage kidney failure and contributing factor for cardio-vascular diseases. A number of genes have been implicated in both syndromic and non-syndromic CAKUT but they explain only a small proportion of all cases.

Our team studies the molecular background of developmental disorders from the viewpoint of the kidney, which is positioned in an intersection of some key developmental pathways. We believe that by performing genetic screening of CAKUT patients, whose renal abnormalities are accompanied with disorders of other organs and systems, we will identify key players in the embryonic development and if possible, help to unravel the complex networks of developmental interactions.

Using both "classical" candidate gene sequencing and high-throughput analyses, aCGH and NGS, we determined the genetic cause of the disease in a number of complex cases whit extra-renal phenotypes such as skeletal abnormalities, mental retardation, glaucoma, facial dysmorphism, hearing loss, etc. Both known and novel changes were found. Among those were point mutations, small insertions, whole and partial gene deletions, large chromosomal aberrations and aneuploidies. The genetic variants accounted for disorders with diverse mode of inheritance – autosomal dominant with incomplete penetrance, autosomal recessive as well as X-linked.

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## OP-23 **SPECTRUM OF MUTATIONS IN THE CFTR GENE OF ALBANIAN CYSTIC FIBROSIS PATIENTS**

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**Background:** Mutation epidemiology in each ethnic group is a crucial step of cystic fibrosis (CF) diagnosis and prevention. The pattern of CFTR mutations in a given population provides important information for the establishment of population-based molecular diagnostics. The whole spectrum of CFTR gene mutations in Albania remains unknown to the scientific community.

**Methods:** Identification of the pattern of mutations causing CF in Albania was performed in 152 unrelated CF patients from Albania. Albanian patients diagnosed by clinical indications and sweat test, were analyzed for CFTR mutations using PCR-OLA, Reverse Blot Strip assay, sequencing and MLPA protocols.

**Results:** Analyzing 304 CF chromosomes in Albania we found 12 different CFTR mutations, which accounted for 84 % (255/304) of CF Albanian alleles. Out of 12 CFTR mutations, five CF mutations: p.Phe508del (70.06%), c.489+1G>T (4.27%), p.Gly542X (3.61%), p.Gly85Glu (1.97%), and c.579+1G>T (1.31%) accounted for about 81% and the other 7 mutations, p.Arg1158X (0.65%), p.Asn1303Lys (0.33%), p.Ser466X (0.33%), p.Ser549Arg (0.33%), p.Glu822X (0.33), p.Arg1070Gln (0.33%) and c.54-5490_273+10250del21kb(CFTRdele2,3 (21 kb) (0.33%), accounted for about 3% of CF mutations in Albania. Based on the new pattern of CF mutations in Albanians and the detection rate of up to 83% our laboratory performed prenatal diagnosis of CF in Albania since 2005.

**Conclusions:** This study presents the largest screening of Albanian CF patients useful to complete the puzzle of Balkan Peninsula CF mutations. Considering that about 1/4 of the Albanian population have migrated in some European countries (Greece, Italy, Germany, France and U.K.), in USA and Canada, our data will contribute in CF genetic testing of immigrants and their families.

## OP-24 **DIAGNOSTIC AND THERAPEUTIC APPROACH IN CHILDREN WITH BILIARY ATRESIA**

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**Introduction:** Biliary atresia is a rare disease, which is presenting in the first weeks of life. The pathogenesis is still unclear, despite the efforts of many researchers. There are different hypotheses including genetic predisposition, infectious or toxic insults during pregnancy, immune dysregulation, but the cause is probably multifactorial, leading to a common anatomical substrate – obliterative extrahepatic cholangiopathy.

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.

Aim of this study is to summarise the results of the management of children with biliary atresia in Bulgaria and to establish a diagnostic and therapeutic algorithm for early diagnosis, introduction of appropriate treatment and preparation for surgery.

**Methods:** We present a retrospective study of clinical data for a period of twenty years.

**Results:** We included 38 children with biliary atresia who underwent liver transplantation with or without Kasai portoenterostomy. All patients presented with typical symptoms – jaundice, pale stool, dark urine, and specific laboratory results – conjugated hyperbilirubinaemia and cholestasis. 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.

**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 national strategy for all rare gastrointestinal and liver diseases.

## OP-25 **RARE DISEASES, NEW GENES, MOLECULAR MECHANISMS AND TREATMENTS**

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**I Background:** he autosomal recessive forms of spondylocostal dysostosis SCD) are caused by mutations in the DLL3 gene, the MESP2 gene (SCDO2) and the LFNG gene (SCDO3).

**Patients, Methods, Results:** The proband is a 12 years old boy with growth deficiency (>3 percentile). The trunk is shortened, the legs and arms are normal. Pectus carinatus and scoliosis are prominent. Joints are not stiff. The intelligence is normal, corneas are not cloudy and sight and hearing are normal. Urine for mucopolisaccharidosis are normal. Ultrasound of the kidneys, liver and hearth are uneventful. X-rays showed multiple, generalised, 'mild' segmentation abnormalities throughout the spine. However, the ribs appear almost normal, perhaps slightly thicker, and there is possibly a point of fusion high on the left and posteriorly, but not at the origin of the ribs. Similar X-ray features are present in the father (38 years) and the uncle (36 years). The grandfather died at the age of 63 from thyroid cancer, but otherwise had the same clinical characteristics. Gene sequencing excluded the DLL3 mutation in the proband. Whole exome sequencing, filtering, SNP analysis implicated a causative effect of a member of the Notch signalling pathway the TBX3 gene/protein. Functional analysis has further demonstrated lower enzymatic activity. It was thus demonstrated that the TBX3 gene alterations are causative in autosomal dominant SCD. In a large cohort of patients with congenital scoliosis (CS) TBX3 gene alterations were also demonstrated in ~7% of patients.

**Conclusions:** This is a family with four members in three generations with an apparent autosomal dominant inheritance where the proband is DLL3 negative. A novel TBX3 gene alteration was discovered and proven causative. The same alteration was found in a significant number of patients with CS. This prenatal diagnosis in CS is possible and gene targeting is feasible.

**II Background:** Mutations in key genes of the phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway have been identified in numerous tumor samples, while loss of PTEN function or activation of AKT1, AKT2 or AKT3 have been implicated in disorders that feature overgrowth and/or hypoglycemia.

**Patients, Methods, Results:** An exome sequencing of DNA from affected and unaffected skin fibroblasts from a patient (C1) with unclassified severe overgrowth of the right leg identified a cancer-associated variant in PIK3CA in DNA from the affected sample that was not present in the unaffected sample (c.3140A>T which predicts p.His1047Leu). The alteration was also found in DNA isolated from other affected tissues from muscle, bone, fibrous and adipose tissue.

These patients did not meet the clinical criteria for Proteus syndrome, matching those of CLOVES syndrome. However, these patients lacked the complex truncal vascular malformations that are commonly found in patients with CLOVES.

**Conclusions:** Thus the spectrum of phenotypes associated with somatic activation of PI3K signaling is expanded and multiple therapeutic targets are suggested.

## OP-26 **STUDY OF ATXN2 REPEAT LENGTH IN C9ORF72 EXPANSION CARRIERS**

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**Introduction:** Hexanucleotide repeat expansion (GGGGCC) in chromosome 9 open reading frame 72 (C9ORF72) is the first gene change found to be the link between amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). C9ORF72 expansion has been also detected in other neurodegenerative disorders, such as Alzheimer's disease (AD) and Huntington disease like (HD like) syndrome, but reason of such phenotypic heterogeneity is still unclear. Recent studies suggest possible role of CAG repeats in ataxin2 (ATXN2) gene in phenotypic expression of C9ORF72 expansion. Intermediate ATXN2 repeats (27-32 CAG repeats) seem to be associated with the risk towards developing ALS, but results are not consistent.

**Methodology:** C9ORF72 hexanucleotide expansion analysis has been performed in large cohort of Serbian patients diagnosed as: ALS (N=280), FTD (N=264), AD (N=159), and HD like (N=135). The number of GGGGCC repeats in C9ORF72 was determined using 2-step protocol. Normal alleles were determined by standard PCR amplification of region containing repeats and fragment analysis on capillary electrophoresis. In the second step, repeat-primed PCR was performed for all apparently normal homozygous samples. Cut-off size for repeat expansion was 30 repeats. Subsequently, number of CAG repeats in ATXN2 was determined for all C9ORF72 expansion carriers by fluorescent PCR and capillary electrophoresis.

**Results:** Normal C9ORF72 alleles ranged from 2-27 repeats. C9ORF72 expansions were detected in 9 (3,21%) ALS, 5 (1,89%) FTD, and 1 (0,74%) HD like patient. One (0.63%) AD patient had borderline number of hexanucleotide repeats. Among C9ORF72 expansion carriers the ATXN2 repeat length ranged from 22-28 repeat units and the most frequent allele was with 22 repeats. Intermediate ATXN2 repeat length was detected in one (11.11%) ALS case carrying C9ORF72 expansion and none in FTD, AD, nor HD like group. In conclusion, further ATXN2 repeat assessment is needed to evaluate the significance of these repeats among ALS patients.

## OP-27 **GENETICALLY VERIFIED TUBEROUS SCLEROSIS COMPLEX (TSC) IN A COHORT OF FIFTEEN BULGARIAN FAMILIES**

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Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder caused by mutations in the TSC1 or TSC2 genes. The TSC diagnostic criteria were divided into major and minor features. The classic manifestation includes epilepsy (80%), intellectual disability (60%), and facial angiofibroma (75%). In addition to these initially described features many other clinical symptoms have been adopted and are included in the diagnostic criteria. The genetic verification of the diagnosis is a major feature. The clinical manifestations vary considerably between and within families. The phenotypic spectrum ranges from minor features as skin lesions, including hypomelanotic macules, facial angiofibromas, ungual fibromas and shagreen patches to therapeutic resistant epilepsy, intellectual disability, and increased morbidity due to cortical dysplasias which includes tubers and cerebral white matter radial migration lines, subependymal nodules and subependymal giant cell astrocytomas, renal insufficiency and lung affections as in our patient.

Here we report on the results of the first molecular testing of 14 Bulgarian patients and one Romanian patient with clinically suspected TSC. Sixty percent of them were positive for mutations in the TSC2 and TSC1 genes (33%). We found five novel mutations: three of them in the TSC2 gene, which are nonsense and frameshift and two in the TSC1 gene also nonsense and frameshift. In addition we detected 10 previously reported mutations; some of them described only ones in the literature. All cases represent the typical clinical features of TSC and met the clinical criteria.

TSC diagnosis was confirmed in all but one of the cases. In 38% of our cases the family history was positive, in one case de novo mutation was detected and in 44% of the cases the parents were not available for genetic testing. Our results add novel findings in the genetic heterogeneity and pathogenesis of TSC.

**Keywords:** TSC1 gene, TSC2 gene, TSC
The study was partially supported by Medical University Sofia, № Д-131/2017

## OP-28 **MOLECULAR PROFILING OF PAPILLARY THYROID CANCER BY RNA EXPRESSION AND NGS SEQUENCING PLATFORMS**

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**Purpose:** Thyroid cancer is a challenging disorder whose frequency is increasing significantly. By 2019 it's expected to be the number three cancer in women and in women aged 35 years and younger it is already the number one cancer.

Our research started by molecular profiling of papillary thyroid cancer in Bulgarian patients at RNA expression level. We continued by NGS analysis of cases with familial form of PTC in families of Swiss origin. The purpose was to identify other variants rather than commonly known ones which are probably predisposing to FPTC.

**Methodology:** We have applied RNA expression platform of Affymetrix on pure tumor samples obtained by laser-captured microdissected slides and NGS sequencing using TruSight Cancer sequencing panel (Illumina). Analysis of the sequencing data was performed using the Softgenetics NextGene Software.

**Results:** We have previously created the first "expression map" of PTC revealing about 150 up-regulated genes and suggesting a targeted gene (RGS4) for cancer therapy. Subsequently we found a plethora of mutational changes in DNA in FPTC cases. We revealed 17 rare variants with a possible damaging effect on protein function, of which 12 were unique for each family, while 5 were common for two of the four families. In all of the families we observed variants in genes belonging to Fanconi anemia (FA) pathway that cause cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair.

Our results suggest a possible role of the revealed mutations in causing genetic predisposition to FPTC. The study has impact on the molecular pathogenesis of FPTC and hopefully will lead to a better management of this type of human cancer.

## OP-29 **GENETIC PROFILING OF ADVANCED LARYNGEAL CARCINOMA BY NEW GENERATION SEQUENCING**

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**Introduction:** 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. 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.

**Material and Methods:** In the current study were included 15 patients with advanced LSCC. The isolated DNA from tumours was used for sequencing on MiSeq (Illumina) using TruSeq Amplicon Cancer Panel, containing 48 tumour-associated genes. The data was analyzed by VarSeq Software (v.1.4.6).

**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). Four new variants in TP35 gene with pathogenic prediction (c.536A>G; c.546C>A; c.193C>T; c.641A>G) were found. The second commonly mutated gene was PIK3CA, with five pathogenic variants in five patients. Two of them were new (c.3118A>G; c.3031delC), and one (c.1633G>C) was related to Vemurafenib resistance. 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). One mutation was found in each FGFR2 (new variant: c.880G>T), HRAS (c.37G>C) and SMAD4 (new variant: c.394C>G).

**Conclusion:** 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.

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## OP-30 **ASSOCIATION OF FC GAMMA RECEPTOR POLYMORPHISMS WITH AUTOIMMUNE HEMOLYTIC ANAEMIA**

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**Purpose:** Autoimmune hemolytic anemia(AIHA) is a second most common autoimmune blood disorders. The etiology of AIHA remains unclear, but both genetic and environmental factors may have a role in the development of the disease. The aim of our study was to investigate a possible role of two polymorphisms in the Fc gamma receptor 2A and 3A(FCGR2A and FCGR3A) in the development of AIHA. 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.

**Methodology:** We have analyzed 70 adult patients with AIHA; 35 patients with idiopathic AIHA and 35 patients with secondary AIHA and chronic lymphocytic leukemia. Controls were 120 healthy individuals. DNA was isolated from peripheral blood mononuclear cells and genotyping was performed by using PCR/RFLP methods. The distribution of genotypes and allele frequencies were compared by using a chi-squared test or Fisher's exact test.

**Results:** Our results demonstrated significantly different genotype distribution for FCGR2A+494A/G in patients with AIHA (n=70; A/A=45, A/G=19, G/G=6) and controls (n=120; A/A=55, A/G=50, G/G=15), p=0.048. There was also significantly higher frequency of the high affinity FCGR2A-131H(+494A) allele in patients with AIHA comparing with controls (66.6% versus 77.8%; p=0.028). Statistical analysis of the genotype distribution for FCGR3A+559T/G showed significant difference between patients with AIHA (n=70; T/T=22, T/G=23, G/G=25) and controls (n=120; T/T=52, T/G=46, G/G=22), p=0.025. We also found significantly higher frequency of the high affinity FCGR3A-158V(+559G) allele in patients with AIHA comparing with control individuals (47.9% versus 37.5%; p=0.007). Our results suggest possible role of both polymorphisms in the etiology and development of autoimmune hemolytic anemia, but further larger prospective studies are necessary to confirm these results.

## OP-31 **ASSOCIATION OF ADORA2A GENE RS2298383 POLYMORPHISM WITH EFFICACY/ TOXICITY OF MTX**

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**Statement of purpose:** Methotrexate (MTX) has been anchor drug in the treatment of RA for more than 30 years, but still there is no efficient way to predict its therapy outcomes. At least part of antiinflamatory effect of MTX is achieved via influence on adenosine cycle. MTX induces intracellular accumulation of adenosine and its increased release in blood. Adenosine exerts its antiinflammatory effects by binding to A2A adenosine receptors (ADORA2A) on mononuclear leukocytes. Therefore efficacy of MTX treatment may be influenced by ADORA2A gene polymorphisms. Our aim was to examine the influence of rs2298383 ADORA2A gene polymorphism on efficacy and toxicity of methotrexate.

**Methodology:** We have genotyped 126 RA patients for rs2298383 polymorphism in ADORA2A gene using KASP genotyping system. Diagnosis of disease was established for each patient accordingly to EULAR criteria. The efficacy of therapy was assessed after 6 months of the therapy based on the EULAR response criteria, with a change in the Disease activity score (DAS28) as the main criterion. We defined patients with good and moderate response as ,,responders" and patients with poor response as ,,nonresponders". Additionally rDAS values were used to establish patients response. Side effects of the drug were recorded and classified as mild, moderate and

severe. We compared differences in the efficacy and toxicity of therapy among patients with different genotypes.

**Summary of results:** Among patients, 114 (90,5%) were responders and 12 (9,5%) nonresponders. Observed frequencies of TT, CT and CC genotypes were 42,1%, 41,3%, 16,7%, respectively. 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. Nonresponders recived corticosteroides more often (75%) than responders (49,1%), although this difference was not significant (0,079). There was no association between rs2298383 and adverse drug effects.

## OP-32 **GENETIC TESTING AND INSURANCE**

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**Summary:** This review presents some aspects of genetic testing-associated problems and health insurance, as well as the complex questions resulting from thi procedure, concerning the relationship between medical practitioners, patients and insurance companies. The issue of defining the terms genetic test, genetic diagnosis and genetic information is considered. Under discussion are the application and significance of genetic testing, when applying for different types of health insurance (e.g. insurance against sickness, "life" insurance, insurance against disability). The status of these issues is presented in an international aspect and in Bulgaria.

**Keywords:** genetic testing, genetic test, genetic information, health insurance

## OP-33 **A RARE DISEASE OF THE MITOCHONDRIAL RESPIRATORY CHAIN – 3-METHYLGLUTACONIC ACIDURIA. APPROACH TO DIAGNOSIS AND REHABILITATION**

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**Introduction:** Hereditary diseases of the mitochondrial respiratory chain are detected late, do not have etiological treatment. The existence of methods for correcting oxidative phosphorylation is a motivation for early diagnostics.

**Purpose:** To study the clinical phenotype of a rare disease, to evaluate the effect of synthropy on the clinical and biochemical features of the pathology for developing a strategy for improving the quality of life of patients.

**Description:** Since 2014, 7997 patients with suspected hereditary metabolic diseases have undergone gas chromatography/mass spectrometry of urine. An increase of the level of 3-methylglutaconic acid was found in 1,070 cases. In 2 patients, its level remained elevated and coincided. Monitoring for the family lasts 5 years. Both patients are siblings 3 and 5 years old. Sick from birth - psycho-motor retardation, muscle hypotonia, pyramidal insufficiency, microcephaly, epilepsy. Excluded are the Rett and Angelmann syndrome. At the examination - nonspecific hyperaminoacidopathy, lactate-acidosis, hiperhomocysteinemia, MTHFR 677 C/T, MTRR 66 G/G (violation of cobalamin E). The treatment strategy is aimed at long-term correction of the revealed disorders in the form of a combination of energotropic and folate therapy, complex rehabilitation with the use of special nutrition (USA) and correction of natural nutrition in accordance with biochemical indicators. The quality of life of patients is progressively improving.

**Conclusions:** Diseases of the respiratory chain of mitochondria are many-sided, rare, in total constitute a great medical and social problem and are subject to individual etiopathogenetic and symptomatic therapy.

## OP-34 **SELECTIVE SCREENING OF MITOCHONDRIAL DYSFUNCTION IN A REGION WITH A HIGH LEVEL OF NEUROLOGICAL DISEASES**

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**Introduction:** Mitochondrial dysfunction is the cause of many pathological disorders that are different their variety and complexity. Intensive study of the mitochondrial (mtDNA) polymorphisms made define it as typical pathological process, for which there is no

nosology and etiological specificity. The frequency of mitochondrial dysfunction in population is 1:3000.

**Purpose:** Determine the frequency of polymorphisms of mitochondrial DNA and biochemical markers of disturbance in the Krebs cycle in patients with neurological pathology.

**Results:** Clinical and genetic characteristics of mtDNA polymorphisms (203 patients) involve disorders of nervous (82.16% of patients), muscle (43.24%), ophthalmic (62.16%), cardiovascular (35.14%), skeletal (38.0%) and digestive system (40.54%). 75 patients (36.5%) had characteristic clinical features of classic mitochondrial syndromes MERRF, MELAS, NARP, Leigh, Kearns-Sayre, Leber (confirmed by molecular diagnostics). In 91 patients (45.31%) we found elements of syntropy.

The more frequent of mtDNA polymorphisms in patient with neurological symptoms were tRNA polymorphisms-lysine: 8697G/A; 8860G; 8701G/A; 8856G/A; 8860 (CRS); 8251G/A; 8472S/T; 8448T/C; 8994G/A; 8337T/C; 8794S/T; 8584G/A; 8701A/G and amino acid substitutions tRNA-lysine (syn, thr/ala, pro/leu, met/val, met/thr, his/tyr, ala/thr). The causes of encephalopathy were associated with polymorphisms tRNA-lysine and new mutations (leucine tRNA) (3624 A/G; 3594S/T, 3705G/A, 3505/G, 3552T/A).

**Conclusions:** The findings suggest the need to assess the state of the mitochondrial genome and metabolites of the Krebs cycle in patients with various neurological diseases, as pathways to pathogenetic therapy.

## OP-35 **PHARMACOGENETIC STUDIES IN PATIENTS WITH CANCER**

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**Introduction:** Pharmacogenetic studies focus on the prediction of the response of tumor tissue to standard therapy by genetic profiling. The role of pharmacogenetic biomarkers is to choose the most appropriate drug at optimal dosage for each patient. Kinase inhibitors may be used as target therapy for non-small cell lung cancer (NSCLC) and papillary thyroid cancer (PTC) with positive mutations in the EGFR gene. Many other pharmacogenetics variants could be associated with adverse drug reactions to chemotherapy. Our aim is to characterize genetic variants with pharmacogenetic effects in patients with cancer.

**Methods:** We performed DNA analysis on 29 patients (19 patients with NSCLC and 10 patients with PTC). NSCLC samples were extracted from formalin–fixed paraffin-embedded tissue (FFPET) and PTC samples were extracted from blood. The samples were sequenced by next generation sequencing (NGS), MiSeq instrument using cancer panel of 94 genes and 284 SNPs. The results were analyzed using pharmgkb database (https://www.pharmgkb.org/) for SNP variants associated with sensitivity to certain drugs.

**Results:** Pharmacogenetic variants were found in different genes: ERCC2 (rs13181) (51.72%), TP53 (rs1042522) (75.86%), XPC (rs2228001) (58.62%), EGFR (rs227983) (44.82%) and ERCC5 (rs17655) (20.68%) in cancer patients. The first 3 variants were associated with sensitivity to chemotherapy with the platinum compounds (cisplatin). The EGFR (rs227983) variant is not included in the real time PCR test for target therapy, but it defines sensitivity to panitumumab, cetuximab and EGFR inhibitors. ERCC5 (rs17655) was found only in PTC patients and determine sensitivity to platinum compounds.

**Conclusion:** Our data shows that cancer panel could reveal significant variants for personalized treatment. It has the potential to improve the therapy outcome and prognosis for distinct types of cancers.

## OP-36 **GENETIC RESEARCH, FAMILY AND FAMILY RELATIONS**

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**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 men and 96 women, the largest age group being within the 20-22 age – 92.

**Results and discussion:** A very large proportion of young people appreciate the importance of a happy family life (92%) and the avoidance of family conflicts (89%). Most of them believe that conducting genetic research will reduce the number of unhappy families (63%) and prevent divorces resulting from the birth of a genetically damaged child (90%). Concerning the role of genetic tests for the arisal of conflicts and the feelings of guilt, the biggest percentage is that of the groups of neutral responses (39% and 43%) and almost equal in size are the groups with a positive (31% and 29%) or a negative response (30% and 28%).

**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.

## OP-37 **ANALYSIS OF THE ASSOCIATION BETWEEN PAI-1 GENE 4G/5G POLYMORPHISM AND EFFICACY OF THROMBOLYTIC THERAPY IN PATIENTS WITH ISCHEMIC STROKE**

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**Statement of purpose:** Ischemic stroke (IS) is a leading cause of death and morbidity worldwide. Timely administered trombolytic therapy with recombinant tissue-type plasminogen activator (rt-PA) to suitable patients with IS might be helpful. The plasminogen activator inhibitor-1 (PAI-1) has important role in fibrinolytic system as the main inhibitor of endogenously synthesized tPA in plasma. Therefore, we aimed to determine whether 4G/5G polymorphism of the PAI-1 gene modulates rt-PA therapy efficacy and occurrence of the hemorrhagic transformation (HT), as the most often rt-PA complication.

**Methodology:** A total of 115 patients with IS who received rt-PA<3h were enrolled in the study. The neurological outcome was measured with National Institutes of Health Stroke Scale (NIHSS) at hospital admission and a month after IS. Modified Rankin scale (mRS) at hospital discharge and 3rd month was used to evaluate functional recoveries after IS. Favorable outcome was defined as 0-2 and poor as 3-6 as well as death. Genotypisation was performed using PCR-RFLP method.

**Summary of results:** Among our patients, observed frequencies of 4G/4G, 4G/5G and 5G/5G genotypes were 34.8%, 41.7% and 23.5%, respectively. Average NIHSS reduction was 8.1±4.7, in 4G/4G group 8.8±4.4, in 4G/5G group 7.1±5.0 and in 5G/5G group 8.1±4.6. There was a significant reduction in NIHSS after thrombolytic therapy ($p<0.001$), but there was no significant difference in NIHSS reduction between genotypes. Total of 62 patients (54.4%) had favorable outcome, according to mRS. There was no significant difference between the groups with different genotypes. Frequency of HT was 11.4%, with no significant difference between genotypes. The effect size (ES) analysis showed very large ES at one month after discharge for NIHSS and after three months for mRS, with the highest values for 4G/4G genotype. Our study did not show significant differences in outcome after rt-PA therapy between patients with different genotypes.

## OP-38 **SCREENING FOR SNPS IN A SET OF DRUG-METABOLIZING ENZYMES IN BULGARIAN POPULATION**

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**Introduction:** Pharmacogenetics is the study of inherited genetic variants in drug metabolism which can affect individual responses to medications, both in terms of therapeutic and adverse effects. Over the last decade, the development of molecular methods and the introduction of GWAS and NGS, enabled the acquisition of a mass amount of data regarding the genotype of numerous pharmacogenetic

variants implicated in inter-individual drug response variability. The aim of our study is to determine the genotypes in the set of genes involved in the metabolism of drugs and other chemical compounds in Bulgarian population.

**Materials and Methods:** 103 Bulgarian probands were genotyped for the 25 single nucleotide polymorphisms (SNPs) in 23 genes and null/non-null allele in 2 genes. The DNA samples were extracted from saliva samples and further genetic testing was done by DNA Life, Nordic Laboratories using the QuantStudio 12K Flex Real-Time PCR System. The results were analyzed using PharmGkb database (https://www.pharmgkb.org/ and https://opensnp.org/) for SNP variants associated with sensitivity to certain drugs as well as population frequency.

**Results:** The preliminarily data suggest that 64% from the tested cohort for the COMT have AA/AG genotype; 7,5% are homozygous for the minor allele T for MTHFR (677C>T) polymorphic variant; 45% from the tested are carriers of null allele for GSTM1 and 20,7% - for GSTT1; 18,8% are homozygous for the 289bp ALU insertion in ACE and 40% - are DD. We found that only 15% are TT homozygous for AGT (rs699), which is twice lower than the data for general population tested, reported in https://opensnp.org/; 34% are carriers of CC genotype in VDR (rs1544410 ) and may be beneficial as compared to patients with the TT genotype when treated with bisphosphonates. The result among the frequency in the other SNPs in the genes: CETP, APOE, CYP1A1, CYP1A2, NQ01, IL-6, TNFA, GSTP1, MTR, MTRR, MnSOD/SOD2, eNOS, COL1A1, PPARG, TCF7L2 and FTO are in progress.

**Conclusion:** These data could be beneficial for the dose evaluation in different drugs in Bulgarians, both for the cancer chemotherapeutics and drugs, used for many common diseases. Future efforts should be directed at expanding this research into specific patient groups and creating evidence basis to support clinical application of pharmacogenetic tests in Bulgaria.

## OP-39 **PLEVEN REGISTRY OF CONGENITAL ANOMALIES IN THE EUROCAT NETWORK**

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Established in 1979, the European network of population-based registries for the epidemiologic surveillance of congenital anomalies (EUROCAT) covers over one third of European births (more than 1.7 million births/year, over 750.000 cases, 55 registries in 33 countries). We aim to present the Pleven Registry of congenital anomalies (CAs) in the EUROCAT network. The registry is established in 1988 and has started collecting data on CAs. Since November 2013, the Pleven registry is an affiliate member of the EUROCAT network. The registration of CAs is based on the criteria according to EUROCAT recommendations. The registry records all cases of CAs in live births, stillbirths (including fetal deaths of $\geq$ 20 weeks gestation) and terminations of pregnancy, following prenatal diagnosis. In the registry structure there are two sections: Registry of CAs - database of all registered CA cases; and Genetic family registry – database of families with CA. The main activities of the registry include: 1) Registration of CAs – based on an active screening of all births in the tree delivery hospitals in the city of Pleven; 2) Genetic counseling – provided to the families revealed through the registration process; 3) Follow up of the families and application of prenatal diagnosis in future risk pregnancies. Based on the data reported of Pleven Registry and published on EUROCAT web site, for period 2008-2012, the established overall total prevalence of CAs was 19.25 per 1000 births. Conclusions: The Pleven registry of CAs provides reliable epidemiological information on CA in Pleven region; gives opportunity to improve the diagnosis and to study the etiology of CA; provides compete genetic information on CA; ensures long-term support of the affected families; enables prevention of CAs and assessment the impact of prenatal screening programs.

**Keywords:** congenital anomalies, Pleven registry, EUROCAT

## OP-40 **NGS APPROACH IN CASES WITH CONGENITAL NEUROMUSCULAR DISORDERS**

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Neuromuscular disorders are genetically heterogeneous group; sometimes clinically ambiguous to differentially diagnose. Next generation sequencing (NGS) is a useful tool in clarifying such cases. Here we report three different cases of congenital neuromuscular

disorders, genetically verified by NGS approach and targeted evaluation of genes associated with the clinical symptoms of the patients. The first case is a child with clinical diagnosis of congenital myasthenic syndrome. By whole exome sequencing (WES), a novel homozygous variant in the VAMP1 gene was found: c.66delT, p.(Gly23Alafs*6). This variant was also detected in both parents in a heterozygous state. The second case is a patient with clinical diagnosis of merosin-deficient congenital muscular dystrophy. By WES, two variants in heterozygous state were found in the LAMA2 gene: c.2901C>A p.(Cys967*) and c.7732C>T p.(Arg2578*). The first variant is inherited from the mother, while the second variant is inherited from the father. Homozygous or compound heterozygous pathogenic variants in the LAMA2 gene are known to cause merosindeficient congenital muscular dystrophy type 1A, which was the differential diagnosis in this case. The last case is a patent with clinical diagnosis of hereditary motor sensory neuropathy. The patient's father has the same clinical symptoms. By NGS (Illumina TruSight One) we identified the splice-site variant c.1056+1G>A in the COL6A1 gene, related to the clinical picture in the family. The segregation analysis showed the same heterozygous variant in the father. 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.

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## OP-41 **AUTOSOMAL RECESSIVE NEUROLOGIC DISORDERS AMONG BULGARIAN MUSLIMS**

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Autosomal-recessive (AR) disorders are more common in closed ethnic or religious minorities. After the identification of more than 15 AR diseases among Bulgarian Roma, in the last five years three disorders, caused by recessive mutations with presumably founder effect were found in the Bulgarian Muslim religious minority:

- Limb-girdle muscular dystrophy (LGMD) 2G in the region of Smolian
- Variant of ataxia teleangiectasia, Louis-Bar disease- in the region of Dospat and Surnica
- Hereditary sensory and motor polyneuropathy type Lom (CMT 4D) in the region of Yakoruda

Mutations in TCAP gene are known to cause autosomal recessive limb-girdle muscular dystrophy type 2G (LGMD2G), congenital muscular dystrophy and dilated and hypertrophic cardiomyopathy. We studied 17 affected individuals from 11 pedigrees, belonging to a Bulgarian Muslim minority from the South-West of Bulgaria, homozygous for the c.75G>A, p.Trp25Xmutation in TCAP gene. The heterozygous carrier rate of p.Trp25X among 100 newborns in this region was found to be 2%. The clinical features in the Bulgarian TCAP group can be described with disease onset in the second to third decade of life, proximal muscle weakness in the lower limbs, followed or accompanied by difficulties in ankle dorsiflexion and involvement of the proximal muscles of the upper limbs 5-9 years after the disease onset. Respiratory and cardiac functions were not affected.

Ataxia-telangiectasia (A-T) is a rare autosomal recessive disorder due to mutations in the ATM-gene. The classical phenotype is characterized by progressive childhood-onset cerebellar ataxia, oculomotor apraxia, telangiectasias of the conjunctivae, hypersensitivity to ionizing radiation, and immunodeficiency. We studies 14 patients, belonging to two big Bulgarian muslim pedigrees, with data of more than 25 affected from four consequent generations. In all the affected a homozygous mutation p.V2716A in ATM gene was found. The age at onset in our group varies between 14 days and 20 years. The main symptoms are dystonic and choreic hyperkinesias, more prominent in the upper limbs and the neck, dystonic disarthria and dysphagia. The clinical course was very slowly progressive. Brain imaging was normal.

CMT 4D, caused by a founder mutation in NDRG1- gene, was described for the first time in Bulgarian Roma patients. This is a demyelinating form of polyneuropathy with an early onset in the first decade of life, hearing impairment and loss of ambulation before the age of 50 years. IVS8-1 G>A and IVS6-2 T>G mutations in NDGR1- gene in homozygous or compound heterozygous state were found in Bulgarian Muslim patient with variable clinical phenotype in terms of severity.

**Keywords:** AR disorders, founder mutations, Bulgarian Muslims

## OP-42 **NPC1 AND NPC2 GENE ANALYSIS IN SERBIAN PATIENTS WITH NIEMANN-PICK DISEASE TYPE C**

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**Introduction:** Niemann-Pick disease type C (NP-C) is a lysosomal lipid storage disorder caused by mutations in either the NPC1 gene (in 95% of cases) or the NPC2 gene. It is an autosomal recessively inherited disease most commonly characterized by hepatosplenomegaly and a severe progressive neurological dysfunction with the age of onset ranging from early infancy to adulthood. The large majority of mutations in NPC1 gene are missense mutations (70%), while nonsense mutation (E20X) appears relatively frequent in NPC2 gene.

**Methodology:** This study evaluated 142 Serbian adult patients with symptoms that meet the NP-C criteria, and their relatives. Our algorithm for genetic testing involved 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 and than in negative cases sequencing of all five exons of NPC2 gene was performed. All analyses were performed on ABI 3500 genetic analyzer.

**Results:** Genetic testing revealed 14 patients to be either compound heterozygotes (n=13) or homozygote (n=1) for mutations in NPC1 gene. Eight missense mutations, one nonsense mutation, one small deletion, one intronic mutation were found in NPC1 gene, alongside with one large deletion of exons 6 to 9 that were detected by Centogene (Rostock, Germany). Only one heterozygous 5\' donor splice-site mutation was detected in NPC2 gene. All mutations are previously described. Heterozygous mutations were identified in 22 relatives of patients. Missense mutations constituted the majority of detected mutations (~ 85%).These results signify the importance of NPC1 sequencing as standard genetic test in NP-C. Our findings better characterize NP-C in Serbian population and facilitate future studies into genotype-phenotype correlations.

## OP-43 **GENETIC FORMS OF AMYOTROPHIC LATERAL SCLEROSIS IN BULGARIA**

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**Introduction:** Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease of the nervous system, whereby most of the cases are dominantly sporadic while about 7% are familial genetic forms with mutations. Until now, 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.

**Objectives:** Since 1990 we have established a hospital register for ALS patients, more than 1000 sporadic and 10 familial cases. 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. Genomic DNA after signed consent form of the probands and their family members was isolated from a blood sample or a saliva swab. Genetic testing of seven ALS-associated genes (SOD1, FUS, ANG, TAR-43, UBQLN1, C9orf72, TBK1), was performed by Sanger sequencing or repeat analysis.

**Results:** Three familial cases do not have DNA test. Three families have proven mutations In the SOD1 gene: Leu106Val, Leu144Phe and Leu38Val. Three families have a G4C2-repeat expansion in the C9orf72 gene. Among the sporadic patients, one patient carried the Cys146X mutation in the SOD1 gene, one sporadic case carried the repeat expansion C9orf72 - G4C2 and one was with a polymorphism (Pro440Leu) in the UBQLN2 gene. One family has mutation in TBK1 gene. Genetic clinical correlations are discussed.

**Conclusion:** Our data presents the mutations found in the Bulgarian ALS population. Maintaining a register allows some sporadic cases to be verified as familial when further relatives from the same family exhibit the disease symptoms or when a follow up of the next of kin and children take place.

## OP-44 **NGS SEQUENCING IN SERVICE OF NEUROGENETICS IN BULGARIA**

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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 and the testing of all contributing genes was challenging, if at all possible. The implementation of the technology for targeted next generation sequencing (tNGS) provided new possibilities for diagnostic genetic testing in neurogenetics.

Twelve patients with neurological diagnosis were referred in 2016/2017 to the Laboratory of Genome Diagnostics for performing tNGS. The analysis was implemented on MiSeq Illumina with TruSight One kit. Seven patients were with differential diagnosis epilepsy, encephalopathy, mental retardation. The rest were referred to the Laboratory with the following diagnosis: myotonia, cataract-ataxia syndrome, Noonan – Costello syndrome, polyneuropathy and a patient with complex phenotype and no differential diagnosis. In some of the patients aCGH and/or mitochondrial genome analysis have been previously performed and results were negative. In half of all analyzed patients with tNGS genetic diagnosis could be made, so the overall detection rate was 50%. Thanks to the genetic testing a clarified diagnosis was made in two of the patients and changed in four of them. In two patients referred with epilepsy, the diagnosis were changed to hyperprolinemia type I and Allan-Herndon-Dudley syndrome. The diagnosis of the patient with Noonan – Costello syndrome was changed to cardiofacio cutaneous syndrome. We discovered a rare case of association of polyneuropathy with Klinefelter syndrome, confirmed with cytogenetic analysis.

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.

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## OP-45 **CHARCOT-MARIE-TOOTH (CMT): ETHNIC DIFFERENCES, GENETIC AND CLINICAL SPECTRUM IN BULGARIA**

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**Background and aim:** 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:** Our cohort consists of 831 patients with genetically confirmed diagnosis of CMT. All the patients have undergone neurological, neurophysiological, and genetic testing.

**Results:** The distribution of patients with genetically confirmed diagnosis among the different ethnicities is: 542 Bulgarians, 258 Roma

and 31 Turks. We diagnosed 16 different types of CMT, caused by mutations in the following genes: PMP22, MPZ, GJB1, YARS, MFN2, NDRG1, CTDP1, HK1, GDAP1, SH3TC2, HINT1, BSCL2, GARS, HSP22 and IGHMBP2. Variety of inheritance patterns was detected: AD CMT account for 55% of all genetically confirmed cases, AR CMT was verified in 33%, and X-linked mode of inheritance was found in 6% of the cases. The clinical spectrum consists of sensory-motor neuropathies, predominantly motor neuropathies and neuropathies with neuromyotonia. The most frequent form in Bulgarians is CMT1A (52.2%), followed by dominant intermediate CMT (DI-CMT), caused by mutations in YARS gene (11.8%). CMT4D was found in a religious minority of Bulgarian Muslims that until recently was described only in Roma patients. Among Roma the most common hereditary peripheral neuropathies are CMT4D (49.6%), CCFDN (31.4%) and CMT4G (12.2%). Mutations in GJB1 gene account for half of the Turks diagnosed with CMT.

**Conclusion:** Significant genetic variety of CMT was established. Several rare forms of CMT were determined in the country. In Bulgarians autosomal dominant forms (86.3 %) are more frequent, while in Roma predominate autosomal recessive forms (95.3 %). These results contribute to the knowledge of genetic spectrum of CMT in Bulgarian population and could give clues to clinicians which genes to test depending on the ethnicity of the affected.

## OP-46 **THE GENETIC EPIDEMIOLOGY OF HEREDITARY SPASTIC PARAPLEGIAS IN BULGARIA**

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**Introduction:** The hereditary spastic paraplegias are a group of clinically and genetically heterogeneous neurodegenerative disorders, that present with progressive lower limb spasticity and weakness, causing gait impairment.

HSPs can be classified into autosomal dominant, autosomal recessive and X-linked forms, depending on their mode of inheritance. Also, based on whether spastic paraplegia appears on its own or in combination with additional neurological signs, they are further classified into 'pure' and 'complicated' forms.

HSP affects males and females of all ethnic groups from around the world, with more than 80 genetic types. The most common AD-HSP worldwide is SPG4, followed by SPG3A, while SPG11 is the most frequent AR-HSP, followed by SPG15.

**Aim:** To describe the genetic epidemiology of HSP among the various ethnic populations in Bulgaria.

**Materials and methods:** We performed genetic testing in 269 patients from 198 affected families belonging to Bulgarian, Turkish and Roma ethnic groups with spastic paraplegia syndrome. The patients also underwent neurological evaluation, magnetic resonance imaging of the brain, nerve conduction studies, neuropsychological and neuroophthalmological evaluations.

**Results:** Genetic studies confirmed the diagnosis in 99 patients from 51 families. AD-HSP was found in 31 families, with mutations in the spastin gene (SPG4) in 27 families and atlastin gene mutations (SPG3) in 4 families. AR-HSP was found in 20 families, with paraplegin gene (SPG7) mutations in 11 families, 2 families with ZFFYVE26 gene mutations (SPG15) and 7 families with mutations in the spataxin gene (SPG11), TUBB4A gene, alsin gene, NT5C2 gene (SPG45), GBA2 gene (SPG46), AP4S1 gene (SPG52) and ATP13A2 gene (SPG78) respectively.

**Conclusion:** The most common form of HSP in Bulgaria is SPG4 and is distributed among the various ethnicities, whereas SPG7 being the second most common form, has been found only in the Roma population. The second most common form of AD-HSP in Bulgaria is SPG3A. A variety of 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.

**Keywords:** Hereditary spastic paraplegia in Bulgaria, SPG4, SPG7

## OP-47 **THE ERA OF PRECISION MEDICINE – A BASIC GUIDE OF HOW TO NAVIGATE THE FIELD**

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Precision medicine defined as "the right treatment to the right patient, at the right dose, at the right time" is the newest therapeutic approach, aiming to tailor medical treatment according to each person's unique clinical, genetic, and environmental information and to replace the extremely outdated "one-size-fits all" treatment model.

Its rapid evolution in the last decade has made it a difficult field to navigate for the regular medical practitioner. Here we present the current trends in personalized medicine - from its goals, through the path of biomarker development and validation, to overview of the most widely applied, EMA approved, personalized treatments for various diseases available in Europe.

Since the advent of precision medicine is largely due to the great innovations in the field of genomics, we focus on the place of genomic technologies and genetic tests in precision medicine. We will review the basic science and principles of the various kinds of commercially available genetic tests, so medical practitioners have the ability to make informed decisions what kind of tests will be most beneficial for improving the chances of successful treatment for any of their patients.

As a result we expect to improve the understanding in the medical community of the basic principles of precision medicine; of its core technologies and approaches – their capabilities and limits; of the regulation bodies governing the field, as well as to provide reliable information sources, allowing medical practitioners to keep up-to-date with the novelties in the field.

## OP-48 **CLINICAL AND GENETIC STUDY OF HUNTINGTON'S DISEASE: THE 9 YEARS OF EXPERIENCE OF OUR TEAM**

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**Objectives:** Huntington\'s disease (HD) is a rare, neurodegenerative disorder characterized by chorea, psychiatric disturbances, and dementia. Our aim is to determine the demographic, clinical, and molecular genetics features of HD in Bulgaria and to create a local database of the patients with the disease.

**Methods:** Clinical, neurophysiological, magnetic resonance/ computed tomography imaging of brain and molecular genetic studies were performed.

**Results:** A total of 80 symptomatic individuals from 60 families were evaluated. They originate from different region of Bulgaria and belongs to the two largest ethnic groups –Bulgarian and Turkic. We have no patients from the third group – the Roma. According to the family history, 60 other members of these families also have signs of HD. Genetic testing was performed in 55 symptomatic and 9 asymptomatic individuals, and the diagnosis was confirmed in all of them. In 28 patients the gene was inherited from the mother and in 18 from the father. In 4 participants there was no known family history, and in 14 the information was missing. Motor onset was most common followed by mixed onset (motor and cognitive signs) and non-motor onset (psychiatric problems). The age at onset varied from 22 to 66 years. Most participants were taking Haloperidol for treatment of the hyperkinesis.

**Conclusions:** The phenotype, and the HD genotype, of our patients was similar to those reported in the 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 to improve the quality of life in our patients.

## OP-49 **GENETIC STUDY OF ACHONDROPLASIA IN SERBIAN POPULATION**

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**Introduction:** Achondroplasia is the most common form of short – limb short stature. The disorder is autosomaly dominant inherited with 80% of de novo mutations. Genetic signature of achondroplasia is recurrent mutation (c.1138G>A, p.G380R) in gene encoding for fibroblast growth factor receptor 3 (FGFR3), found in more than 98% of cases. According to literature, in another 1% cases mutation FGFR3 (c.1138G>C, p.G380R) is detected, while few other neighboring changes in the same gene are observed extremely rare. The aim of our study was to investigate incidence of these mutations in Serbian cases suspected of achondroplasia, and to establish genetic testing of this disorder in our country.

**Methodology:** Our study comprised nine children postnataly diagnosed with achondroplasia – like short stature, and six cases of prenatal diagnosis. One prenatal diagnosis was in affected pregnant woman previously confirmed for (c.1138G>A, G380R) mutation; in another cases fetal growth retardation (FGR) suspect to achondroplasia was observed. After DNA extraction from appropriate material, the target region of FGFR3 gene was PCR amplified and direct Sanger sequencing on ABI 3500 genetic analyzer was performed.

**Results:** FGFR3 mutations were found in five out of 15 cases suspected to achondroplasia. FGFR3 (c.1138G>A, p.G380R) mutation was detected in three postnatally diagnosed children and in one fetus with FGR. In one case of postnatal diagnosis, a rare FGFR3 (c.1123G>T, p.G375C) mutation was found. All detected mutations were in heterozygous state. No mutation found in prenatal diagnosis of affected woman, and healthy child was born.

**Conclusion:** In our study we state that 80% of patients affected with achondroplasia in Serbian population have FGFR3 (c.1138G>A, p.G380R) mutation. A very rare FGFR3 G375C mutation we have detected in one of patients confirming the heterogeneity of the genetic basis of this disease in our population.

## OP-50 **MOLECULAR DIAGNOSTICS AND GENETIC SCREENING OF PRIMARY IMMUNE DEFICIENCIES**

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Molecular genetics techniques are an essential diagnostic tool for primary immunodeficiency diseases (PID). The latest represent a heterogeneous group of inborn defects of the immune system that result in recurrent and severe infections. Although the condition is rare, early data from pilot screening programs suggest that one in 30,000–50,000 infants may be born with severe combined immune deficiency (SCID). The clinical complications and the associated economic burden of a delayed diagnosis, as well as the potentially fatal outcome if not treated promptly, emphasize the importance of early identification of the affected new-borns. Population-based screening is the only means to detect SCID before the onset of life-threatening infections in most patients. The benefit of a neonatal screening for SCID is best exemplified by the extremely effective treatment for this condition, with approximately 90% survival following hematopoietic stem cell transplantation before 3-6 months of age. Here we present a proposal for National pilot SCID screening project based on detection of T-cell receptor excision circles (TRECs) on dried blood spot (time-resolved fluorescence resonance energy transfer based detection technology). This project is a logical continuation of PID molecular diagnostics progress in our department. Presenting a review on our studies that have investigated mutations among patients with different types of PID we demonstrate variety of strategies for molecular diagnostic: from Sanger sequencing of candidate genes to Next-generation sequencing technology.

## OP-51 **CARDIAC INVOLVMENT IN PATIENTS WITH HEREDITARY TRANSTHYRETIN AMYLOIDOSIS ASSOCIATED WITH GLU89GLN MUTATION AND ITS IMPACT ON PROGNOSIS**

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Hereditary transthyretin-related amyloidosis (ATTR) is a rare genetic disease with autosomal dominant inheritance, caused by a mutation in the transthyretin gene. Two main phenotypes are described - neurologic and cardiac.

**Aim:** The aim of the study was to evaluate cardiac involvement in patients with ATTR with the most common mutation in our country (Glu89Gln) and its impact on prognosis.

**Patients and methods:** 63 patients, (29 males) at mean age 58±7 years with genetically verified Glu89Gln mutation were included in the study. A clinical examination, 12-lead ECG, and Echocardiography were performed. The patients were followed for 31 months on average, in a range from 1 to 72 months.

**Results:** Cardiomyopathy and peripheral polyneuropathy were present at diagnosis in all evaluated patients. A significant increase in wall thickness of both left and right ventricles (septum – 17,9±3,4 mm; posterior wall – 17,2±2,8 mm; RV free wall – 8,2±2,0 mm) was found with restrictive left ventricular (LV) filling pattern in 25 (39,7%) patients and a reduced LV ejection fraction in 12 (19%) patients. Pathological ECG was present in 51 (81%) patients, most common being low voltage in 22 (34,9%), pathological Q wave in 34 (53,1%), first degree A-V block in 15 (23,8%), left anterior fascicular block in 24 (38,1,3%), atrial fibrillation in 6 (9,5%). 15 deaths (24%) occurred during follow up (10 patients with advanced heart failure and severe LV dysfunction, 2 patients from ischemic stroke, 3 patients died suddenly). 1 (1,6%) patient suffered from non-fatal ischemic stroke, 19 (30,2%) had symptoms of worsening heart failure, 3 (4,8%) patients were with new onset atrial fibrillation (AF) and one patient with A-V block III degree.

**Conclusion:** A significant cardiac involvement was found in the evaluated patients with ATTR associated with Glu89Gln mutation. Heart failure and rhythm and conduction disturbances were the main causes of death.

## OP-52 **TRANSTHYRETIN FAMILIAL AMYOLOID POLYNEUROPATHY IN BULGARIA (TTR-FAP)**

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**Background:** TTR-FAP is a rare autosomal-dominant disease found all over the world due to a mutation in the TTR gene, sequenced in 1985, with more than 100 point mutations with variable penetrance. Val30Met is the most often all over the world accepted as endemic.

**Aim:** To present current data about the epidemiology of the disease in Bulgaria: different mutations have been found, formed endemic focuses for every one of the mutations.

**Methods:** Patients suspected for TTR - FAP were clinically and instrumentally tested for symptoms of the systems essential for the diagnosis and subsequent DNA sequencing in enough clinical reason.

**Results:** Some of the mutations have expressed endemic focuses. The mutation and family numbers are: 64 with Glu89Gln. The patients originate from a 200 kms lenght focus in the south-westernt part, long along the current political border between BG & MK from Dupnitza to Petrich and Melnik cyties. 7 with Ser77Phe originated extremely only from v. Vakarel and its surrounding. 9 with with Val30Met. It is „rare" till now. The spread of this mutation is a little bit scattered, with small focus in the region of Smolyan & Blagoevgrad city, might be region of Plovdiv city and south Bulgaria (Rodopa mounthin region). Three families with Val30 originated from regions now in northen Greece. 2 with Gly47Glu and 1 with Ser52Pro, originated from regions of north BG. All patients have bulgarian enthnicity except those with Gly47Glu, who are gypsies. Different mutations expres clinically-genetic correlations.

**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 in neighbor countries.

## OP-53 **ХОМОЗИГОТЕН MYD88 ДЕФИЦИТ – СЪОБЩЕНИЕ НА СЛУЧАЙ И ОБЗОР НА ЛИТЕРАТУРАТА**

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Автозомно рецесивният дефицит на Миелоидния Диференциращ фактор 88 (MyD88) е рядка форма на първичен имунен дефицит, засягащ Toll-like рецептор и Интерлевкин 1- рецептор - медиирани компоненти на вродения имунитет. Описан е през 2008г. и протича с рецидивиращи пиогенни инфекции, причинени от ограничен спектър бактерии и със слаб възпалителен отговор на макроорганизма.

**Цел:** Представя се първия у нас генетично потвърден случай на хомозиготен MyD88 дефицит.

**Описание на случай:** Касае се за дете от мъжки пол, родено доносено от шеста неусложнена бременност чрез секцио. Инфекциозните прояви стартират от едномесечна възраст с вирусен ентероколит, последван от сепсис с чернодробна недостатъчност и ДИК синдром на двумесечна възраст, тежък миоперикардит на 9-месечна възраст, пневмония с рецидив на перикардита на едногодишна възраст, изява на хепатомегалия с оформени огнищни лезии в черния дроб – на 18-месечна възраст, рецидивиращи супуративни лимфаденити, двустранен мастоидит, грануломатозен менингоенцефалит. Изолирани причинители са E. faecium и S. aureus. Лабораторните изследвания установяват левкоцитоза с умерена до тежка еозинофилия, тромбоцитоза, изразена възпалителна констелация и хипергамаглобулинемия с високи IgE до 1089 g/l, намаляващи при овладяване на инфекциите. Чернодробната биопсия демонстрира грануломатозен тип възпаление с централна некроза и наличие на хифи. След изключване на паразитни заболявания, туберкулоза, грануломатозен хепатит, други по-чести форми на имунен дефицит, чернодробен тумор е проведен NGS с панел за първичен имунен дефицит във Виена, Австрия, който потвърждава MyD88 хомозиготен дефицит. Описва се терапевтичния подход и се дискутират различията в клиничното протичане и етиологичния спектър на инфекциите в сравнение със съобщените в литературата серии.

**Заключение:** Съобщаваме рядка причина за първичен дефект във вродения имунитет, протичащ с необичайно тежки бактериални инфекции и в един етап от хода на болестта дори мимикиращ чернодробен тумор. Диагностицирането му е възможно единствено генетично поради дори компенсаторно стимулирания клетъчен и хуморален имунитет, определен чрез стандартните имунологични тестове.

## OP-54 **НОВИ ВЪЗМОЖНОСТИ ЗА ПЕРСОНАЛИЗИРАНА ПРОФИЛАКТИКА ПРИ ПАЦИЕНТИ С ХЕМОФИЛИЯ А С ADVATE®**

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Профилактиката с коагулационен фактор VIII е с доказана ефективност за снижаване на честотата на кървене при пациенти с хемофилия А, но въпреки това, по-голяма част от тях продължават да получават кръвоизливи. Прилаганите понастоящем стандартни профилактични режими са базирани на килограм т.м. и не отговарят на реалните нужди и очаквания на пациента.

Презентацията е обзор на персонализирания подход към съвременната профилактика, включващ оценка на комплекс от параметри (възраст, фенотип на кървене, индивидуална фармакокинетика (ФК), ставно здраве, съпътстващи заболявания и придържане към терапията). Представени са възможностите за повишаване на ефективността чрез електронното медицинско устройство на Shire – myPKFIT. Анализирани са резултати от единственото засега рандомизирано проучване за оценка на ефективността и безопасността на ФК-индивидуализирана профилактика при пациенти с тежка хемофилия А с рекомбинантен коагулационен фактор VIII (rFVIII) Advate®. Дискутира се превъзходството на персонализирания подход и софтуерната му версия като клинична алтернатива на стандартния профилактичен режим.

## OP-55 **ФЕЙБА-40 ГОДИНИ ПРИЛОЖЕНИЕ ПРЕГЛЕД НА ТРОМБО-ЕМБОЛИЧНИТЕ УСЛОЖНЕНИЯ ПРИ ПАЦИЕНТИ С ВРОДЕНА ХЕМОФИЛИЯ**

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Feiba® е регистриран през 1978 г. продукт. От тогава досега са проведени десетки клинични изпитвания, които доказват терапевтичната ефективност, ефикасност и безопасност на продукта. Редица постмаркетингови проучва-

ния и мета-анализи доказват ползите и предимствата на FEIBA при индикациите, за които продуктът е разрешен. Тази презентация обобщава всички спонтанни и описани в медицинската литература случаи на тромбоемболични усложнения, възникнали при прилагането на Feiba при пациенти с вродена хемофилия, документирани в глобалната база данни на Shire за безопасност/ Shire's global safety base/.Повече от 7 милиарда международни единици Feiba (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.). Докладваната честота на тромбоемболични усложнения е сравнима с публикуваните до момента данни и потвърждава профила на безопасност на Фейба 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.

## OP-56 **FEIBA – НАДЕЖДНИЯТ ПАРТНЬОР В ХЕМАТОЛОГИЧНАТА ПРАКТИКА ПРИ ВРОДЕНИ И ПРИДОБИТИ ИНИХИБИТОРНИ ХЕМОФИЛИИ – ОБЗОР НА КЛИНИЧНИТЕ ПРОУЧВАНИЯ**

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FEIBA е човешки плазмен протеин, заедно с активност заобикаляща инхибитора на ф VIII. Принадлежи към т.нар By pass – лекарствени продукти и е регистрирана в над 60 страни. Индикациите на FEIBA включват, както овладяване на кръвоизливни епизоди при вродена и придобита инхибиторна хемофилия А и В, така и тяхната профилактика за продължителен период от време, вкл при имунотолерансна терапия.

Разгледаните са резултатите от богата клинична програма проучвания , обхващаща значителен брой пациенти и и кръвоизливни епизоди в над 20 международни центрове . Получените резултати в проучванията и данните от клиничната практика са аналогични и еднопосочни: висока статистически достоверност по отношение на ефикастността и ефективността при овладяването на кръвоизлвините епизоди, както в профилактичен режим или оп– demant приложение. Резултатите от 2 проспективни, рандомизирани, клинични проучвания дават неоспорими доказателства, че профилактика с FEIBA 3 - 4 пъти седмично значимо понижава общата честота на кръвоизливите и ставното кървене, като ограничава развитието на нови таргетни стави в сравнение с лечението при нужда. По отношение на безопастността - към днешна дата няма съобщения за трансмисия на вирусите на hepatitis A, B, и C или HIV инфекциия, дефинитивно свързани с FEIBA. Анализът от приложението на над 7 билиона единици FEIBA при над 2 000 000 инфузии показва красноречиво - тромбоемболичните усложнения са рядкост, а по време на Pro-FEIBA and PROOF проучванията, въобще не са наблюдавани.

FEIBA е утвърден и сигурен лекарствен продукт от десетилетия за овладяване и профилактиране на кръвоизливните епизоди при пациентите с инхибиторна хемофилия. Чрез FEIBA не само се подобрява качеството на живот , но и се осигурява оптимална семейна, професионална и социална реадаптация на тази изключително малка група пациенти.

## OP-57 **FIRAZYR® (ICATIBANT) FOR ON-DEMAND TREATMENT ACUTE ATTACKS OF HAE**

*Beleva-Popova T*

Shire

Firazyr is indicated for symptomatic treatment of acute attacks of HAE in adults with C1-INH deficiency. Firazyr is a selective competitive bradykinin B2 receptor antagonist. Firazyr 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. The efficacy and safety of Firazyr for the treatment of acute HAE attacks were evaluated in three multicentre, double-blind, randomised, controlled Phase III trials: FAST-1, FAST-2 and FAST-3. Firazyr started working within an hour to relieve painful HAE attacks. Firazyr one-dose efficacy brings symptom relief in 9 out of 10 patients. Firazyr is indicated for subcutaneous treatment for acute HAE attacks in adults, licensed for self-administration.

# OP-58 **IDENTIFICATION OF CYP2C19*2 ALLELIC VARIANT IN HEALTHY ALBANIAN POPULATION**

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**Background:** The polymorphic P450 isoenzyme, CYP2C19 metabolize many important drugs, such as omeprazole, colpidogrel, mephenytoin, diazepam, citaloparam, imipramine, amitriptyline and clomipramine. Some allelic variants, which modify the enzymatic activity are found in human populations, as CYP2C19 *2 and CYP2C19 *3. This polymorphisms gives rise to important inter-individual and interethnic variability and plays a clinical role in therapeutic agents effectiveness. CYP2C19*2 allele, is a poor metabolizer (PM) allele and was found in Balkan at various frequencies from 8 to 16%. The aim of this study is to determine the frequency of CYP2C19 genotype and CYP2C19*2 allele frequency and to compare the data provided with previous findings in other Caucasian populations. Identifi cation of CYP2C19*2 allele frequency in Albanianpopulation represent new genetic data, that complete the puzzle of Balkan region for CYP2C19 gene variants.

**Methods:** We analyzed 100 unrelated, healthy Albanian individuals randomly selected from blood donors. In our study we have male and female in equal proportion aged 37±14.48 years old. The polymorphism of CYP2C19 was carried out by the molecular technique PCR-RFLP based in DNA amplification (PCR) and digestion by restriction enzyme SmaI.

Results: By analyzing CYP2C19 gene, 59 subjects (59%) were homozygous for CYP2C19*1 allele, one (1%) was homozygous for CYP2C19*2allele 40 subjects (40%) were heterozygous carriers of the two alleles. The frequency of wild type allele CYP2C19 *1 was found to be 79.0% while the frequency of CYP2C19*2 allele was found to be 21.0%.

**Conclusions:** These results suggested a particular pattern of CYP2C19*2 allele in Albanian population. In our knowledge the frequency of CYP2C19 *2 allele in Albania is the higher found in European populations. About 1/3 of Albanian population are intermediate and poor metabolizers which should be taken in consideration by clinicians in determining the drug dosage of their patients based in genotyping of CYP2C19gene.

**Keywords:** drug metabolism, CYP2C19, genetic polymorphism, Albanian population

# POSTER PRESENTATIONS / РЕЗЮМЕТА НА ПОСТЕРИ

## PP-01 MULTIDISCIPLINARY APPROACH AND SPEECH, OCCUPATIONAL, AND PHYSICAL THERAPY IN A CASE OF SYNDROMA JOUBERT

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**Background and aims:** Purpose: 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.

**Methodology:** 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 nerous 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.

## PP-02 THE CASE OF THE TERMINAL DELETION OF LONG ARM OF THE CHROMOSOME 3

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Last years we have seen an increase of the number of patients with complaints complaining of mental, physical retardation and speech delay who get an primary medical and genetic consultation in specialized Center of Medical Geneticsof the National Children\'s Specialized Hospital \"OKHMATDYT\". Among different syndromes which we detect in process of working, there are approximately 50% - aneuploidy, 30% - microdeletion syndromes confirmed by fluorescence hybridization in situ, and 20% - unbalanced chromosomal rearrangements.

**Purpose:** to improve the diagnostic of different microdeletion syndromes during genetic examination.

**Methods:** clinical, genealogical, standart chromosome analysis.

**Results:** The proband (child, 19 month) with complaints on chronic pneumonia, which was resist to the treatment, retardation of psychomotor development and difficulty feeding 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 - 5370 g (-4 SDC), body length - 73 cm (-2 SDC), head circumference - 38 cm (-3 SDC). 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 cryptorchidism. Abnormalities of the internal organs were not found. Standart 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. it is known more than 36 cases of 3q28 monosomy, and since 2001 it is called the syndrome.

For children with coarse delay physical and mental development recommended cytogenetic screening involving molecular diagnostic methods.

## PP-03 **MOLECULAR ANALYSIS OF AZF (AZOOSPERMIA FACTOR) GENE MICRODELETIONS WITH QUADRUPLEX REAL TIME POLYMERASE CHAIN REACTION METHOD IN INFERTILE TURKISH MALES**

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**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. 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.

**Results:** This 6 infertile male patients have Y chromosome microdeletion on different regions of AZF gene. A1 and A2 patients have AZFa microdeletion. AZFa microdeletions can cause the maturation arrest.In this situation, couples can have a children with TESE method.

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. 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 I did.

## PP-04 **FCGR2A AND FCGR3A VARIANTS ARE NOT ASSOCIATED WITH RESPONSE TO RITUXIMAB IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Purpose:** 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. com. 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.

**Methodology:** 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(34-173x109/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%). DNA was isolated from peripheral blood mononuclear cells and genotyping was performed by using PCR/RFLP methods. The distribution of genotypes was compared by using a chi-squared test or Fisher's exact test.

**Results:** 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.

## PP-05 **CYTOTOXIC T-LYMPHOCYTE ANTIGEN-4 GENE POLYMORPHISM AND THE RISK FOR CHRONIC LYMPHOCYTIC LEUKEMIA IN MACEDONIA POPULATION**

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**Purpose:** 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.

**Methodology:** We have 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.

**Results:** Our results did not demonstrate significantly different CTLA-4 genotypes distribution in patients with CLL (G/G=16, A/G=53, A/A=61) comparing with controls (G/G=10, A/G=39, A/A=51), p=0.777 and p=0.464 for allele frequencies. 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).

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 it 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.

## PP-06 **TUMOR NECROSIS FACTOR GENE POLYMORPHISMS AND THE RISK FOR CHRONIC LYMPHOCYTIC LEUKEMIA IN MACEDONIA POPULATION**

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**Purpose:** 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.

**Methodology:** We have analyzed 130 patients with chronic lymphocytic leukemia and 120 healthy controls. Thirty of these 130 CLL patients have also autoimmune hemolytic anemia (AIHA). Genotyping was performed by using PCR-RFLP methods.

**Results:** Our results did not demonstrate significantly different distribution of the TNFβ+252 G/A genotypes and allele frequencies in patients with CLL (G/G=18, A/G=43, A/A=69) comparing with controls (G/G=16, A/G=35, A/A=69), p=0.764 and p=0.544. We also didn't found significant differences in the genotype distribution or allele frequencies for TNFα-308G/A between CLL patients (G/G=97, G/A=29, A/A=4) and controls (G/G=95, G/A=23, A/A=2), p=0.612 and p=0.318.

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. In conclusion, 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 are more frequent in CLL patients with AIHA suggesting that these two polymorphisms may predispose to the development of AIHA in CLL patients.

## PP-07 **BETA-1 ADRENERGIC RECEPTOR GENE POLYMORPHISMS WITH OBSTRUCTIVE SLEEP APNEA SYNDROME IN EAST OF TURKEY**

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**Aim:**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.

**Results:** 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.

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 ($P < 0.05$).

**Keywords:** ADRB1 gene polymorphisms; Obstructive sleep apnea syndrome Body mass index; Restriction fragment length polymorphism; Turkish population;

## PP-08 **SHORT-BOWEL SYNDROME IN THE NEONATAL PERIOD**

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Short-bowel syndrome is a disease, clinically characterized by malabsorption, diarrhea, steatorrhea, fluid and electrolyte imbalance and malnutrition. The outcome in all cases of the syndrome is functional or anatomical loss of large segments of the small intestine, resulting in serious compromise of the absorptive capacity. We present a brief review with physiology of the small and large intestines, etiology of the syndrome, adaptation, treatment, complications and prognosis. We report six clinical cases from our practice. Five of the children are prematurely born in the 33rd to 35th gestational week, one is delivered on term. In five of the children there is an anatomical defect: in two - gastroschisis, the other three - small intestinal atresia at different levels with a subsequent microcolon. The sixth child is premature, developing a complication in the early neonatal period - necrotizing enterocolitis with small intestine perforation. After the first surgical intervention in all infants, adaptation is problematic, enteral nutrition - incomplete, parenteral nutrition - life-saving. Complications are recorded. A clinical picture of malnutrition and severe energy deficiency is developed. Four of the patients died in early infancy without reaching a second stage of surgical intervention. Two children survive and receive a surgical correction, one of which is diagnosed afterwards in childhood with cerebral palsy and cystic fibrosis, and the other child develops to date without any problems.

## PP-09 **DIAGNOSIS OF MYELOPROLIFERATIVE NEOPLASMS USING MOLECULAR METHODS FROM SINGLE CENTRE EXPERIENCE**

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Myeloproliferative neoplasms (MPNs) are clonal disorders of the multipotent hematopoetic stem cells which result in uncontrolled produce of the myeloid line of cells. This group of diseases consist of the following entities: Chronic myelogenous leukemia (CML),

Polycytemia vera (PVR), 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.

The aim of this study was evaluation of the hematological, biochemical and molecular diagnostic parameters in patients with MPNs.

We have analyzed hemoglobin level, erythrocytes, leukocytes and thrombocytes count in 143 patients (63 men and 80 women) with median age of 61 (range 16-88) diagnosed with MPNs in our Clinic in the last 5 years. We have evaluated LDH values and LAP score in all patients. The presence of BCR-ABL oncogene was investigated by RT-PCR methods. JAK2V617F mutation was analyzed by allele-specific PCR and confirmed with RFLP method.

The highest haemoglobin values and erythrocyte count were detected in PV patients. The leukocyte count was highest in CML patients. The highest values of trombocytes were determined in ET patients. High LDH values were more common in patients with CML and PMF. Low LAP score was specific for CML patients. Molecular analysis confirmed the presence of BCR-ABL oncogene in all CML patients. B3/a2 transcript was present in 64,3%, while b2/a2 was present in 35,7% of CML patients. . JAK2V617F mutation was positive in 62,4% of patients with BCR-ABL negative myeloproliferative neoplasm JAK2V617F mutation was detected in 86,4% of patients with PV and 59,2% of patients with ET.There was a significant correlation between the diagnosis and the presence of JAK2617F mutation. In conclusion, the molecular testing of bcr/abl oncogene and the JAK2V617F mutation are mandatory for the diagnosis and the therapy of patients with MPNs.

## PP-10 **CYTOTOXIC T-LYMPHOCYTE ANTIGEN-4 GENE POLYMORPHISMAND THE RISK FOR TYPE 2 DIABETES IN MACEDONIA POPULATION**

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**Purpose:** Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a CD28 homologue which plays an important role in negative regulation of T cell response. It is transitly expressedon 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 andType 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 thisCTLA-4 polymorphism with the risk and susceptibility for type 2 diabetes in Macedonia.

**Methodology:** We have examined this CTLA-4 polymorphism in 95consecutive patients with type 2 diabetes and 120 healthy controls. Genotyping was performed by using PCR-RFLP methods.

**Results:** Median age of our patients with type 2 diabetes was 64.8±7.6 years and 43 (41%) were males. Median duration of diabetes was 12.5±5.8 years. Number of patients on insulin at the moment of analysis was 43(45%). Median duration of insulin treatment was 5.6±5.1 years. Average HbA1c was 8.5±2.0%. Number of patients with diabetic retinopathy was 54(57%).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 patientswith type 2 diabetes and controlsin Republic of Macedonia.

## PP-11 **METABOLISM OF VITAMIN D SYSTEM IN CHILDREN WITH AUTISM SPECTRUM DISORDERS**

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**Introduction:** The frequency of autistic spectrum disorders (ASD) over the past 5 years has increased from 1:110 to 1:68 and continues to grow. An important role in etiopathogenesis of ASD is given to metabolic disorders, in particular, to the disorder of metabolism of vitamin D system, which plays one of the leading roles in maintaining the epigenetic health of the organism.

**Purpose:** To study the state of vitamin D metabolism in children with ASD for the development of examination algorithms and pathogenetic treatment.

**Results:** We examined 130 children: 86 with ASD and 44 neurotypical. The polymorphism BsmI of the VDR gene was studied: bb (pathological homozygote) – 13 (15.20%) and 0 (0%), respectively, Bb - 43 (50.00%) and 26 (59.09%), BB - 29 (33.72%) and 18 (40.90%). 95% of children with ASD showed a decrease of 25-OH-D level in the blood, whereas in the control group in only 9% cases. polymorphic variants of the genes mutilation cycle were studied: an increase in the frequency of MTHFR 677 C/T polymorphisms (47.67% and 36.36%, respectively), MTHFR 677 T/T (9.30% and 6.82%), MTRR 66 G/G (31.40% and 25.00%), MTR 2756 A/G (44.19% and 34.09%).

**Conclusions:** The specific weight of vitamin D3 deficiency in children with ASD occupies one of the leading places; diagnosis and correction of vitamin D deficiency in children with ASD should be part of the examination and treatment algorithms.

## PP-12 **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.

**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.

## PP-13 **ESTIMATION OF RELATIVE TELOMERE LENGTH IN DIFFERENT MSCS**

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**Purpose:** The aim of this study was to estimate and compare prolifretive capacity of various human mesenchimal stem cells (MSTs) by evaluating relative telomere length (RTL).

**Methodology:** For estimation of telomere length was used DNA isolated from periphral blood (PB-MSCs), umbilical cord (UC-MSCs), adipose tissue (AT-MSCs), dental pulp (SHEDs), periodontal ligament (PDL-MSCs), and bone marrow stromal cell line (HS-5) as reference sample. Using quantitative real-time PCR was determined RTL by ordering dd Ct value.

**Summary of results:** A statisticaly significant difference in RTL values was observed between various MSCs, as well as at lower ($p=0.04$) and higher ($p=0.009$) passages among analyzed MSCs. The median RTL values showed decresing trend from the lower to higher passages in the following order: from 13.03 to 10.46 in PB-MSCs, from 6.06 to 3.60 in UC-MSCs, from 3.35 to 2.08 in PDL-MSCs, from 2.3 to 1.4 in SHEDs, and from 2.14 to 1.52 in AT-MSCs. According to our data, a statisticaly higher RTL value in PB-MSCs compared to others analyzed MSCs might suggest to their greater prolifertive capacity. Contrary, a lower telomere length at MSCs from UC, PDL, SHEDs, and AT might indicate to their reduced proliferative capacity. However, shortening of telomere length probably lead to loss of self reneval potency, with specific rate in different MSCs and their passages, and that characteristic could be usefull marker in estimation of MSCs in clinical protocols.

## PP-14 **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 biohidric 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 HR 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:** Venous blood was taken from 5 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 (from 0.1 to 20 μl/ml) or L-Ascorbic acid. 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.

**Results and Discussion:** The results of the trypan blue staining test show that the administration of a 0.1 mM solution of L-ascorbic acid 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.

In the MTT test, the values obtained vary considerably, requiring more extensive research for their accurate interpretation.

## PP-15 **THE MLL REARRANGEMENTS WITH ADDITIONAL CHROMOSOMAL ABBERATIONS IN PATIENTS UNDER ONE YEAR OLD WITH AML (TWO CASES)**

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**Purpose:** Pediatric acute myeloid leukemia (AML) is a rare clinically and genetically heterogeneous disease with incidence of 7 cases per 1000000 children.

Patient's initial response to treatment and prognosis is largely determined by the presence of cytogenetic abnormalities and genetic lesions. Recurrent cytogenetic abnormalities, such as 11q23/MLL-rearrangements, predict outcome. Depending on the method of detection, the overall incidence of 11q23 abnormalities among children with AML ranges from 15% to 25%. In children younger than 2 years, the peak incidence of 11q23/MLL gene rearrangements is 50%-60%. By contrast, the incidence of 11q23 abnormalities in adults with AML is approximately 5%, and these abnormalities are rarely seen in patients older than 60 years. > 60 different translocation partners have been identified, and new partners are still being reported to add to the diversity of MLL-rearranged leukemia. Additional cytogenetic aberrations (ACA) occurred in about one-half the MLL-rearranged AML cases and have independent prognostic significance in pediatri 11q23/MLL-rearranged AML, and the mechanism underlying these prognostic differences should be studied. Considering the rarity of the disease, 2 new cases of the 11q23/MLL-rearranged AML, associated with ACA and complex karyotype (3 abberrations) in children < 2 years can supplement knowledge about this form of leukemia.

## PP-16 **RING CHROMOSOME 15 SYNDROME IN A NEWBORN BABY FROM BULGARIA – A CLINICAL CASE**

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Ring chromosome 15 /r(15)/ syndrome is a rare structural chromosomal abnormality, described for the first time by Jacobsen in 1966. Up to now less than 50 patients have been reported in the world, and to our knowledge there is no reported case in Bulgarian literature.

Ring chromosome 15 syndrome is associated with a wide spectrum of anomalies, with the common main characteristics: growth retardation (100%), variable mental retardation (95%), microcephaly (88%), hypertelorism (46%) and triangular face (42%). Excluding a few reported cases of ring (15) diagnosed prenatally, based on some ultrasound markers, most of the described in literature cases are of old patients or children at average age of 8.1 years.

We report a case of r(15) syndrome, diagnosed in a newborn female Bulgarian baby. The patient, product of the first pregnancy to 24 year old mother and 30 year old father, is born per vias naturalis at 38 weeks' gestation with low neonatal parameters: birth weight 2500g, height 43 cm. She presented dysmorphic features: microcephaly, triangular face, hypertelorism. Chomosomal analysis found karyotype 46, XX, ring (15)(p11.2-q26.3). The karyotypes of her parents were normal. The follow up of the girl till now reveals at 9 months age she has feeding difficulties and growth delay.

Most of the reported cases of r(15) are diagnosed at late age of the patients (mean age 8.1 years). We succeed to diagnose r(15) syndrome in newborn baby based on the presence of growth delay and dysmorphic features at birth. The following reexamination of patient would reveal the dynamic of phenotypic features of the syndrome over the time.

**Keywords:** ring chromosome 15, newborn baby, growth delay

## PP-17 **CHROMOSOMAL ABNORMALITIES AND Y CHROMOSOME MICRODELETIONS IN BULGARIAN MALE WITH AZOOSPERMIA OR SEVERE OLIGOSPERMIA**

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Male infertility is a complex disease with participation of many different causal factors. Genetic abnormalities are thought to play important role in multifactorial etiology of male infertility. Aim: In order to investigate the proportion of genetic causes in etiology of male infertility, genetic examination for chromosomal abnormalities (CA) and microdeletions of Yq chromosome (delYq) was carried out in Bulgarian males with severe infertility. Materials and methods: For the study period (2007-2016), cytogenetic analysis by GTG-banding was carried out in 137 infertile men (58 patients with azoospermia and 79 patients with severe oligozoospermia) and molecular testing for AZF region microdeletions of Y chromosome by multiplex PCR, was carried out in 109 infertile men (including 63 patients with azoospermia and 46 patients with severe oligozoospermia). Results: Chromosomal abnormalities were found in 16.8% (23/137) of all investigated infertile men. The frequency of CA was 20.7% (12/58) in subgroup of patients with azoospermia and 13.9% (11/79) in patients with oligozoospermia. The established frequency of delYq was 5.5% (6/109) in overall group of infertile male and higher - 9.5% (6/63) in subgroup of patients with azoospermia. No delYq was detected in subgroup patients with oligozoospermia. The overall proportion of the two genetic factors was higher 30.2% in patient with azoospermia and 14% in men with oligozoospermia. In conclusion, chromosomal abnormalities and Yq microdeletions are important genetic factors in etiology of male infertility, accounting for about 22% of cases with severe infertility in Bulgarian men. Genetic testing should be a routine part of examinations in infertile males, giving helpful information about the cause of the problem. Along with genetic counseling, by assessing the possible genetic risk they provide opportunity for the best choice of an appropriate technique for assisted reproduction of the couple.

**Keywords:** male infertility, genetic causes, chromosomal abnormalities, Y chromosome microdeletions

## PP-18 **КЛИНИЧЕН СЛУЧАЙ НА НОВОРОДЕНО МОМИЧЕ С ICHTHYOSIS CONGENITA, РЕЗУЛТАТ ОТ МУТАЦИЯ В ALOX12B ГЕНА**

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Понятието Автозомно рецесивна вродена ихтиоза (АРВИ) се отнася за група от редки заболявания на кератинизацията, класифицирани като несиндромна форма на Ихтиоза. Тази група е изключително хетерогенна, както клинично /включва Lamellar Ichthyosis, Congenital Ichthyosiform Erythroderma, Harlequin Ichthyosis и др./, така и генетично (открити са девет гена, асоцииращи с АРВИ). Най-често АРВИ се изявява клинично при раждане под формата на Collodium baby /CB/, с честота 1:50000 до 1:100000 раждания за Европейските популации. За Българската популация липсват публикувани данни относно честотата и мутационния спектър на АРВИ. Постнатални усложнения и смърт се описват съответно в 40% и 11% от случаите на CB.

Представяме случай на новородено момиче с Collodium baby. Бебето е родено преждевременно в 36-37 г.с. с опъната, лъскава кожа и малформативни стигми: ектропион, малки лошо моделирани уши, къса шия, брахидактилия с недоразвити нокти. Кожният статус показва типична за вродената ихтиоза (ВИ) еволюция: десквамация на повърхностния слой на едри ламели с подлежаща ранима кожа, образуване на дълбоки, сълзящи рагади, с лимфна течност. В следващите дни състоянието на бебето се влошава поради развилия се стафилококов сепсис и детето екзитира на 5-ия ден след раждането.

Поради бързия ход на усложненията, завършили с летален изход, при детето не е взет билогичен материал за генетична диагностика. Молекулярно-генетичният анализ при майката, започнал с директно секвениране на TGM1 гена, който е с най-голям дял в етиологията на АРВИ (по литературни данни), не установи генетични дефекти. Изследването на следващия по честота ген - ALOX12B, показа наличие на мутация c.1562A>G (в хетерозиготно състояние). Същият молекулен дефект се потвърди и при бащата.

Поставянето на точна клинична и генетична диагноза при случаи на ВИ, дава възможност за навременна и адекватна генетична консултация и ефективна профилактика (пренатална диагностика) на заболяването в засегнатите семейства.

Представеният от нас случай ще допринесе за обогатяване на знанията, относно честота и генетичния спектър на ВИ, за Българската популация.

**Ключови думи:** вродена ихтиоза, Ichthyosis congenita, Collodium baby, ALOX12B

## PP-19 **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 among 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 who meet the criteria for diagnosis of possible FXTAS.

**Methodology:** The study included 100 patients, 67 men and 33 women, with clinical picture of degenerative ataxia, tremor and Parkinsonism with 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 allels 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.

Fragile X-associated tremor/ataxia syndrome should be considered in the differential diagnosis of patients with movement disorders and dementia, especially when there is a strong family history of movement disorders/diseases from the spectrum of autism. Parkinsonism and gait ataxia may also be seen in individuals with 41 CGG expansions.

## PP-20 **THE ANALYSIS OF ENOS 4A/B POLYMORPHISM IN SURGICAL PATIENTS WITH SECONDARY PERITONITIS**

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**Purpose:** Secondary peritonitis is a peritoneal inflammation caused by perforation, necrosis or penetrant injuries of gastrointestinal tract. Although significant progress in surgical treatment and intensive care has been made secondary peritonitis still remains a life threatening condition. For identification of patients who have high risk of developing severe sepsis and multiple organ disfunction syndrome (MODS) genetic information might be of great importance. Under conditions of sepsis and endotoxemia nitric oxide (NO) is syntetized by endothelial nitric oxide synthase eNOS. NO acts as a central vasoactive supstance important for maintaining an appropriate blood flow to vital organs such are lungs, kidneys and liver. Also, NO production has been associated with the upregulation of proinflammatory cytokines. The aim of our study was to analyze the association of eNOS 4a/b VNTR polymorphism with the outcome of surgical patients with secondary peritonitis.

**Methodology:** Our study included 70 surgical patients treated at the Clinic for Emergency Surgery due to the gastrointestinal perforation and secondary peritonitis. We analyzed biochemical parameters: leukocite count, neutrophil count, serum C reactive protein level and procalcitonin level and surgical outcome: development of sepsis, severe complications and mortality. eNOS 4a/b VNTR polymorphism genotypes were detected by simple PCR method.

**Results:** The analysis of eNOS 4a/b VNTR polymorphism showed no significant association between analyzed biochemical parameters, morbidity and mortality in surgical patients with secondary peritonitis. However, when we analyzed only patients with Gram positive infections 4b/b genotype was significantly associated with ICU stay ($p=0.036$). We also observed that only patients with 4b/b genotype have developed MODS and acute respiratory distress syndrome (ARDS) but the results were not statisticaly significant. Our results suggest that eNOS 4a/b polymorphism could influence the risk of severe complications in patients with secondary peritonitis however these preliminary results should be confirmed on a larger group of patients.

## PP-21 **THE ROLE OF MOLECULAR GENETIC ANALYSIS IN THE DIAGNOSTICS OF CONGENITAL HEART DISEASES**

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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.

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 gene locus 22q11.21 responsible for DiGeorge syndrome was found. 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. 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. The first step is DNA analysis which aims to detect the most common chromosomal abnormalities: trisomy 21, 18, 13, and aneuploidies concerning the sex chromosomes. The second step is MLPA analysis for DiGeorge critical regions followed by screening for large deletions/duplications along the genes associated with congenital heart defects. In case of hypoplastic left heart syndrome we proceed with sequencing of the genes GJA1 and NKX2-5.

Moreover, by NGS we clarified two CHD cases. The first case is a patient with dilated cardiomyopathy, heterozygous for p.Gln1916X in

the MYH7 gene. The segregation analysis in the family showed the same heterozygous variant in the patient's father who died before the age of 40 with diagnosis of Emery-Dreifuss muscular dystrophy. The second case is a patient with clinical diagnosis Long QT syndrome, heterozygous for p.Ala561Val in the KCNH2 gene.

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.

## PP-22 **PRESENCE OF HIGH RISK HUMAN PAPILLOMA VIRUSES IN NONINVASIVE URINE SAMPLES WITH MOLECULAR PROFILE FLUCTUATIONS FOR PROSTATE CANCER, CYTOLOGICALLY CONFIRMED**

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The aim of the study is to investigate noninvasive urine specimens in suspected prostate cancer (PCa) patients by a combinative panel of PCA3, TMPRSS2-ERG fusions and GSTP1 promoter hypermethylation. Furthermore, we tested urine specimens for a presence of high-risk Human Papilloma Viruses (HPV) as an inflammatory cofactor in the complicated origin of PCa. A total of 44 patients with elevated PSA and/or PCa physiological symptoms were analyzed. Real-time RT-PCR, DNA sequencing, bisulfite conversion of DNA followed by PCR, cytological preparations and staining were applied. Molecular fluctuations as neoplastic GSTP1 allele, PCA3 strongly elevated expression or hyperexpression were registered in most of the patients. Only in 4 cases a positive T2-ERG status was detected. High-risk HPV types were detected in 34,1% of our urine specimens, obtained from patients at high risk of PCa based on their molecular profiles. Approximately 96% of detected high-risk HPVs are: 16, 33, 35, 31, distributed in the subgroup with highest oncogenic potential and experimentally proven association with malignant transformation of the prostatic epithelium. The estimated frequency of high-risk HPV types in control male samples with urothelial infection is significantly lower (11%).

The pathological examination on cytological slides from high-risk HPV positive urine specimens showed inflammation; variable adaptations of cellular growth and differentiation and partially viral cytopathic effect. In a proportion of patients with molecular PCa disturbed profile precancerous conditions (increased primitive cells with disturbed maturation; enlarged hyperchromatic nucleus and condensed chromatin) were found. Our molecular PCa findings, were confirmed on the cellular level with cytological findings of high grade alterations: 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.

The present study concerns novel data for Bulgarian PCa patients.

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## PP-23 **GENETIC TESTING IN COELIAC DISEASE – OUR EXPERIENCE IN BULGARIA**

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Celiac 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 celiac disease is estimated to 1% in Europe, there are no available data according precisely to the Bulgarian population.

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.

A simplified SSP-PCR (Sequence specific PCR) version has been applied by our team. HLA genotyping allows clinical risk assessment

for celiac 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.

We preformed SSP-PCR HLA genotyping of 139 individuals in total presenting with celiac related complaints. Eighty nine 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). Seventeen individuals were assigned as moderate risk patients 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.

## PP-24 **MOLECULAR DIAGNOSTIC APPROACH IN NEURO-ONCOLOGY**

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Brain tumors are among the most lethal and aggressive ones. Because of the invasive nature of central nervous system (CNS) gliomas and difficulties to resect surgically all tumor cells, relapses are frequently occurred. The current option for diagnostic based on biopsies may not reflect the heterogeneity of brain malignancies.

More than a decade, significant part of scientific efforts have been focused on the post-transcriptional gene regulation and functional role of non-coding RNAs, in particular microRNAs as a potential non-invasive biomarker.

MicroRNAs have been found in variety of body fluids like blood, urine, saliva and cerebrospinal fluid (CSF). Furthermore circulating microRNAs have also been shown to correlate with the tumor stage, subtype, recurrence and patient response to chemotherapy. Thus, circulating microRNAs represent a potential source of biomarkers for early detection and monitoring of tumor response to treatment.

In the present study, we aimed to collect Bulgarian DNA and RNA bio bank of patients' samples with different CNS cancers. Highly specific molecular method as qRT-PCR was optimized for different tissues, plasma and/or CSF. The ongoing genetic approach is focused on selection of unique microRNA expression profile panel, its optimization and normalization. Based on this approach we are planning to propose a diagnostic methodology which will be constructed for correlation between chosen miRNAs signature and differentiation of primary and metastatic tumors; their grades; probability of recurrence; drug resistance or efficacy prediction and future personalized therapy as well as virus encoded miRNAs contributing to neuro-inflammatory and malignancy process.

## PP-25 **ARSA GENE TESTING: METACHROMATIC LEUKODYSTROPHY OR ARSA PSEUDODEFICIENCY?**

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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. Direct sequencing of the ARSA gene was performed in order to screen for germline mutations in three index patients with suspected diagnosis MLD. The molecular genetic testing showed already reported ARSA deficiency mutations (c.684+1G>A; c.763G>A; p.Glu255Lys; c.1150G>A, p.Glu384Lys) and three known ARSA-PD variants (c.*96A>G; c.1055A>G, p.Asn352Ser; c.1178C>G, p.Thr393Ser). In the first patient 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 second MLD patient 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. The third patient was heterozygous only for the p.Asn352Ser ARSA-PD variant and MLD was not genetically confirmed. 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.

**Keywords:** ARSA, arylsulfatase A, MLD

## PP-26 **ROLE OF THE FTO GENE POLYMORPHISM RS9939609 IN BULGARIAN OBESE ADULTS IN THE DEVELOPMENT OF PREDIABETES**

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Diabetes mellitus (DM) is a widespread disease that has developed over the last decade as an epidemic worldwide. The number of newly diagnosed patients with type 2 DM is increasing annually. The analysis of the literature suggests a link between obesity and the development of the disorder. In 2007, FTO (fat mass and obesity-associated gene) was first discovered in a genome-wide association study (GWAS) for type 2 DM and, almost simultaneously, two other teams independently reported that the FTO gene was associated with obesity.

The aim of our study is to test if risk genotypes AA and AT of rs9939609 are more common in obese adults (BMI>25) with increased homeostasis model assessment insulin resistance index (HOMA-IR) compared to obese controls with normal HOMA-IR. Analysis was done on a total of 217 patients, 120 of them with impaired HOMA-IR. DNA samples were analyzed by PCR - direct sequencing.

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.

Our conclusion is that FTO could be one of multiple susceptibility genes for the development of impaired glucose tolerance and prediabetes in obese adults.

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## PP-27 **SCREENING OF BRCA1 AND BRCA2 GENES IN BULGARIAN OVARIAN CANCER PATIENTS**

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**Background:** According to the National Cancer Register in Bulgaria 838 women have developed ovarian cancer (OC) for 2012. Between 10 and 15 percent of these cases are caused by mutations in the BRCA1 and BRCA2 genes.

**Materials and methods:** We have screened 125 Bulgarian patients with OC for germline mutations in the BRCA1/2 genes. The mutation analysis was performed by Next Generation Sequencing (NGS) with Ion Torrent PGM System, Sanger Sequencing and MLPA (Multiplex Ligation Dependent Probe Amplification).

**Results:** Ninety six OC patients, unselected for histological subtype were screened by direct sequencing for the recurrent BRCA1/2 mutation in the Bulgarian population. Two of the mutations were found in the OC patients: c.5263_5264insC and c.9098_9099insA with frequencies of 10.42% and 1.04%, respectively.

We further analysed the coding sequences of BRCA1 and BRCA2 genes in 29 patients selected by the following criteria: high grade serous carcinoma, disease progression and platinum sensitivity. In total, 11 (37.48%) pathogenic mutations were found of which 9 in BRCA1 and

2 in BRCA2. The most prevalent mutation observed with frequency of 17.24% was c.5263_5264insC, followed by c.2019delA (3.44%), c. 5212G>A (3.44%), c.139T>C (3.44%), c.5333-1G>T (3.44%) and c.5496G>A (3.44%) in BRCA1. The other two mutations in BRCA2 c. 8059_8063delGTTCT and c.3545_3546delTT were detected with frequency of 3.90% each.

**Conclusion:** The most prevalent mutation in Eastern Europe c.5263_5264insC was prevalent among the studied Bulgarian OC patients with 11.2% (14/125) overall frequency and appeared to have a founder effect in the Bulgarian population. Altogether the seven frequent mutations in our study were responsible for the inherited predisposition of 24.13% (7/29) of the OC patients selected for family history and histological subtype. The results are relevant to clinical practice and personalized treatment of patients with OC.

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## PP-28 **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. 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. In the blood – increase level of iron, AST, ALT, lactate, methionine. In the neurological status expressed manifestations of subcortical, akinetic-rigid syndromes. Ultrasound examination – signs of portal hypertension, 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.

**Conclusion:** 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.

## PP-29 **THE CASE OF RARE DISEASE – BENIGN RECURRENT INTRAHEPATIC CHOLESTASIS (BRIC)**

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**Introduction:** Benign Recurrent Intrahepatic Cholestasis (BRIC) – is a rare genetic disorder. Characterized by causeless cholestasis, by increase of transaminases level, but liver structure and function damages are absent. Type of inheritance is autosomal recessive.

**Purpose:** To study an increase of transaminases activity, this can be of relatively benign nature.

**Methods:** Among 3000 patients with an increase of transaminases, examined in the center, BRIC syndrome is registered for the first time.

**Results:** In metabolic disorders, the liver is involved in the process at 95% of patients, so often we face with high level of transaminases. After correction of the underlying disease, liver values approach to the reference values. We give an example where treatment did not lead to the expected results.

Patient S., 15 years complained of general weakness, fatigue, and periodical skin itch.

From the anamnesis it is known that at 12 years the biochemical indices were monitored for the first time, a 10-fold increase of

transaminases level, GGT values, alkaline phosphatase were noted, bilirubin was not significantly increased and mild hepatomegaly.

A search for markers of viral hepatitis was carried out, the result was negative. Bacterial, TORCH-infection, toxic damage were also excluded. The diagnosis is: acute hepatitis of undefined etiology.

In the wake of rising, causeless periods of jaundice, itching began to arise. During the incursion period there was a high level of transaminases, alkaline phosphatase, total bilirubin, but GGT values increased slightly. Dynamics excludes autoimmune hepatitis, liver biopsy did not reveal pathological changes.

**Conclusions:** Analyzing this clinical case (wave-like course, absence of complaints during the inter-attack period, absence of liver changes with a significant increase in transaminase activity, absence of markers of viral hepatitis), we came to the conclusion that the patient had the BRIC syndrome. To search for molecular target, a mutation in ATP8B1 gene is searched.

## PP-30 **PRE- AND POSTNATAL DIAGNOSTICS OF GENOMIC DISORDERS IN CHROMOSOMES 17 AND 22**

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High resolution chromosomal microarray (CMA) is now worldwide first tier diagnostic tool for detection of genomic rearrangements, as genetic cause of developmental disorders and multiple congenital anomalies. Non- recurrent genomic rearrangements often result from reparative mechanisms such as non-allelic homologous recombination (NAHR) during meiosis.

In the present study we performed array Comparative Genomic Hybridi¬zation (aCGH) using Agilent Microarray Kits, 4x180K and 4x44K in four samples- two patients with unclear syndromic conditions and two prenatal samples of fetuses with indications for genomic disorders.

aCGH analysis revealed deletion and duplication spanning different regions of chromosome 17- 17p13.3p13.2 and 17p12. The deletion in 17p13.3p13.2 is 4.4 Mbp in size and was found in fetal material with ultrasound indication for lissencephaly. Deletions in this region are associated with Miller-Dieker lissencephaly. The duplication in 17p12 (1.37 Mbp) was detected in 29 years old boy with developmental delay, skeletal anomalies and facial dysmorphisms.

In 5 years old girl with microcephaly, developmental delay and dysmorphism 2.45 Mbp microdeletion in 22q11.21 region was detected. The aberration affects 38 genes, 11 of which are associated with disease. Additionally, 1.23Mb deletion in the same region was found in chorionic villus sample with ultrasound image of abnormal heart development. Deletions in this region are genetic cause of 22q11.2 microdeletion syndrome, while duplication are associated with 22q11.2 duplication syndrome (OMIM: 608363) and Cat Eye Syndrome (OMIM: 115470).

This study demonstrates the feasibility of aCGH for pre- and postnatal diagnosis, which will improve the subsequent genetic counseling and will clarify the risk for future pregnancies in the affected families. It is important to use more extensive genomic approaches, such as CMA in order to clarify the actual genetic causes of the growing number of syndromic conditions.

The study was supported by and DUNK 01-2/2009 of NSF, Ministry of Education and Science.

## PP-31 **MUTATIONAL BURDEN IN BULGARIAN SCHIZOPHRENIA AND BIPOLAR DISORDER PATIENTS: A BIOINFORMATICS ANALYSIS**

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**Purpose:** 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.

**Materials:** We studied the genetic burden of 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. The cohort consisted of Bulgarian BAD and schizophrenia patient samples collected in the Molecular Medicine Center as part of current and previous projects looking into the basis of psychiatric disorders. After NGS sequencing of the complete coding region of interest the frequency of mutation was corrected for the gene size and the expected intolerance of each gene to variation. The RVIS public database was used for this task.

**Results:** The result was an objective measure of mutational burden that was used to determine the most affected genes in the cohort. Using graphical exploratory analysis we defined a group of seven genes that showed more variation than expected under a linear distribution model. A GO enrichment test showed a significant enrichment of aldehyde-lyase activity related genes in the result. Three of the seven genes form or take part in voltage-gated ion channels and have been related to epilepsy or other seizure disorders. Another one of the genes has been linked to Alzheimer's Disease. These results suggest that part of the mutational burden of the studied disorders may be concentrated in ion channel proteins, similar to epilepsy.

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## PP-32 **BUDD-CHIARI SYNDROME IN A PATIENT WITH MUTATIONS IN JAK-2, FACTOR V AND MTHFR**

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Primary 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. Mutation in Factor V Leiden is detected in about 53% of patients with BCS, and is the most common prothrombotic disease associated with the disorder. Mutations in the MTHFR gene also show slight association with venous thrombosis. 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 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 factor II. The combination of these mutations is rare, with only a few patients reported in the literature. The present case illustrates the need to identify mutations in the prothrombotic factors in patients with Budd-Chiari syndrome in order to improve diagnosis and therapy.

## PP-33 **DE NOVO C.721A>C, P.(THR241PRO) MUTATION IN BRAF GENE IN PATIENT WITH RASOPATHY**

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Dysregulation of the RAS-MAPK signaling pathway has been recognized as the molecular cause underlying a group of clinically related disorders – Noonan, Neurofibromatosis type 1, Costello LEOPARD, and cardio-facio-cutaneous syndromes. Due to mutations in genes encoding RAS proteins (KRAS and HRAS), downstream transducers (RAF1, BRAF, MEK1 and MEK2) or pathway regulators (PTPN11, SOS1, NF1 and SPRED1), these disorders are defined as RASopathies. We present a case of a child with supravalvular pulmonary stenosis, mild hypertrophic cardiomyopathy, short stature, facial dysmorphism and motor development delays. Target sequencing revealed that the patient has a de novo missense heterozygous, mutation c.721A> C p.(Thr241Pro) in the BRAF gene. According to

Rauen, pathogenic mutations in BRAF molecularly define cardio-facio-cutaneous syndrome. 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.

## PP-34 **BRUCK TYPE 1 SYNDROME DUE TO A MUTATION C.831DUPC IN FKBP10 GENE IN BULGARIAN ROMA**

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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 E. u Clemens M., 1997). It is caused by homozygous mutations in FKBP10 gene on 17q21 chromosome.

The disease was found for the first time in Bulgaria among inbred Roma families belonging to Wallachian group of basketmakers in Kostenetz, Dolna Banya and Maritza, Sofia region. We identified 10 affected individuals, 6 female and 4 male. 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.

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), myasthenic reaction, ECG and echocardiography, ventillatory assessments, X-rays of thoracic spine, lung and heart, and neuro-ophthalmology assessment were performed. Blood or saliva were taken from all patients to isolate DNA and perform a molecular genetic analysis

The clinical examination in all the patients revealed short stature and neck with limited mobility, short body, pectus excavatus, pronounced right-convex thoracolumbar scoliosis with a formed gybus in the proximal thoracic spine, 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 deformations of the feet with pronounced plantar hygromy.

NCS, EMG, neuro-ophthalmological evaluation and metabolic screening were normal in the evaluated patients.

Ventillatory assessment revealed respiratory failure 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.

## PP-35 **NOVEL FORM OF COMPLICATED HEREDITARY SPASTIC PARAPLEGIA (SPG78), DUE TO MUTATIONS IN THE ATP13A2/PARK9 GENE**

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**Introduction:** Hereditary spastic paraplegias (HSP) are heterogeneous neurodegenerative disorders characterized by progressive spasticity and weakness of the lower limbs due to degeneration of the corticospinal tracts. Molecular defects in ATP13A2-gene have been associated with Kufor-Rakeb syndrome and neuronal ceroid lipofuscinosis.

**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. Neuroimaging investigations revealed pronounced vermian and hemispheric cerebellar atrophy. The affected were carrying homozygous p.Thr512Ile (c.1535C4T) mutation in ATP13A2. 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.

**Conclusion:** HSP phenotype should be added to the spectrum of ATP13A2- related neurological disorders.

**Keywords:** ATP13A2 mutations, hereditary spastic parapegia

## PP-36 **CLINICAL AND GENETIC HETEROGENEITY OF MYOPATHIES IN BULGARIA**

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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) 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. 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.

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.

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.

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.

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.

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) 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 laminin-211 lead to congenital muscular dystrophy type 1A (MDC1A), identified in 5 patients.

**Keywords:** myopathies, clinical heterogeneity, epidemiology

## PP-37 **AUTOSOMAL-RECESSIVE CONGENITAL CEREBELLAR ATAXIA IS CAUSED BY MUTATIONS IN METABOTROPIC GLUTAMATE RECEPTOR 1**

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Autosomal-recessive congenital cerebellar ataxia was identified in Roma patients originating from a small subisolate with a known strong founder effect. 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. Exome sequencing, used for linkage analysis on extracted SNP genotypes and for mutation detection, identified two novel (i.e., not found in any database) variants located 7 bp apart within a unique 6q24 linkage region. Both mutations cosegregated with the disease in five affected families, in which all ten patients were homozygous. The mutated gene, GRM1, encodes metabotropic glutamate receptor mGluR1, which is highly expressed in cerebellar Purkinje cells and plays an important role in cerebellar development and synaptic plasticity. The two mutations affect a gene region critical for alternative splicing and the generation of receptor isoforms; they are a 3 bp exon 8 deletion and an intron 8 splicing mutation (c.2652_2654del and c.2660þ2T>G, respectively. The functional impact of the deletion is unclear and is overshadowed by the splicing defect. Although ataxia lymphoblastoid cell lines expressed GRM1 at levels comparable to those of control cells, the aberrant transcripts skipped exon 8 or ended in intron 8 and encoded various species of nonfunctional receptors either lacking the transmembrane domain and containing abnormal intracellular tails or completely missing the tail. The study implicates mGluR1 in human hereditary ataxia. It also illustrates the potential of the Roma founder populations for mutation identification by exome sequencing.

**Keywords:** congenital ataxia, Roma, GRM1, exome sequencing

## PP-38 **ЗАХАРЕН ДИАБЕТ И ЛОШ АПЕТИТ – КАКВА Е ДИАГНОЗАТА?**

*Колева Р*

ДКЦ I гр.Стара Загора

Захарният диабет е 2-то 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,кодиращ 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.

## PP-39 **PROGRAMMABLE AND AUTOMATED BEAD-BASED MICROFLUIDICS FOR VERSATILE DNA MICROARRAYS FOR GENOMIC ANALYSES UNDER ISOTHERMAL CONDITIONS**

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Advances in modern genomic research depend heavily on applications of various devices for automated high- or ultra-throughput arrays. Micro- and nanofluidics offer possibilities for miniaturization and integration of many different arrays onto a single device. Therefore, such devices are becoming a platform of choice for developing analytical instruments for modern biotechnology. This paper presents an implementation of a bead-based microfluidic platform for fully automated and programmable DNA microarrays. The devices are designed to work under isothermal conditions as DNA immobilization and hybridization transfer are performed under steady temperature using reversible pH alterations of reaction solutions. This offers the possibility for integration of more selection modules onto a single chip compared to maintaining a temperature gradient. This novel technology allows integration of many modules on a single reusable chip reducing the application cost. The method takes advantage of demonstrated highspeed DNA hybridization kinetics and denaturation on beads under flow conditions, high-fidelity of DNA hybridization, and small sample volumes are needed. The microfluidic devices are applied for a single nucleotide polymorphism analysis and DNA sequencing by synthesis without the need for fluorescent removal step. Apart from that, the microfluidic platform presented is applicable to many areas of modern biotechnology, including biosensor devices, DNA hybridization microarrays, molecular computation, onchip nucleic acid selection, high-throughput screening of chemical libraries for drug discovery.

## PP-40 **RARE GENETIC VARIANTS IDENTIFIED BY NGS CONTRIBUTE TO BOTH SCHIZOPHRENIA AND BIPOLAR DISORDER**

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Schizophrenia (SCZ) and bipolar affective disorder (BAD) are complex psychiatric disorders with significant social impact, with overlapping clinical symptoms and shared genetic risk factors. Genes involved in neurotransmission, connectivity, and plasticity have been shown to contribute to BAD and SCZ, but the molecular basis of these disorders is not yet well understood. Targeted NGS of a panel of genes was performed to investigate the genetic architecture of Bulgarian patients with BAD and SCZ.

A total of 204 individuals with BAD, 131 with SCZ diagnosed based on DSMIV and ICD-10 as well as 184 healthy controls were

recruited. The samples were sequenced on the Ion Torrent PGM platform. The sequencing panel comprised of 187 genes previously identified in GWAS or linkage studies. Only samples with coverage of at least 95% of the target regions at 20x were included in the analyses. Bioinformatics tools (SIFT & PolyPhen2) were used to identify variants that were likely to be pathogenic.

In total 5373 rare variants have been detected, of which 1230 new. A total of 2826 nucleotide changes were found in patients, 1831 singletons and 995 recurrent. While the majority of the variants were detected in 43 genes, common for both in SCZ and BAD patients, there were also some singletons specific for BP (in 35 genes) and SCZ (18 genes). In total 14 rare LOF variants, 889 MS variants, 243 of which potentially damaging; 109 splice site variants and 32 in regulatory regions were discovered.

In summary, we could identify both recurrent and unique rare variants with potential functional relevance in genes associated with neurotransmission, neurogenesis, synaptogenesis and plasticity, signaling cascade pathways. The data add to the accumulating evidence of considerable overlap between the genes contributing to both schizophrenia and bipolar disorder.

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## PP-41 **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 остеопереза (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 ѝ 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.

**Методология:** 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 PCR в реално време.

**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.

## PP-42 **CLINICAL SYMPTOMATOLOGY AND GENETIC INVESTIGATION IN PATIENTS WITH LEBER'S HEREDITARY OPTIC NEUROPATHY**

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**Abstract:** 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 G11778A in MT-ND4 gene, G3460A in MT-ND1 gene and T14484C in MT-ND6 gene 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 results of genetic investigation in Bulgarian patients with LHON.

**Materials and methods:** On the basis of the clinical evaluation and genetic investigation 12 patients - 9 males and 3 females, age ranged between 7 – 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, direct ophthalmoscopy, optic - coherence tomography, evaluation of ocular motility and genetic investigation was performed in the patients.

**Results and discussion:** Seven patients from 2 unrelated families had family history of LHON and the remaining 5 patients were isolated cases in their families. 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. Visual field examination revealed the most common finding of bilateral central scotoma. In 3 patients, examined during the acute stage of optic neuropathy, ophthalmoscopy revealed an optic disc hyperemia and retinal vessels tortuosity and in the remaining 9 patients examined after the acute stage bilateral optic atrophy was found. The genetic testing revealed the following gene mutations responsible for the disease: G11778A, MT-ND4 gene in 3 patients, G3460A, MT-ND1 gene in 2 patients, G3635A, MT-ND1 gene in a family with 4 patients; G11778A, MT-ND4 gene and T14484C, MT-ND6 gene in a family with 3 patients.

**Conclusion:** Except the most common mutations G11778A in MT-ND4, G3460A in MT-ND1, T14484C in MT-ND6 in our patients, a rare mutation G3635A in MT-ND1 gene was found in a family with 4 affected. Interestingly a digenic inheritance of G11778A and T14484C was detected in another family with 3 affected. A genetic investigation of the whole mitochondrial genome is necessary in all patients suspected for having LHON.

## PP-43 **RESULTS FROM CYTOGENETIC STUDIES IN PATIENTS WITH REPRODUCTIVE FAILURE (A 20-YEAR EXPERIENCE)**

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**Background:** Among many other physiological, immunological and environmental factors, chromosomal abnormalities contribute to infertility and repeated spontaneous abortion.

**Aim:** To determine the incidence, prevalence and types of chromosomal abnormalities in patients with reproductive failure.

**Materials and method:** 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 reproductive failure (recurrent spontaneous miscarriage, missed abortion or infertility). Karyotype was established on GTG-banded metaphases on stimulated with PHA cultures of peripheral blood T-lymphocytes according to standard protocol and for each patient at least 11 metaphases were analyzed.

**Results:** A total of 113 pathologic karyotypes were identified (2.22%), 59 in men (2.29%) and 54 in women (2.15%) respectively. Most frequent (66/58.4%) from whole pathology were translocations – 58(88%) balanced reciprocal chromosomal rearrangement and 8(12%) Robertsonian, both in men (31/53%) and in women (35/65%), followed by gonosomal abnormalities (23/20.3%) – mainly numerical pathology (16/14.2%) – 47,XXX – 3, 47,XXY - 11; 47,XYY - 2. In addition - 5 cases with XX male syndrome (8.5% from pathology in male) and 2 cases with del(Y) was found. The inversions were present in 12.4% and mosaic karyotype in 0.09% of patients

**Conclusion:** 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.

**Keywords:** chromosomal abnormality, reproductive failure, infertility, cytogenetics

## PP-44 **APPLICATION OF MOLECULAR KARYOTYPING IN THE DIAGNOSIS OF 10Q MICRODELETION SYNDROME**

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**Introduction:** Terminal microdeletons are rare chromosomal aberrations leading to congenital anomalies and intellectual disabilities. Birth defects are responsible for a high percentage (33%) of neonatal mortality and are therefore a serious clinical and diagnostic problem. We present a clinical case of congenital anomalies syndrome (10q-deletion), including low stature, facial dysmorphism, clinodactyly, limited elbow extension, cryptorchidism, hyperkinesia, behavioral problems, intellectual disability, failure to thrive, heart and genitourinary system defects.

**Materials and Methods:** Microarray comparative genomic hybridization was applied in the present study. We used CytoChip Oligo (BlueGnome, Cambridge, UK) version 1.1, 4x44k., with a resolution of 70kb. The software analysis was made by BluefuseMulti v.4.2.

**Results:** We identified pathogenic terminal deletion in chromosome 10 – del(10)(q26.12q26.3), covering 12,629,370 bp.

**Discussion:** The detected pathogenic deletion - del(10)(q26.12q26.3), covers 85 HGNC and 49 OMIM genes, including FGFR2 (fibroblast growth factor receptor 2) and CTBP2 (C-terminal-binding protein 2). It is assumed that haploinsufficiency of one or more genes located in the 10q26.12-q26.13 region, may contribute to the pathogenesis of the syndrome, therefore they are considered as a serious candidate genes. The FGFR2 gene encodes a protein, with important role in cell division, regulation of cell growth and maturation, neovascularization, wound healing and embryonic development. The CTBP2 gene encodes two different proteins. The first regulates the expression of other genes, and the second is a component of synapses in the nervous system. Other associated genes are: ADRB1 (adrenergic receptor), DPYSL4 (Dihydropyrimidinase-related protein 4) encoding protein and DRD1IP (dopamine receptor-specific protein vesicular protein), respectively, in mediating the physiological effects of adrenaline and noradrenaline with a possible association with heart failure, neuronal differentiation and in mediating memory and learning processes. Accurate diagnosis through high-resolution methods allows the early application of appropriate therapy, a better assessment of recurrent family risk and adequate prophylaxis.

## PP-45 **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 the various protocols. PCR amplification was carried out on GeneAmp PCR System 9700, using the AmpFlSTR 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:** 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.

## PP-46 **CASES OF WOLF-HIRSCHHORN SYNDROME DIAGNOSED THE LABORATORY OF MEDICAL GENETICS – VARNA FOR THE PERIOD 2008-2017**

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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. 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.

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.

**Aim:** A presentation of three cases of WHS (1 boy and 2 girls), all carrying de novo 4p rearrangements, age 0–1 years.

**Methods and materials:** A high resolution GTG cytogenetic analysis (550 bands) is used in the presented cases.

**Results:** The first case is about a child, who was referred to us with a clinical diagnosis of Malformative syndrome. The cytogenetic analysis found a karyotype 46, XY, del(4)(p15.1), corresponding to Wolf-Hirschhorn syndrome. The second case was about dysmorphic face and hypotension and a karyotype 46, XX, del (4)(p15.2) was found. In the third case there was a typical malformative stigmas for Wolf-Hirschhorn syndrome and was found a larger deletion of the short arm of the 4th chromosome - 46,XX,del(4)(p14).

**Conclusion:** 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.

**Keywords:** Wolf-Hirshhorn syndrome, terminal deletion of the short arm of chromosome 4, 4p-

## PP-47 **GENETIC STRUCTURE OF ALBANIAN GIPSY POPULATION BY 15 STR IDENTIFILER MARKERS**

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**Background:** In the frame of the Projects for complete registering of Albanian Gipsy communities living in Albania, Gjenoma Laboratory have collaborated with one NGO Legal Studio, in Tirana, to perform genetic tests for paternity, maternity and familial identification using 15 STR Identifiler kit. Gipsy individuals have signed the consent form for each genetic test and the results of the tests were presented in the Civil Courts of various regions of Albania in the period 2013-2016.

**Materials and methods:** This study included 70 unrelated adult Albanian gipsy from various regions of Albania, presented to perform DNA identification tests requested by the civil courts. DNA from bucal swabs was isolated by the Chelex protocol. PCR amplification was carried out on GeneAmp PCR System 9700, using the AmpFlSTR Identifiler PCR amplification kit (Applied Biosystems, USA), according to the manufacturer's instructions. STR typing was performed in an ABI 3130 Genetic Analyser (Applied Biosystems); STRs allele calling was performed through GeneMapper IDX v1.1.1 software, using manufacturer's allelic ladders, bins and panels.

Allele frequencies and other statistical parameters commonly used in forensic and paternity tests were calculated with PowerStats software v1.2.

**Results:** The exact population differentiation test performed using Arlequine software v3.5 revealed significant differences between our sample of Albanian Gipsy and other Gipsy populations in the Balkan Region. We consider our data valuable for an Albanian Gipsy 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 Albanian Gipsy living in Albania.

## PP-48 **GENETIC MARKERS FOR IMMUNE INTOLERANCE IN REPRODUCTIVE FAILURE – A 5 YEARS' EXPERIENCE IN VARNA**

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**Aim:** During the past decades research on immunopathogenesis denotes that idiopathic reproductive failure isassociatedwithanunbal ancedmaterno–fetallimmunological tolerance. The purpose of this study was to analyze whether there is a correlation between the genetic biomarkers for immunologic intolerance in pregnant women and reproductive failure.

**Methodology:** Inthisstudy, we analyzed 82patients, referred to the Genetic counseling in MBAL "St. Marina"- Varna with the history of recurrent miscarriages. Genomic DNA was extracted from peripheral blood samples. The HLA-G 14 bp INS/DEL polymorphism (rs66554220) was amplified by PCR and visualized by electrophoresis on a 3% agarose gel. PCR products were either 224 or 210bp, or both 224 and 210bp, depending on the insertion/deletion of the 14bp in exon 8.

Genotyping of IL1A-889 C>T and IL1B+3954 C>T polymorphisms was performed by PCR and direct sequencing.

**Results:** The mean age of the patients tested was 33.2 years. All of them were tested due to reproductive failure of unknown origin. Of all 82 women,53 were homozygotes Ins/Insorheterozygotes Ins/Delfor HLA-G allele. 31 of the patients were heterozygotes C/T or homozygotes T/T for IL1A-889 and ILB-3954. These genetic biomarkers display a negative impact on the prognosis of pregnancy outcome.A 14-bpinsertion/deletion polymorphism in exon 8 could play a possible role in recurrent miscarriages, because the presence of a 14-bp insertion allele is associated with a lower HLA-G expression, thus leading to a weaker protective effect against reproductive failure.

Single nucleotide polymorphisms (SNP) located in the promoter region of IL-1 gene cluster may modulate transcription of the IL – 1 gene protein. As a result there will be lower levels of IL-1 protein production. This finding also correlates with a worse chance of a successful pregnancy outcome.

The results of this study might be of significance for clinicians and those involved inunderstanding infertility and recurrent miscarriage.

## PP-49 **GENETIC MARKERS FOR THROMBOPHILIA IN PATIENTS WITH CEREBROVASCULAR ACCIDENT AND PERSISTENT FORAMEN OVALE**

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**Aim:** The incidence of persistent voramen 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 for ascertainment of a correlation between PFO and ischemic stroke (IS).

Genotype profiling of patients with PFO was performed, seeking for association with ischemic stroke.

**Methodology:** 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). 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:** 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. 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). 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. 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.

**Conclusion:** Patients with PFO and stroke carry more often genetic mutations, associated with thrombophilia than patients with PFO without stroke, although we could not find a 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.

## PP-50 **PRELIMINARY RESULTS OF GENETIC VARIANT CYP1A2 1*F, INVOLVED IN CAFFEINE INTOLERANCE, IN ALBANIAN POPULATION**

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**Background:** 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. 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. Up to know we have not population data about the frequency of CYP1A2 1*F allele in Albania. Each ethnic population presented a particular pattern of CYP1A2 genetic variants and their identification in Albanian population could be of genetic and clinical interest. Our aim was the analysis of 100 healthy Albanians for the identification of CYP1A2 1*F allele frequency.

**Methodology:** In our preliminary study we analyzed 50 healthy individuals and used PCR- RFLP protocol, based on DNA amplification (PCR), digestion by restriction enzyme Apal of PCR products and the control of the fragments by gel electrophoresis. Using this method we determine directly the genotype for two most diffused alleles CYP1A2 1*A ( Wild type) dhe CYP1A2 1*F.

**Results:** From DNA analysis we found the genotypes and using standart formulas of population genetics we calculate the frequency of YP1A2*1A allele at 80% and the frequency of CYP1A2 1*F allele at 20%. The frequency of CYP1A2 1*F allele of 20% means that about 4% of Albanians have homozygote genotypes and should be intolerant to caffeine. Persons who demonstrated disturbs when consumed café or other drinks containing caffeine should performed the test of CYP1A2 1*F variant identification.

## PP-51 **BECKWITH-WIEDEMANN SYNDROME IN FOUR UNRELATED CASES – FROM THE GENETIC COUNSELING IN MBAL "ST. MARINA" VARNA**

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**Aim:** The authors report on 4 cases of Beckwith-Wiedemann syndrome (BWS) in patients, referred to the Genetic Counseling of the Laboratory of Medical Genetics in Varna. The aim of this study is to compare the clinical manifestation and the genetic findings between the patients.

**Methodology:** Pedigree analysis shows that all cases are sporadic. The four unrelated patients are two females and two males, aging from 2 months old to 2 years old. All of them clinically presented with sufficient, though variable clinical signs of BWS and were eligible for performing mutational analysis.

Methylation test in region KvDMR1 (gene KCNQ1OT) of chromosome 11p15.5 and gene H19DMR with MLPA analysis was performed. Indirect DNA analysis with polymorphic markers D11S1984, D11S922, TH, D11S4088 and D11S1346 was used for identifying UPD11p15.5.

**Results:** Beckwith-Wiedemann syndrome (BWS) 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 hyperinsulinsm and unusual ear creases or pits. Children with BWS may also have hemihyperplasia.

The authors' present detailed clinical information for each case and the corresponding results of the mutational analysis - abnormal methylation at region KvDMR1 (gene KCNQ1OT) of chromosome 11p15.5 (hypomethylation to demethylation), or hypermethylation of H19DMR, and in one case paternal uniparental disomy 11p15.5 with focus on phenotype – genotype correlation.

## PP-52 **GENOMIC DISORDERS IN THE CONTEXT OF PRENATAL AND POSTNATAL DIAGNOSTICS IN BULGARIA**

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**Purpose:** Routine implementation of array comparative genomic hybridization (aCGH) analysis in the diagnostics of patients with suspected genomic disorders.

**Methodology:** For the period of 1 year, 52 samples were screened by microarray-based copy number analysis in the Laboratoty of Genomic diagnostics, MUS. Among them, 20 samples were obtained from fetuses with anomalies. Most of the postnatal patients were indicated because of developmental/mental retardation, autistic spectrum disorders, epilepsy, dysmorphological features and suspected genetic syndromes. Prenatal cases had some of the following indications: multiple structural fetal anomalies (MSFA), intrauterine retardation, repeated fetal heart and brain anomalies, marker chromosome, increased nuchal translucency and other soft ultrasound markers.

**Results:** Array CGH confirmed clinical diagnosis in some of postnatal cases and changed the clinical diagnosis in one of suspected syndromes – the patient was diagnosed as having Silver-Russel syndrome, but aCGH revealed 17p12 microduplication, characteristic for Charcot-Marie-Tooth type 1A syndrome. Array CGH revealed the following results in cases with repeated fetal anomalies: 17p13.3 microdeletion in lysencephaly; 1p36 microdeletion in a syndrome of MSFA; 22q11.21 microdeletion in cono-truncal anomaly; 22q11.1 microduplication in cat-eye syndrome; combined 10q26.3 microdeletion and 17q25.3 microduplication in a syndrome of developmental delay; 22q11.21 microduplication in a case of mosaic marker chromosome of the mother. We can conclude that there is a high probability (about 30%) to reveal prenatally microstructural genomic aberrations by array CGH, in cases of recurrent anomalies.

In this presentation we discuss the disclosing of genomic results in pre- and postnatal settings, what types of results go on reports, how do we present and manage this information in the clinic, the highly focused report on fetal samples. We highlight the role of pre-test education, genetic counseling and informed consent in our communication with patients, and registries with systematic annotation of genotype-phenotype correlations regarding national level of genomic diagnostics.

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## PP-53 **GENETIC DIAGNOSTIC SURVEY ON CHILDREN WITH CONGENITAL AND HEREDITARY DISEASES FOR A PERIOD OF TWO YEARS**

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**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 of 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 applied. The children were classified according to indications for referral, indicative unit and verified diagnosis.

**Results:** The total number of children is 560(45%) out of 1246 genetically consulted patients for this period (the number of women for biochemical screening excluded). 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%). 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 clinics and 82(14,8%) were outpatients. Diagnostic genetic and metabolic investigations were performed in 536 children in our laboratory and in other genetic laboratories in Bulgaria and abroad. Based on these analyses genetically determined pathological conditions were diagnosed in 119 (22.2%) pediatric patients. The spectrum of all genetic diagnoses is being presented and analyzed.

This survey shows the importance of genetic counselling in childhood, when congenital or hereditary pathology is suspected. Genetics labs are centres for high specialized health care and are focused on rare diseases in children, which need appropriate genetic investigations and long lasting follow up.

## PP-54 **CLINICAL VARIABILITY OF CONGENITAL MYASTHENIC SYNDROME TYPE IA DUE TO MUTATION 1267DELG IN 100 CASES**

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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 muscle weakness.

**Results:** One hundred affected with genetically confirmed disease were examined. All the patients are from Roma ethnicity and 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. Depending on the clinical course three groups of patients were determined: mild clinical course - 47% (47/100); moderate clinical course - 32% (32/100); severe clinical course - 21%.

Spirometry was performed in seated and laid back positions. Results for forced vital capacity for 14 patients show more than 10% difference between seated and laid back position due to diaphragm weakness. Nine of these 14 patients have mild clinical course, 3 – moderate clinical course, 2 – severe clinical course. None of the other patients used non-invasive or invasive ventilation, or ever had respiratory crises.

Patients in our study that have bulbar weakness leading to swallowing difficulties and choking on food/drink have mostly moderate or severe clinical course.

**Conslusion:** CMS type Ia is characterized by a neonatal onset and variety in the clinical course despite the genetic homogeneity of the study group. The clinical course varies from mild ptosis and ophtalmoparesis to severe muscle weakness. 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. Further examinations are needed to determine modifying genes, which are responsible for the pathogenesis of the disease.

## PP-55 **ANALYSIS OF SOMATIC MUTATIONS IN LUNG ADENOCARCINOMAS AND SQUAMOUS CELL CARCINOMAS WITH TARGETED NGS**

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**Purpose:** The most frequent histologic subtypes of non-small cell lung cancers (NSCLC): adenocarcinoma (AC) and squamous cell carcinoma (SCC) could be discriminated at molecular level by specific "driver mutations" in many tumour-associated genes. Targeted next generation sequencing (NGS) allows simultaneous analysis of many genes and more complete characterization of the somatic mutations in tumours.

**Methodology:** In the current study DNA isolated from fresh-frozen tumour tissues of 8 patients with AC and 6 with SCC were included. Somatic mutations in a panel of 48 tumour-associated genes (TSACP) were analysed by NGS platform MiSeq (Illumina).

**Results:** The performed bioinformatic analysis showed that the most frequently mutated gene in the studied samples wasTP53with 10 different pathogenic variants (52%) found in 5 patients with SCC and in 5 patients with AC. Four of them were present in more than one patient. The second most commonly mutated gene wasKRAS(18%) in which four activating pathogenic variants were found. The mutation p.Gly12Val was detected in four (28.5%) samples(3 SCC, 1 AC).The BRAF V600E variant leading to decreased sensitivity to TKI-gefitinib treatment and good response to therapy with vemurafenib or dabrafenib was detected in one AC.Other pathogenic mutations were found in the genes GNAS, STK11 APC and RB1 with frequency of 7% each. EGFR and ALK mutations were not observed. Fifty % of the samples harboured pathogenic mutations in two different tumour-associated genes.

Our results suggest that the NGS analysis of NSCLC using a panel of tumour-associated genes is able to detect somatic mutations that are currently used as predictive biomarkers for targeted therapies. In addition it reveals the spectra of "driver" mutations in the lung tumours andprovides opportunities for the discovery of new therapeutic targets and personalized treatment.

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## PP-56 **A RARE MUTATION OF SCN8A GENE IN A PATIENT WITH EARLY INFANTILE EPILEPTIC ENCEPHALOPATHY**

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**Introduction:** Early Infantile Epileptic Encephalopathy (EIEE) 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. Episodes may occur more than a hundred times per day. Those who live past the age of 2 years manifest with severe psychomotor deficits. The genetic basis of the disease is complex with defects in more than a 265 genes related to neuronal dysfunction or brain dysgenesis.

**Methods:** In this study targeted next-generation sequencing was performed to elucidate the genetic basis of early-onset epileptic encephalopathy in our patient. NGS library was prepared using TruSight One gene panel, allowing to provide comprehensive coverage of known genes related with the EIEE. Sequencing was performed on an Illumina MiSeq system. For variant filtering and downstream analysis we were using Variant Studio and GenomeBrowse softwares.

**Results:** We identified an extremely rare heterozygous missense mutation in the gene SCN8A: NM_014191.3:c.5616G>A, NP_055006.1:p.Arg1872Gln. The variant is known and has been observed in two patients similarly affected with an early epileptic encephalopathy and development delay. Pathological variants of the SCN8A gene are phenotypically expressed as an autosomal dominant form of EIEE-13 and thus affected individuals are heterozygous for the variant.

**Conclusion:** The identification of causative mutation in our patient delivered crucial insights into etiology of the disease. Considering the wide variability in severity and penetrance of the disorders from the epileptic spectrum, there is large genetic heterogeneity in their inheritance. This underlines the usability of TruSight One gene panel in order to successfully detect rare defects in patients with EEIE.

## PP-57 **MOLECULAR MARKERS WITH DIAGNOSTIC AND PROGNOSTIC VALUE FOR TREATMENT OF PEDIATRIC SOLID TUMOURS**

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The high incidence of pediatric solid tumours (PST), their great malignancy potential and high mortality rates warrants timely and accurate diagnosis in order to offer the patients reliable therapy. We aimed to investigate the chromosomal aberrations on chromosomes 1, 2, 3, 4, 7, 9, 11, 12, 14 and 17 in PST, estimate their frequency and potential use as predictive/prognostic biomarkers.

The pilot study included analysis of large genomic deletions/duplications in 24 patients with MLPA kits P251, P252 and P253. The group involved 9 patients with neuroblastoma (NB), 8 with nephroblastoma/Wilms tumour (WT), 4 with rhabdomyosarcoma (RS) and 3 with Ewing sarcoma (ES). DNA was isolated from FFPE tumour tissue.

Deletion of any of 1p, 11q or amplification of 17q define the Segmental Chromosomal Alteration (SCA) type in NB, characterised by high risk of recurrence. Half of the NB cases belong to this subtype.

In 4 of the Wilms tumours aberations in chromosomes 1, 3 and 11 were found. Two of them were with duplication of the whole 1q, one with concurrent deletion in 11q (ATM-THY1), amplification of 2p, 7 and 12. In four patients (2 neuroblastomas, 1 rhabdomyosarcoma and 1 Wilms tumour) duplications of 2p, including the MYCN gene were found, which is negative prognostic factor. Deletion of 9p was found in 4 cases (2 NB; 1 ES and 1 WT). CDKN2A is located on 9p21 and its inactivation is detected in many tumours including NB and ES. In two patients this was the only aberation observed. Another frequent rearrangement involved chromosome arm 4p (deletion in 2 NB and 1 WT and amplification in 2 WT).

In conclusion MLPA is effective method to detect chromosomal rearrangements in PST and could be used in diagnostic setting for defining precise molecular subtypes and risk stratification.

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## PP-58 **IDENTIFICATION OF CYP2C9*2 ALLELIC VARIANT IN HEALTHY ALBANIAN POPULATION**

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**Abstract:** CYP2C9 enzyme is part of cytochorome P450 enzyme family involved in metabolization of different endogenous and exogenous compounds including some medications with narrow therapeutic index such as warfarin. The CYP2C9 gene encoding for CYP2C9 enzyme is highly polymorphic. CYP2C9*2 and CYP2C9*3 are the most common variants associated with reduced CYP2C9 enzymatic activity in comparison with the wild-type allele, CYP2C9*1. In Balkan populations CYP2C19*2 allele frequencies was estimated to vary from 10 to 16%. There are no data available for Albanian population regarding allelic variants of CYP2C9 gene and the aim of these study is to determine the frequency of CYP2C9*2 allele in Albanian population and to compare the data provided with previous findings in other Caucasian populations.

**Methods:** We analyzed 100 unrelated, healthy Albanian individuals randomly selected from blood donors. In our study we have male and female in equal proportion aged 37±14.48 years old. We applied a PCR-RFLP protocol, based in DNA amplification (PCR) and digestion by restriction enzyme AvaII.

**Results:** CYP2C9 genotype and allele frequencies were in accordance with Hardy-Weinberg. We have concluded that 74 subjects (74%) were homozygous for CYP2C9*1 allele, three (3%) was homozygous for CYP2C9*2 allele 23 subjects (23%) were heterozygous carriers of the two alleles. The frequency of wild type allele CYP2C9*1 was found to be 85.5% while the frequency of CYP2C19*2 allele was found to be 14.5%.

**Conclusion:** Frequencies of CYP2C9 allelic variants in Albanian population were similar to those found in other Caucasian populations ($p<0.05$). About 1/4 of Albanian population are intermediate and poor metabolizers for medications metabolised by CYP2C19 enzyme. These results should be taken in consideration by clinicists in determining drug dosage of their patients based in their genotypic profile.

With these findings we have a clear idea about frequencies of CYP2C9*2 genetic variant in Balkan region.

**Keywords:** drug metabolism, CYP2C9, genetic polymorphism, Albanian population

## PP-59 **CREATING HAEMOPHILIA COMPREHENSIVE CARE CENTRE AT ST. MARINA UNIVERSITY HOSPITAL – VARNA**

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The Haemophilia Comprehensive Care Centre at St. Marina University Hospital in Varna was opened in November 2013. In the beginning of 2014 along with the Haemophilia Centre at the University Hospital in Bonn (Germany) were included in the World Federation of Hemophilia's (WFH) Twinning Program. For a short period of time the Centre met all the European certification criteria of the European Haemophilia Network (EUHANET) and in April 2014 It received the status of a European Haemophilia Comprehensive Care Centre. One year later It was recognized by the Ministry of Health as the first Expert Rare Diseases Centre in Bulgaria. In 2016 the Centre became part of the European Reference Network on Rare Hematological Diseases (EuroBloodNet) and was awarded the "Twins of the Year – 2015" by the WFH.

## PP-60 **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.

**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 8sub-types of LGMD were diagnosed.The most common type wasLGMD2C due toCys283Tyr mutation in Gamma-sarcoglycan gene(founder for 57Gypsi/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 wasLGMD2G, with mutation in the TCAP gene- 17 patients, 16of whom were homozygous for the c.75G>A, p.Trp25*mutation. They belonged to the religious minority of Bulgarian Muslims. Three patients withLGMD2Z 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 50years(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.

## PP-61 **NEUROFORMA – INNOVATIVE APPROACH OF REHABILITATION FOR NEUROLOGICAL DISEASES AND INJURIES**

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**Abstract:** Neuroforma is an innovative technological digital solution for motor and cognitive rehabilitation, developed by neurorehabilitation and neuropsychology professionals. Neuroforma is designed for patients with neurological diseases and for those recovering from injuries. It's particularly suited for the rehabilitation of patients with multiple sclerosis, those recovering from a brain stroke or brain injury and people suffering from neurodegenerative diseases.

Performa uses virtual reality technology and real-time motion capture in 2D and 3D, with biofeedback elements, proven to be useful in neurorehabilitation by scientific research. It combines more than 20 cognitive and physical exercise modules, which offer a comprehensive rehabilitation regime, adaptable to the specific needs of each patient. They engage both motor and cognitive skills, which can improve movement precision, eye-hand coordination, joint mobility, muscle strength and endurance, perception and decision making processes, attention and memory. All this in an attractive, interactive and motivating way.

Neuroforma has been localized for Bulgaria and will be available to Bulgarian patients in the newly established Centre for psychosocial rehabilitation for people with rare diseases (CPRD) in Sofia from the 15th of September 2017. There is clinical research confirming that Neuroforma have is effective in patients with Huntington's disease, Multiple sclerosis, brain stroke and brain injuries.

**Autor:** Bulgarian Huntington Association (BHA) is a NGO in public benefit, established in February of 2014, as a result of the partnership between healthcare professionals and families affected by Huntington's Disease. In its brief existence, BHA has managed to organize several successful public events, such as a bike tour dedicated to HD, Bulgarian cinema screenings of a documentary dealing with HD, as well as a seminar for professionals dedicated to HD. BHA created and translated several materials concerning HD, submitted and managed several projects, which offered psychosocial support to people affected by rare diseases and just recently established the Centre for psychosocial rehabilitation for people with rare diseases.

## PP-62 **BLOOD BIOMARKERS FOR RISK PREDICTION OF AGGRESSIVE PROSTATE CANCER PATIENTS – PRELIMINARY STUDY OF A ROMANIAN COHORT**

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**Purpose:** Prostate cancer 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. Several single nucleotide polymorphisms (SNPs) in genes were associated with the risk of prostate cancer. The objective of this study was 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. The diagnosis of prostate carcinoma was confirmed by clinical and laboratory examination. Genomic DNA was isolated from whole blood samples and genotyping was performed by allelic discrimination using a classic allele-specific Taqman assay for the following SNPs: rs2735839 (KLK2-KLK3), rs12422149 (SLCO2B1), rs4149117 (SLCO1B3), rs138213197 (HOXB13), rs4054823 (17p12).

**Results:** For rs2735839 (KLK2-KLK3), 6% of patients were homozygous and 15% heterozygous for the high-risk allele (A). 6% of patients exhibited homozygosity and 17% heterozygosity for the A allele (high risk) of rs12422149 (SLCO2B1). However, in the case of rs4149117 (SLCO1B3), only 4% of patients presented homozygous wild type alleles (GG), while 23% were heterozygous and 73%

homozygous for the T variant. All patients were heterozygous (CT) for rs138213197 (HOXB13). The percentage of patients harbouring TT (high risk) genotype at rs4054823 (17p12) was 32%, while 43% were heterozygous.

The observed genotype distribution of the five studied gene polymorphisms and the differences in frequency of risk alleles may contribute to characterizing the genetic diversity of prostate cancer. Our preliminary results in this small patient cohort show no statistically significant association of the investigated SNPs with the biochemical markers and tumor stage.

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## PP-63 **ОРТОДОНТСКО ЛЕЧЕНИЕ ПРИ ПАЦИЕНТ СЪС СИНДРОМ НА ТЪРНЪР: ПРЕДСТАВЯНЕ НА КЛИНИЧЕН СЛУЧАЙ**

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**Цел:** Пациентите със синдром на Търнър имат отличително изражение на орофациалния комплекс. Въпреки че такава аномалия изисква специализирано ортодонтско третиране, на пациентите се оказва намалено внимание, а също липсват конкретни насоки за лечението им в литературата по дентална медицина. Целта е да се разгледа и опише характерната кранио-фациална манифестация на заболяването, съчетана с прояви в устната кухина, както и да се създадат указания за работа при конкретния клиничен случай.

**Методи:** След преминало две годишно интерсептивно ортодонтско лечение с незадоволителен резултат, при шестнадесет годишен пациент със синдром на Търнър е започнато късно ортодонтско лечение с фиксирана техника на диагноза отворена захапка с четири екстракции- на горна челюст първи премолари и на долна челюст втори премолари .

**Резултати:** При преглед на пациента се наблюдава, че във фронта горните фронтални зъби не припокриват долните с разстояние между тях 3 до 4 мм, което създава неудобство при дъвкателния акт, но същевременно и при дишане. Създават се насоки за лечение, като се подчертава необходимостта да се подобри екстраоралния вид на лицето. Прави се корекция на зъбите на горна челюст, което включва деротиране, нивелиране и дистализиране на зъбите. Като втори етап следва корекция на зъбите на долна челюст в същите стъпки. Като последен етап се синхронизират горна и долна зъбна редици.

**Заключение:** 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.

## PP-64 **FIRST SYSTEMATIC GENETIC STUDY OF BULGARIAN PATIENTS WITH PARKINSON DISEASE**

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Parkinson disease (PD) is a common neurodegenerative disease resulting from the interplay of multiple genes with environmental risk factors. Monogenic forms are rarely found, inherited in an autosomal dominant, autosomal recessive, or X-linked manner. The main goal of the current study is to evaluate the frequency and type of mutations in genes associated with Parkinson disease in Bulgarian patients

Altogether, 69 patients with PD and detailed clinical assessment and 108 healthy controls, matched to the patients by age, gender and ethnicity (NC) were recruited. All individuals were analyzed with a custom panel including known and candidate PD genes on an Illumina NGS platform. All pathogenic variants were confirmed with Sanger sequencing.

In 52% (36/69) of the patients were identified variants in LRRK2, PARK2, PINK1, PARK7, ATP13A2, FBXO7, PSEN1, PSEN2, CHMP2B, GRN, MAPT, EIF4G1. Of then, 14 were novel (10 missense, 2 splice site, 2 frameshift). Four pathogenic, including two novel ones, were identified in PARK2, PARK7, PSEN2. For 14 variants the significance is uncertain (13 missense, 1 splice site).

This is the first study in Bulgaria including big cohort of 69 well characterized patients that shows distribution of novel and already reported variants in genes associated with Parkinson disease. Our findings contribute to a better understanding the molecular basis of Parkinson disease and have implications for diagnostic testing and genetic counseling in Bulgarian population. The identification of mutations in genes linked to Parkinson disease in substantial part of our patients strongly supports NGS studies as an approach for a more efficient and cost effective genetic testing.

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## PP-65 **WHOLE-GENOME SEQUENCING IN NEWBORN SCREENING – PRELIMINARY RESULTS ON MEDICAL PROFESSIONALS' ATTITUDES AND OPINIONS IN BULGARIA**

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**Background:** Newborn screening (NBS) is a public health service aimed at detecting apparently healthy infants with severe congenital disorders, for which there is available cost-effective identification and effective treatment. NBS is considered to be the longest running and most successful population screening activity worldwide. Whole-genome sequencing (WGS) is viewed as a major vehicle for translating genetic and genomic advances into population health gains.

**Objective:** The aim of this study was to explore the attitudes and opinions on the potential use of WGS in conjunction with the traditional NBS. We conducted an online survey among paediatricians and geneticists from Bulgaria.

**Results and discussion:** While half of the paediatricians surveyed supported population-based non-selective WGS in NBS, 65.2% of the geneticists expressed concerns. Most participants underlined that ethical issues were as important as medical ones and called for a stricter protection of affected individuals against any abuse of their personal data. Extensive genetic counselling and psychological support to families were mentioned as key elements in this potential activity. Nevertheless, both paediatricians and geneticists considered that NBS in Bulgaria could be further developed, with selective WGS being suggested as a potential option.

**Conclusion:** While non-selective WGS for all newborns is not currently perceived as feasible, paediatricians and geneticists do believe that selective WGS could strengthen current NBS programmes. Cross-border project collaborations may set the stage for generating experience and evidence on these complex issues.

## PP-66 **MOLECULAR DETECTION OF VIRUS HERPES SIMPLEX TYPE 1 IN PATIENTS WITH PERIODONTAL DISEASE**

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**Background:** 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 between the presence of this virus and the stage of periodontal disease.

**Methods:** Supragingival and subgingival dental plaque samples were taken from a total of 89 patients with periodontitis for DNA extraction. 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 disease. Using the PCR (Polymerase chain reaction) method molecular detection of HSV-1 was performed.

**Results:** HSV-1 virus was detected in 22/89 (24.7%) patients with periodontal disease. It 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 2/12 (16.7%) in the subgingival plaque samples, $p<0.05$. In patients with advanced stage of the disease the HSV-1 virus was detected in 10/35 (28.6%) patients, of which in 6/10 (60%) patients in supragingival samples and in 6/10 (60%) subgingival plaque samples, $p>0.05$. Interestingly, in two patients with moderate and two patients with advanced stage of the disease the HSV-1 was concomitantly detected in both, supra and subgingival plaque samples.

**Conclusion:** HSV-1 was present in supragingival and subgingival dental plaque in the patients with periodontitis. The frequency of

detected HSV-1 virus was higher in the patients with advanced periodontal disease compared to moderate periodontal disease. We suggest that the presence of HSV-1 is related to the degree of periodontal tissue damage and manifestation of the different degree of periodontal destruction.

**Keywords:** periodontal disease, HSV-1, molecular analysis

## PP-67 **CRITICALLY ILL MSUD PATIENT - CHALLENGES, WAYS TO PREVENT AND OUTCOME**

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Maple Syrup Urine Disease (MSUD) is an autosomal recessive disorder caused by abnormal oxidative decarboxylation of branched amino acids – leucine, isoleucine and valine. Their and the corresponding ketoacids accumulation leads to encephalopathy and progressive neurodegeneration. Catabolic events such as severe infections require timely management in an intensive care setting and monitoring of amino acid concentration.

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 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 patient started normal enteral feeding.

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.

Keywords: Maple Syrup Urine Disease (MSUD), Branch amino acids (BCAA), intensive care, haemodialisis

## PP-68 **A 17-YEAR-OLD BOY WITH LEOPARD SYNDROME**

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We present a 16-year-old boy with short stature, multiple lentigines, electrocardiographic anomalies, ocular hypertelorism, anteverted ears and pectus excavatum, who present to us for the first time. Our clinical diagnosis is LEOPARD syndrome. The very rare LEOPARD syndrome (LS, about 200 cases in literature) is an autosomal-dominant multisystem disease, which was first described in 1969. LEOPARD is an acronym for lentigines, ECG-changes, ocular changes (mostly hypertelorism), pulmonary stenosis, abnormal genitalia, growth retardation, and deafness. Mutations in the protein tyrosine phosphatase, nonreceptor type 11 gene (PTPN11, gene map locus 12q24.1) have been associated with the LS, but distinct mutations within the same gene are related to the Noonan syndrome. The current diagnostic criteria, proposed by Voron et al, are fulfilled by lentigines and 2 of the minor criteria (cardiomyopathy, deafness, ECG-changes, and hypertelorism). On physical examination, our patient was 148.7 cm high, body weight 36.6 kg with multiple lentigines on the skin. . The heart rate was 78 per min and the blood pressure was 97/56 mmHg, puberty – Tanner stage 2 with normal penile development. Growth was grossly affected – current height was 10 cm below the 3th centile (mid-parental height 172 cm, target range …). The IGF-1 level was 261 ng/ml (t.r. 57-426). Bone age showed 3 years delay, and ECG – prolonged QT. EchoCG showed apical aneurysm, Ao insufficience 0-1, thickening of the wall heart chambers. The boy is under follow-up by pediatric endocrinologist and pediatric cardiologist without treatment for the moment. DNA studies were undertaken.

## PP-69 **RESULTS FROM CYTOGENETIC ANALYSIS IN CHILDREN WITH SUSPECTED CHROMOSOMAL DISORDERS (A 10-YEAR EXPERIENCE). THREE CYTOGENETICS CASE REPORTS**

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**Background:** 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. As a result of this rearrangement the developmental delays, sexual development disorders, congenital malformations, and mental retardation are obtained.

**Aim:** To describe the prevalence of different chromosomal abnormalities in Bulgarian children referred for cytogenetic analysis and to present the most complicated three cases for the last10 years.

**Materials and method:** Retrospective study was carried out to identify the frequency and pattern of chromosomal aberrations among patients referred to the Cytogenetic Laboratory. 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:** A total of 448 pathologic karyotypes were identified (17.6%) with different chromosomal abnormalities. The most common were trisomy 21, Down Syndrome - 226 (50.4%), sex chromosomal abnormalities -101 (22.5%) and deletion and structural were 121 (27 %), respectively. The cytogenetic result of the three most complicated cases were as follows:

46,XY,inv(15)(q15 q21.2)mat

mos 46,XX,del(16)(q22)[13] /.../46,XX mat[65]

And the karyotype - 46,XX,t(2;7)(q14;q35) who was revised after DNA analysis and cytogenetic result on mother wich was: 46,XX,t(2;7)(q13;q36)inv(2)(p23q13)

**Conclusion:** 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.

**Keywords:** chromosomal abnormality, cytogenetics, karyotypes

## PP-70 **THE BULGARIAN CENTENARIAN GENOME PROJECT**

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**Introduction:** We initiated the Bulgarian Centenarian Genome Project in early 2017. Its main goal is to discover genetic factors responsible for the maintenance of longer and healthy lifespan in centenarians, as well as to establish the molecular pathways and interactions regulating the processes of ageing and health deterioration.

**Methods and Materials:** Data for all living centenarians within Bulgaria (>300) was obtained. Centenarians are visited in their homes and are presented to the topic of the project. Those who are willing to participate are given an informed consent form and fill up a detailed questionnaire regarding their lifestyle and medical history. Buccal swabs are taken, from which DNA is later isolated. At least 20 DNA samples of centenarians with good medical history will at first be analysed by whole-genome sequencing. The data of the centenarians will be compared to a cohort of young controls from the Bulgarian population using a wide range of bioinformatics tools and computer models in order to find genetic loci promoting healthy longevity and disease prevention. Rare candidate variants enriched in the centenarian cohort will be tested amongst the other centenarians from the DNA bank and other controls via targeted NGS sequencing.

**Results and Discussion:** We are currently in the process of building a DNA bank containing samples from as many people above 100 years as possible. The centenarian genome is the gold standard in genomics, as it is expected not only to be devoid of pathogenic variants with significant penetrance, but also to harbour protective alleles against different environmental risk factors uncommon for the general population. We expect to uncover some of the genetic factors predisposing to healthy longevity, as well as to pave the way for the development of novel drug targets and personalised longevity strategies and healthcare.

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## PP-71 **КЛИНИЧНИ СЛУЧАИ НА ПАЦИЕНТИ С МНОЖЕСТВЕНИ МЕТАХРОННИ НЕОПЛАЗИИ**

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**Въведение:** В последното десетилетие се наблюдава тенденция за нарастване броя на пациентите с множествени метахронни неоплазии. Успехът на новите терапевтични подходи и увеличената обща преживяемост на пациентите е предпоставка за поява на нови генетични нарушения, както и за други клинични прояви на съществуващите. Пациенти и методи: Ретроспективно са проучени здравните досиета на 2316 болни с онкологични заболявания, верифицирани хистологично за периода 2012–2016 г. и лекувани в Клиниката по медицинска онкология на УМБАЛ „Св. Георги“ – ЕАД. Сред тях са регистрирани 22 болни с множествени злокачествени заболявания. Съчетанията включват солидни тумори с онкохематологични неоплазии. Представяме клинични случая на пациент с три метахронно протичащи злокачествени заболявания, както и пациент с метахронно и синхронно протичане.

*Клиничен случай 1:* Бял мъж (71 г.) с фамилна обремененост - майка с колоректален карцином и брат с остеосарком. Рискови фактори - 18 г. трудов стаж в оловно-цинкова мина. През декември 2012 г. се доказва Първична миелофиброза съгласно WHO критериите. Пациентът остава под наблюдение. През юли 2014 г. по повод на дизурични смущения е диагностициран ацинарен Аденокарцином на простатна жлеза, интермедиерен риск, T2bN0M0, G2, Gleason 3+4=7. Остава на диспансерно наблюдение. През декември 2015г. след биопсия на периферен лимфен възел се верифицира хистологично M. Hodgkin - лимфоцитно преобладаване, IIIB кл.ст. Пациентът провежда химиотерапия (6 курса ABVD) и лъчетерапия. Постигната ремисия, остава под активно наблюдение.

*Клиничен случай 2:* Бял мъж (75 г.), без анамнеза за фамилна обремененост. През 1994г. по повод M. Hodgkin–нодуларна склероза, IIIA кл.ст. е получил цитостатична терапия, лъчелечение и спленектомия. През 08.2013 г. след фиброколоноскопия (ФГС) по повод долно-диспептичен синдром хистологично се верифицира аденокарцином на ректума, T2N0M0, G2. След няколко последващи ревизии се доказва И гастроинтестинален стромален тумор -синхронно протичащи тумори. След оперативна интервенция започва терапия с тирозинкиназен инхибитор (Imatinib). През август 2016 г. при ФКС се установява локален рецидив на ректалния карцином - има реоперация с последваща лъче-, и химиотерапия. Пациентът продължава терапията с Imatinib като провежда рестадиращи изследвания.

**Заключение:** Лечението на пациенти с две или повече метахронно или синхронно протичащи неоплазии представлява определено терапевтично предизвикателство. От значение за успеха му е навременната диагностика и индивидуалният подход.

**Ключови думи:** синхронни неоплазии, метахронни неоплазии, лечение

## PP-72 **СИНХРОННО И МЕТАХРОННО ПРОТИЧАЩИ НЕОПЛАЗИИ**

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**Въведение:** Честотата на пациентите с множествени първични злокачествени тумори нараства значително поради разширените диагностични възможности и увеличената преживяемост на тези болни. Пациенти, преживели едно злока-

чествено заболяване, имат 20% по-висок риск за развитие на втора първична неоплазия в същия или друг орган, сравнено с общата популация.

**Пациенти и методи:** Ретроспективно са проучени здравните досиета на 2316 болни с онкологични заболявания, верифицирани хистологично за периода 2012-2016 г. и лекувани в Клиниката по медицинска онкология на УМБАЛ „Св. Георги" – ЕАД. От тях са регистрирани 22 болни с множествени злокачествени заболявания. При подбора на болните приложихме критериите на Warren и Gates. За статистическа обработка се използва алтернативен анализ. Пациентите бяха разделени в две групи - със синхронни и метахронни неоплазии съгласно утвърдените критерии.

**Резултати:** Честотата на случаите с множествени злокачествени заболявания е 0.9%. Метахронните неоплазии са по-чести от синхронните (съотношение 2,7:1). При 63% от тези 22-ма пациенти появата на второ и следващо малигнено заболяване е предшествано от химио-/лъчетерапия за първото. При 18% се срещат по три неоплазии, а при останалите - по две. При 13 % от болните има фамилна обремененост за онкологични заболявания. Най-честото малигнено хематологично заболяване, което се асоциира с други е хронична лимфоцитна левкемия (7).

**Дискусия:** В литературата се посочват вариабилни стойности за честотата на множествените неоплазии (от 0.73% до 11.7%) като при аутопсирани случаи нараства до 36%. Резултатите от нашето проучване съответстват на посочените от други автори данни.

**Заключение:** Зачестяването на множествени (метахронни и синхронни) злокачествени заболявания има мултифакторна етиология и обективни предпоставки. Тези пациенти поставят сериозни терапевтични предизвикателства и изискват интердисциплинарен подход.

**Ключови думи:** синхронни неоплазии, метахронни неоплазии, лечение

## PP-73 **АТИПИЧНА КЛИНИКА ПРИ ИНФЕКЦИОЗНА МОНОНУКЛЕОЗА**

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**Въведение:** Инфекциозната мононуклеоза се причинява от вируса на Ебщайн-Бар, като засяга юноши и млади хора между 15 и 35 години. Известна е като „болест на целувката". Клиничната картина е характерна и включва фебрилитет, главоболие, отпадналост, загуба на апетит и увеличени лимфни възли по цялото тяло, особено по шията („жлезна треска"). В много редки случаи инфекцията протича необичайно.

**Цел:** Представяме клиничния случай на 31-годишна жена от гр. Варна, която постъпва във Второ вътрешно отделение на УМБАЛ „Св. Марина", с направление от личния лекар, за уточняване на диагнозата. Единственият симптом, за който съобщава, е фебрилитет от 5 дни (37,5-39,4 °C), който не се повлиява медикаментозно.

**Материали и методи:** В диференциалната диагностика участват редица квалифицирани специалисти. Извършени са кръвни (ПКК, натривка), образни (рентгенологично изследване, ехография на корем и ехокардиография) и микробиологични изследвания. Последните включват носен секрет, гърлен секрет, урокултура и хемокултура. По искане на лекуващия лекар биват извършени консултации с УНГ и АГ специалист.

**Резултати:** Огледът и физикалните изследвания не установяват патологична находка. Акценти от кръвната картина при постъпването биват: левкопения (Leu 3.28, Neu# 1.70), моноцитоза (0.86, 26.20%) тромбоцитопения (Tr 117), чернодробни ензими (АлАТ 47.1; АсАТ 54.2). Ехографията на корем показа леко уголемена слезка (127/36 мм). Серологичните изследвания са отрицателни. Повторната кръвна картина (след 5 дни престой) установява увеличение на АлАТ (105.0) и АсАТ (65.0). В серума бива вирусът на Ебщайн-Бар. На база резултатите от лабораторните изследвания и без поява на нови симптоми освен фебрилитета, се поставя диагноза инфекциозна мононуклеоза. Пациентката бива приведена за лечение в инфекциозно отделение.

**Дискусия:** Поради неспецифичната клиника, пациентката е красноречив пример за неясно фебрилно състояние. Отхвърлени са били възпалителни, неопластични и автоимунни процеси.

**Заключение:** Случаите с атипично протичане на инфекциозна мононуклеоза често могат да останат недиагностицирани. Това води със себе си до редица излишни диагностични процедури, както и до продължително лечение с антибиотици и др. медикаменти, с евентуални тежки последици за пациента.

## PP-74 **NIEMANN-PICK DISEASE TYPE B AND TYPE C IN BULGARIA – GENETIC AND CLINICAL CHARACTERISTICS**

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Niemann-Pick disease is a multisystem disorder with a wide range of symptoms that vary in severity. Niemann-Pick disease is divided into four main types: type A, type B, type C1, and type C2. These types are classified on the basis of genetic cause and the signs and symptoms of the condition.

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. NP-C is associated with a highly heterogeneous spectrum of visceral, neurological, and psychiatric manifestations that can start at any age, which is often the cause for the under recognition of the disease and substantial delays in diagnosis. Currently in Bulgaria 16 patients 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.

Niemann-Pick disease type B (NP-B) is resulting from mutations in the sphingomyelin phosphodiesterase 1 (SMPD1) gene. We present a genetically homogeneous group of 25 Gypsy patients with intermediate NPB, where we observed a surprising diversity of neurological features. All affected subjects were homozygous for the same ancestral mutation, W391G in SMPD1, yet displayed the entire spectrum of phenotypic variation observed previously in unrelated affected subjects of diverse ethnicity and disease-causing mutations, ranging from subclinical retinal involvement to severe ataxia, cognitive deficits and psychiatric disorders.

**Keywords:** Niemann Pick disease, multisystem involvement

## PP-75 **IDENTIFICATION OF HLA B57:01 ALLELE, CONFERRING HYPERSENSIBILITY REACTION TO ABACAVIR, IN HLAB57 INDIVIDUALS OF ALBANIAN POPULATION BY AS-PCR**

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**Background:** One of the most successful applications of pharmacogenetics research is the genetic screen ing for HLA-B*57:01, strongly associated with an increased risk to develop hypersensitivity reaction (HSR) in HIV-positive patients following abacavir administration. The Caucasian group has a HLA-B*57:01 prevalence of 5–8%. Identification of HLA B57 allele was performed in Albanian population by Laboratory of Immunology, QSUT, Tirana. From individuals analyzed only 1,5 % results HLA B57 genotype. The identification of HLA B57:01 allele was not performed previously and we intend to apply a fast, easy to use and 'low-cost' molecular assay AS-PCR protocol.

**Methodology:** Taking into account the previous experience in the study of HLA region in the diagnostic practice of Laboratory of immunology, 13 blood samples were conserved from HLA B57 Albanian individuals. We extracted DNA from 13 individuals and used

primers for the specific amplification of HLA-B*57 region and its subtype HLA-B*57:01 according published protocols. We decided to use the direct PCR and gel electrophoresis as the first-line protocol to detect the positivity/ negativity for HLA-B*57. For the specific detection of HLA-B*57:01 allele, a nested PCR has been used.

**Results:** The direct-PCR trial proved to be able to confirm HLA-B*57-positive individuals, identified by other methods. All 13 samples results positive for HLA-B*57, and were further typed for the presence of HLA-B*57:01 sequence by nested PCR. All individuals have the presence of HLA-B*57:01 sequence. We confirm that 100% of HLA B57 genotypes have the presence of HLA-B*57:01 allele. In this context the availability of an inexpensive, sensitive, pharmacogenetic predictive test for HSR prevention may represent a good strategy to improve the use of a highly efficient drug like abacivir. To this purpose the development of our direct PCR represents a fast, easy to use and 'low-cost' molecular assay employing HLA-B*57:01 as a predictive pharmacogenomic biomarker.

## PP-76 **СИНДРОМ НА ПРАЗНАТА СЕЛА – ОПИСАНИЕ НА КЛИНИЧЕН СЛУЧАЙ**

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Синдромът на празната села е вроден дефект на диафрагма селе. Обикновено се установява като случайна неврорадиологична находка, но може да бъде и симптоматична – с главоболие, зрителни нарушения, доброкачествена интракраниална хипертензия, питуитарна хипофункция.

Целта на настоящата работа е да бъде описана рядка асоциация на пациент с генерализирано разстройство на психичното развитие-детски аутизъм и данни за синдром на празната села.

Приложени са следните методи: клинични – анамнеза, фамилна анамнеза, неврологичен статус, консултация със специалисти, невроизобразяващи –ЯМР 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, витамин В12.

Авторите представят момче на 6 години с данни за триъгълна брадичка, антимонголоидни очни цепки, ниско поставени ушни миди, хипертрихоза по гърба, генерализирано разстройство на психичното развитие, синдром на празната села, дисхармонична костна възраст, хиперпролактинемия, повишени аланин и глутамат. Проведе се лечение с мултивитамини, логопедична терапия, седивитакс.

## PP-77 **RARE SYNDROMES WITH UNUSUAL DENTAL MANIFESTATIONS (REVIEW)**

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The aim of this poster is to draw attention to rare syndromes with unusual dental manifestations.

**Methods and materials:** A systematic review of available literature in Pub Med was provided.

**Results:** Oculo-facio-cardio-dental 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.

**Keywords:** oral manifestations, rare syndromes

## PP-78 **СЪОБРАЖЕНИЯ И РИСКОВЕ, СВЪРЗАНИ С ДИАГНОСТИКАТА И ЛЕЧЕНИЕТО НА ТЕЖКО ОСТРО КОМБИНИРАНО ОТРАВЯНЕ С ПСИХОТРОПНИ ЛЕКАРСТВА**

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**Резюме:** Отравянето с лекарствени средства, медикаменти и биологични вещества понякога е причинено умишлено с цел самонараняване или самоубийство.Признаците и симптомите на интоксикацията с лекарствени средства, медикаменти и биологични вещества варират в зависимост от дозата и вида на веществото.Тези симптоми често могат да бъдат разделени на различни токсидроми, които са специфични клинични прояви характеризиращи острото отравяне. В клиничната практика все по-рядко се среща остра интоксикация само с един медикамент от определена фармакологична група. През последните години преобладават комбинираните остри отравяния с медикаменти с различна фармакологична принадлежност. Този факт повлиява експертизата на токсичните синдроми или променя тяхната специфична характеристика.

Представеният пациент е с тежко остро отравяне с невролептици и бензодиазепини, клинично изявило се с животозастрашаващи токсидроми.

Случаят е интересен предвид рядко наблюдаваното съчетание от специфичните прояви на всяко едно от употребените психотропни вещества.

**Ключови думи:** остро отравяне, токсидроми, психотропни лекарства

## PP-79 **НЕВРОЛОГИЧНА СИМПТОМИ ПРИ ПАЦИЕНТ С ПЕРИЦЕНТРИЧНА ИНВЕРЗИЯ НА 9-ТА ХРОМОЗОМА**

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Перицентричната инверсия на 9-та хромозома представлява структурен хромозомен вариант на нормалния кариотип. В литературата са описани малък брой пациенти с вродени аномалии и инфертилитет, носители на мутацията.

Целта на настоящата работа е да се представи неврологична симптоматика при пациент с перицентрична инверзия на 9-та хромозома.

Използвани са клинични методи, консултация със специалисти, ЕЕГ, КТ и МРТ на глава, рентгенография на китка, ЕхоКГ, капилярно-зонова електрофореза, МПЗ в урина, G-binding метод, MLPA за микроделеции, субтеломерни делеции и дупликации, ДНК за анализ на мутации в FMR1 ген и др.

Авторите представят пациент на 11 години с инверсия на 9-та хромозома, който се представя с пилорна стеноза, умбиликална херния, конвергиращ страбизъм, дизлексия, дискалкулия, нарушение във фината моторика, лека степен на умствена изостаналост. ЕЕГ-силно абнормен запис с дифузни бавновълнови промени и изразена пароксизмална активност. Нормална находка от МРТ.

Проведено беше лечение с депакин, ноотропил, мултивитамини, логопедична терапия.

## PP-80 **ЧЕРНОДРОБНО ЗАБОЛЯВАНЕ СВЪРЗАНО С МУКОВИСЦИДОЗА – КЛИНИЧНИ СЛУЧАИ**

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Повече от 20 % от пациентите с мусковисцидоза развиват чернодробна болест свързана с муковисцидозата (ЧБСМВ). Портална хипертония и свързаните с нея усложнения са причина за фатален изход при тези пациенти. ЧБСМВ в световен мащаб е третата причина за смъртност, след белодробните и посттрансплантационните усложнения.

Патогенезата на заболяването е многофакторна, като се отдава значение на билиарната обструкция, оксидативния стрес и фиброгенезата. В резултат се развиват множество фенотипни вариации на чернодробното засягане, най-честата от които е огнищната (фокална) билиарна цироза.

Клиничната презентация на заболяването също е вариабилна, като по-голямата част от пациентите със симптоми на чернодробна дисфункция, вече са развили необратими морфологични изменения. Диагнозата се поставя на базата на клинични, клинико-лабораторни и ехографски критерии, като в повечето случай чернодробната биопсия остава стандарт за доказване. Разработени са ехографски и клинични скали за поставяне на диагнозата ЧБСМВ, в основата на които са поставени резултатите от чернодробна биопсия. Златен стандарт в лечението на заболяването е УДХК в дози от 20-30 мг/кг дн.

Представяме два случая на ЧБСМВ – К.Й.П на 15 г. с диагноза муковисцидоза, при която заболяването бе установено при клиничната констелация на хипоалбуминемия, удължено ПВ и признаци на портална хипертония. данни за хепатомегалия и хетероехогенност при ехография и повишена активност на аминотрансферази съчетана с увеличение на билирубин.

А.Н.Ч. 14 г. Също с доказана МВ, с данни за хетероехогенност на чернодробния паренхим и умерено увеличение на амитрансферазната активност.

Двата описани случая представят част от клиничните изяви на ЧБСМВ, като представят диагностични затруднения

## PP-81 **ПРОУЧВАНЕ НА НАГЛАСИТЕ НА МАГИСТЪР-ФАРМАЦЕВТИТЕ ОТНОСНО ВЪЗМОЖНОСТИТЕ ЗА ПРИЛАГАНЕ НА ФАРМАЦЕВТИЧНИ ГРИЖИ ЗА ПАЦИЕНТИ С РЕДКИ БОЛЕСТИ В БЪЛГАРИЯ – ПИЛОТНО ПРОУЧВАНЕ**

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Настоящата разработка се финансира от СМН на МУ София по проект №42/2016 на тема: „Проучване на качеството на живот, амбулаторната терапия на пациенти с редки заболявания в България и на техния достъп до лечение и до фармацевтично обслужване"

**Цел:** Изследване мнението на магистър-фармацевтите относно възможността за имплементиране на фармацевтичните грижи (ФГ) за пациенти с редки заболявания (РБ) в България.

**Методология:** Проведено е пилотно, анкетно проучване сред магистър-фармацевти, работещи в аптеки за обслужване на населението в град София. Анкетата, включва въпроси относно възможността за въвеждане на ФГ за пациенти с РБ в практиката, очакваните ползи, необходимостта от заплащане за оказваните грижи, както и нивото на квалификация на фармацевтите в областта на РБ. За целите на обработване на анкетите е приложена дескриптивна статистика с помощта на статистическата програма STATISTICA Version 13.

**Резултати:** Пилотното проучване обхваща 48 магистър-фармацевти, практикуващи в аптеки за обслужване на населението в град София. 88% от анкетираните са жени, като преобладават лицата на възраст между 25 и 40 години (47%), следвани от 40-50 годишните (29%). Повече от половината (52%) са запознати с дефиницията за РБ, като статистически значимо повече магистър-фармацевти (98% спрямо 2%, $p<0,05$) смятат, че не притежават достатъчно познания относно РБ. 96% проявяват интерес към провеждане на продължаващо обучение за РБ. 20% отговарят, че пациентите с РБ никога не са се допитвали до тях за консултация, а 75% споделят, че това се случва рядко. Най-честите въпроси са относно лекарствената терапия (60,42%), законодателните и административни процедури (31,25%). 89,6% са запознати с концепцията за ФГ и повече от 70% биха инвестирали за имплементирането им в практиката. 30% не смятат, че трябва да се заплаща допълнително за ФГ, а 56,24% посочват сума над 10лв. Основните проблеми са трудна комуникация (40%), липса на време и пространство (79,2%) и недостатъчно персонал в аптеката (66,6%). Очаквани ползи от ФГ са подобряване качеството на живот на пациентите с РБ (87,5%), повишаване доверието към магистър-фармацевта (81,3%) и финансови постъпления за аптеката (21%).

**Заключение:** Магистър-фармацевтите са най-достъпните здравни специалисти, които имат необходимите знания и желание да участват ефективно в цялостната грижа за пациентите с РБ. Възможностите за имплементиране на ФГ са провеждането на продължаващи обучения сред фармацевтите и оптимизирането на работата на магистър-фармацевта в аптеката с насоченост към индивидуализирана грижа за пациента.

## PP-82 **ПРОУЧВАНЕ МНЕНИЕТО НА ПАЦИЕНТИТЕ С РЕДКИ БОЛЕСТИ ОТНОСНО КАЧЕСТВОТО НА ФАРМАЦЕВТИЧНОТО ОБСЛУЖВАНЕ – ПИЛОТНО ПРОУЧВАНЕ**

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Настоящата разработка се финансира от СМН на МУ София по проект №42/2016 на тема: „Проучване на качеството на живот, амбулаторната терапия на пациенти с редки заболявания в България и на техния достъп до лечение и до фармацевтично обслужване"

**Цел:** Изследване удовлетвореността на пациентите с редки заболявания (РЗ) от фармацевтичното обслужване и отношението им относно прилагане на фармацевтичните грижи (ФГ) в България.

**Методология:** Проведено е пилотно, анкетно проучване сред пациенти с РЗ в град София. Въпросите включват данни, относно заболяването, удовлетвореността от терапията, наличието на пречки при получаване на лекарствените продукти, достъпа и качеството на фармацевтичното обслужване. За целите на обработване на анкетите е използвана статистическата програма STATISTICA Version 13.

**Резултати:** Пилотното проучване обхваща 35 пациенти с РЗ сред които 29 с акромегалия, 5 с болест на Кушинг и 1 с болест на Помпе. Преобладаващите респонденти са от женски пол (74%) като преобладават лицата над 50 годишна възраст (65%). Средното време за диагностициране на заболяването е 2,07 години (SD=2,59) като най-дълго време е отнело поставянето на диагноза на пациента с болест на Помпе – 11 години. Висока удовлетвореност от прилаганата терапия е посочена от около 57% от пациентите, което доказва ефективността на лекарственото лечение в реални условия. Административни пречки във връзка с получаване на необходимите лекарства са докладвани от 11% от анкетираните, което потвърждава улеснения достъп на тази група пациенти до фармакотерапия след преминаване на финансирането към НЗОК. Достъпът до фармацевтично обслужване на пациентите с РЗ не е затруднен вероятно поради достатъчния брой аптеки и фармацевти в големите градове. Независимо от високото ниво на удовлетвореност от качеството на фармацевтичното обслужване (85,7%), около 60% от пациентите с РЗ не са склонни да се допитат до магистър-фармацевт и да получат консу лтация поради недоверие в квалификацията и познанията им относно РЗ.

**Заключение:** Пилотното проучване демонстрира адекватен физически и финансов достъп на пациентите с РЗ до лечение, както и удовлетвореност от оказваното фармацевтично обслужване. Нивото на доверие към магистър-фармацевтите остава ниско, което обуславя необходимостта от информиране на обществото относно ролята на фармацевтите и доказване ползата от прилагане на ФГ не само за често срещаните, но и за редките заболявания.

## PP-83 **МЪРТЪВ ПЛОД С DEL (7) (Q 11.23; Q 21.2) ОТ ПРЕКЪСНАТА БРЕМЕННОСТ ПО МЕДИЦИНСКИ ПОКАЗАНИЯ – КЛИНИЧЕН СЛУЧАЙ**

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Представяме клиничен случай на мъртвороден плод от от прекъсната бременност по медицински показания, по данни от Плевенски регистър за вродени аномалии /член на EUROCAT/. Касае се за I-ва бременност на 25 годишна жена, в 11 г.с и 5 дни проведен биохимичен серумен скрининг с ниски рискове за изследваните хромозомни аномалии, но предвид ниските стойности на PAPP-A и Free-bCG, е препоръчано специализирано ултразвуково изследване за търсене на някои допълнителни маркери за хромозомна патология. При проведената фетална морфология в 22 г.с, са установени множество малформации на плода: голям лицев дефект- едностранна цепка на горна устна, асоциран с голям дефект на твърдото небце, патологична сърдечна находка, както и асиметрична интраутеринна ретардация на плода и др. УЗ белези

насочващи към вероятна хромозомна аномалия. Препоръчана е генетична амниоцентеза и Фетална Ехокардиография, но предвид множеството малформации на плода с лоша прогноза, родителите вземат решение да прекъснат своевременно бременността по медицински причини . В 23 г.с. е роден мъртъв плод от мъжки пол с тегло 420 гр., ръст 27см. , обиколка на главата 20 см. От изпратената за цитогенетичен анализ кръвна проба , взетата от пъпна връв, се установява кариотип 46, XY, del (7) (q11.23; q21.2). Последващото хромозомното изследване на родителите установява нормални кариотипи.

Окончателната диагноза доказва, че се касае за хромозомна аномалия на плода с ненаследствен характер (резултат de novo). На семейството е проведена генетична консултация, разяснени са рисковете и дадени препоръки, с оглед планиране на следващи бременности.

**Ключови думи:** del (7) (q 11.23; q 21.2) , прекъсната бременност, хромозомна аномалия

## PP-84 **ПЕРИФЕРНОСЪДОВИ И МИКРОЦИРКУЛАТОРНИ НАРУШЕНИЯ ПРИ ВИБРАЦИОННА БОЛЕСТ ОТ ЛОКАЛНО ВИБРОВЪЗДЕЙСТВИЕ**

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Съдовите нарушения са водещи в клиничната картина на вибрационна болест от локално вибровъздействие (ВБ ЛВВ) наред с периферонервните и мускулно-скелетните.

**Цел:** изследване на периферносъдови и микроциркулаторни промени при болни с ВБ ЛВВ.

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 соногрaфия (ДДС), лазерна доплерова флоуметрия (ЛДФ) и видеокапилароскопия (ВКС).

**Резултати:** 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. Нарушена кожен кръвен ток в пръстите на ръцете воларно и снижена кожна перфузия беше установена при 39.2% чрез ЛДФ. При преобладаващия дял (78.4%) от изследваните се установяват функционални промени в нутриционните кожни микросъдове чрез ВКС: дистонни с преобладаване на спастичните (30.4%), спастични (44.8%) и предимно атонични (3.2%) капилярни бримки, а структурно променени капиляри с аневризми при 21.6%. Периферносъдовите и микроциркулаторни нарушения са в положителна корелация със стадия на заболяването.

**Заключение:** Установяват се ангиоспастични промени в дисталните периферни съдове и кожните нутриционни и терморегулаторни микросъдове на пръстите на ръцете при ВБ ЛВВ. Ранното диагностициране на ВБ ЛВВ с оглед подобряване на прогнозата и качеството на живот на болните изисква познания на личните лекари и специалистите по ортопедия, ревматология, неврология, ангиология, обща медицина върху релевантните професии с вибрационна експозиция и основните клинични прояви за своевременно насочване към специалистите по професионални болести и адекватно терапевтично и превантивно поведение.

## PP-85 **FROM CLINICAL TO GENETIC DIAGNOSIS IN AN 11-YEAR OLD BOY WITH FAMILIAL HYPERCHOLLESTEROLEMIA: A CASE REPORT**

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Familial hypercholesterolemia (FH) is a disorder that causes severe dyslipidemia due to a genetic mutation inherited in an autosomal dominant manner, affecting chromosome 19 and the synthesis of the LDL receptor. Although recent studies have shown that heterozygous FH is not as uncommon as previously thought, homozygous forms account for about one in a million cases in the US as well as in European countries. It is associated with an early onset of generalized atherosclerosis and a high risk of target organ damage in young adulthood.

We present a case of an 11-year old boy, who first sought medical attention for the appearance of xanthomas on both knees and elbows. Blood analysis revealed extremely elevated total and LDL cholesterol levels (18mmol/l and 15mmol/l respectively) with normal triglycerides and HDL. Upon further examination the ratio ApoB/ApoA1 was 2,9 (reference <0.7). Arcus cornealis was present in both eyes of the patient. These findings were further supported by family history of coronary artery disease and sudden cardiac death, consistent with an autosomal dominant pattern of inheritance. Genetic analysis revealed that the patient is in fact a compound heterozygote for two mutations - c.1591A>G; p.(Lys507Glu) and c.2403_2406del, a condition that is equivalent in severity to the homozygous form of the disease. Treatment with a statin was initiated in the patient, later on the dose was doubled and a cholesterol absorption inhibitor (ezetimibe) was added with the overall effect of achieving a 50% decrease of LDL cholesterol levels, even though the target level of 3,5 mmol/l was not reached. In addition to a strict cardio-vascular follow-up and recommendations for lifestyle changes, our team is planning to include the patient into a program for receiving a PCSK9 inhibitor. LDL apheresis is currently not available in Bulgaria.

## PP-86 **ПЪРВИЧЕН ЗЛОКАЧЕСТВЕН МЕЛАНОМ НА ЖЕНСКАТА УРЕТРА**

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**Въведение:** Първичният, злокачествен меланом на урогениталният тракт е рядко заболяване. Най-често засяга уретрата и обикновено се открива случайно. Жените с тази локализация са само 0,2% от всички злокачествени меланоми. Уретралният малигнен меланом се среща предимно в бялата раса и е три пъти по-чест при жени отколкото при мъже.

Обективният преглед установява кървене, секреция понякога трудно уриниране. Откриването на уретралните меланоми в ранен стадий е трудно поради оскъдната симптоматика, което води до късно диагностициране и лоша прогноза.

Клиничен случай: Представяме Ви рядък случай на първичен злокачествен меланом на женската уретра. Пациентката Н.Д. е на 55 годишна възраст. Насочена за консултация поради неинтензивно кървене от областта на гениталиите. При физикален преглед се открива туморна формация около 3см в диаметър, черна на цвят, кървяща при допир ангажираща терминалната част на уретрата,част от малките срамни устни участъци от предна влагалищна стена.КАТ данни за единичен ингвинален лимфен възел в ляво.

Извършихме вулвектомия, парциална уретеректомия и лимфна дисекция в лява ингвинална област.

Патологоанатомичната диагнозата е злокачествен меланом на уретрата като не се установяват положителни лимфни възли. След около пет месеца се извърши трануретрална резекция на меланомни метастази в пикочният мехур, лимфна дисекция в дясна ингвинална област като и от двете интервенции се потвърди хистологично основното заболяване. В момента пациентката провежда курс лъчетерапия

**Дискусия/Заключение:** Уретралният злокачествен меланом има по-лошо прогноза от този на кожата.В цитираният случай играе роля и установяването му в авансирала фаза на вертикален растеж , установени местази в лимфните възли и пикочния мехур. Макроскопски уретралните меланоми са често полипоидни и могат да се объркат с други малигнени заболявания, дори и с бенигнени като уретрални полипи или пролапс на уретралната лигавицата. Лечението зависи най-вече от местоположението и клиничният стадий. Включва ексцизия, уретректомия с или без цистектомия, последващо лъчелечение на оперативната зона и регионалните възли или по-радикален подход – първична вулвектомия, уретректомия и дисекция на лимфни възли.

## PP-87 **КОМПЛЕКСНО ЛЕЧЕНИЕ ПРИ ПАЦИЕНТИ С БОЛЕСТА НА WILSON**

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**Основа:** Болестта на Wilson е 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.

**Предмет:** Представяме пациентка на 46 години с болеста на Wilson, посетила клиничните зали на ФДМ- Пловдив с необходимост от комлексно лечение. С прекаран исхемичен инсулт.

**Методи и резултати:** Комплексното лечение започна с екстракция на зъби под местна анестезия на пациентката, поради което се направиха пълна кръвна картина и биохимия с изследване на INR. Направено бе хистологично изследване, за установяване наличието на мед в лигавица и кост.След проведеното хирургично и терапевтично лечение се пристъпи към протетично лечение, целящо възстановяване на говора, функцията и естетиката. Планът на лечение включи снемаеми и неснемаеми конструкции без съдържание на мед в тях.

**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.

**Ключови думи:** morbus Wilson, INR, пръстен на Кейзър-Флейшър, комплексно лечние68

## PP-88 **ПРОЯВИ И ЛЕЧЕНИЕ ПРИ НЕВРОФИБРОМАТОЗА**

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Болестта на Recklinghausen е генетично заболяване и се унаследява автозомно доминантно. Различните типове се характеризират с кожни пигментации,тумори в централната и периферната нервна система,мозъчно-съдови увреди и различни патологии от страна на вътрешните органи. Засегнатите структури произхождат предимно от ембрионалната ектодерма и/ или мезодерма. Неврофиброматоза тип1 се диагностицира при деца, най-често до десетата година. Върху кожата се забелязват светлокафеви петна ( cafe au lait) ,голямо количество лунички,подутини причинени от туморите, аномалии по костите, може да бъде засегнат и n.opticus. Мозъчни инсулти с различна локализация също се забелязват при тези пациенти. Неврофиброматоза тип2 се среща по-рядко от предходната.Характерно за нея са доброкачествените бавнорастящи тумори обхващащи nn.acustici на двете уши. Проявите са предимно от страна на слухово-равновесния апарат-загуба на слух и равновесие. Понякога се появяват шваноми засягащи и краниалните,спиналните,оптични я или даифрагмалния нерв. Много рядко се развиват менингиоми и астроцитоми. Шваноматозата е много рядка и при ния не се обхващат нервите отговорни за пренасянето на информацията от вестибуларния и слуховия апарат до мозъка. Причинява хронична болка,изтръпвапия,слабост и загуба на мускулна маса. Локализирани са най-често на трети,пети,осми и девети черепно-мозъчни нерви. Заболяването не може да бъде излекувана напълно,но е възможно прилагане симптоматично лечение. Мониториране на състоянието се осъществява с цел проследяване прогресията на болестта. При влошаване на състоянието се пристъпва към хирургично отстраняване на туморните формации,лъчетерапия ,химиотерапия и медикаментозна терапия за овладяване на болката. При тип2 е възможна стереотактична радиохирургия позволяваща директното въвеждане на радиацията в тумора и запазване на слуха. Друго предимство на процедурата е,че не се изисква нарушаване на целостта на кожата. За подобряване на слуха се използват и кохлеарни и брейнстем импланти.

## PP-89 **ПНЕВМОРАХИС**

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**Въведение:** Пневморахис е остро настъпило постъпване на газ в гръбначномозъчния канал. Обикновено е следствие на травма или ятрогенно. Описани са случаи и на пневнморахис по съседство (при пневмомедиастинум или пневмоторакс). В литературата открихме един единствен случай на пневморахис в следствие на сепсис. Ние описваме случай на пневморахис по съседство при ретрофарингеален абсцес.

**Клиничен случай:** Касае се за пациент на 61 години с новооткрит, не лекуван ИЗЗД I, който постъпва в клиниката по УНГ в УМБАЛ "КАСПЕЛА" поради силна болка в гърлото, невъзможност за приемане на течности и храна, фебрилитет до 39,8°С, засилващи се в хода на една седмица. От физикалния статус се установява: foetor ex ore, изтичане на слюнка от устата премесена със сивкаво черен ексудат, липса на тризъм, при мезофарингоскопия - хиперемия и подуване на задна фарингеална стена с малка язва, от която изтичаше некротичен субстрат. WBC: 38x109/l, glu: 26,4 mmol/l

КТ на шията установи газови колекции ретрофарингеално, латерофарингеално, предимно в ляво, в областта на тила -

под вратната мускулатура, в спиналния канал от нивото на върха на аксиса до 2ри торакален, освен това и субклавикуларно и около купулите на белите дробове двустранно.

Бе инцизирано ретро и латерофарингеалното пространство и започната терапия с Clindamicyn, Metronidazole, Meropenem, Amikacin. Пациента бе настанен в интензивно отделение с рефрактерен срив на хемодинамиката, полиорганна недостатъчност и екзитус леталис.

**Заключение:** Макар и рядко пневморахис може да настъпи по съседство в следствие на агресивна инфекция в областта на устната кухина и фаринкса. Той е много лош прогностичен белег.

**Ключови думи:** Пневморахис, ретрофарингеален абсцес, парафарингеален абсцес

## PP-90 **БОЛЕСТ НА WHIPPLE**

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**Въведение:** Болеста на Whipple е рядка, хронична, системна инфекция, с многообразни клинични прояви, причинена от Tropheryma whipplei, грам-положителен вътреклетъчен бацил. Често се манифестира като хроничен серонегативен олигоартрит или полиартрит, които могат да имитират различни ставни заболявания (ревматоиден артрит или спондилоартрит).

**Клиничен случай:** Пациент на 68 г. с диагностициран Ревматоиден артрит /RF отрицателен/ през месец април 2016 г. по повод полиартралгия. Изготвен протокол за лечение с Adalimumab в клиника по Ревматология, което не беше започнато. Хоспитализиран в клиника по Гастроентерология поради фебрилитет, загуба на апетит, редукция на тегло и диариен синдром, появил се е след започнато лечение с метотрексат. При хоспитализацията /06.2016г/ се установиха желязодефицитна анемия, хипопротеинемия, завишени острофазови белтъци. Регистрираха се ежедневно субфебрилитети до 38°C. Резултатът от колоноскопията беше нормален и биопсията също така. Резултатът от гастроскопията показа дифузна чревна лимфангиектазия. Бяха взети дълбоки дуоденални биопсии със следващата хистологична находка – в серийно изследвани фрагменти на тънкочревна мукоза, включваща и мускулната лигавична пластинка, в ламина пропия са налице множество макрофаги чието съдържимо е PAS- позитивно и диастаза резистентно. Установяват се и мастни вакуоли. Находката насочва към болест на Whipple. PCR анализ за откриване на T. whipplei не беше направен. Предвид гореописаните симптоми и хистологична находка приехме диагнозата болест на Whipple. Стартира се лечение с Ceftriaxon 2гр, i.v. две седмици с последваща перорална терапия с Trimethoprim and Sulphamethoxazole с клинично подобрение на втората седмица.

Актуално състояние: В добро общо състояние. На 9-ти месец от лечението.

Актуална терапия: Бисептол 2х960мг/дн.

Параклинични изследвания: В референтен интервал.

**Заключение:** Болеста на Whipple трябва да се подозира при всички пациенти, диагностицирани с Ревматоиден артрит, частично контролиран или неконтролиран с anti-TNF-α , чието състояние се влошава след лечението.

**Ключови думи:** Болест на Whipple, Tropheryma whipplei Ревматоиден артрит, алфаTNF

## PP-91 **ФАРМАКОГЕНОМИКА НА АНТИХИПЕРТЕНЗИВНАТА ТЕРАПИЯ**

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**Въведение:** 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.

**Цел:** Настоящият обзор обобщава наличните данни за генетичните сигнали, свързани с терапевтичния отговор и нежеланите последствия от приложението на основните групи антихипертензивните лекарства.

**Метод:** Литературен обзор.

**Резултати:** 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. Тестване 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. NEDD4L кодира белтък, свързан с натриевия транспорт в бъбрека, а ADRB1 кодира бета-адренергичния рецептор. Намерените полиморфизми са от значение както за предразположението към хипертония, така и за терапевтичния отговор. Все още няма готовност за практическото им използване. Фармакогеномиката на калциевите антагонисти и алфа-блокерите е много малко изследвана. В кардиологията има генетично-базирани насоки за лечение с клопидогрел, аспирин, 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 към клиничната практиката.

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.

**Ключови думи:** фармакогеномика, хипертония, ренин-ангиотензинова система, бета-блокери, тиазиди

## PP-92 **СИНДРОМ НА ФЕЛТИ – КЛИНИЧЕН СЛУЧАЙ**

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Синдромът на Фелти (Felty syndrome – FS), първоначално описан през 1924, е комплекс от симптоми асоцииран с тежка форма на протичане на серопозитивен ревматоиден артрит. Характеризира се с триадата: ревматоиден артрит, спленомегалия и гранулоцитопения. Въпреки безсимптомното протичане при много пациенти със FS, някои от тях могат да развият животозастрашаващи вторични инфекции поради намалената имунна резистентност. Контролът и лечението на основното заболяване (ревматоиден артрит) е в основата на успешните терапевтични стратегии при лечението на FS. Едва 1% от болните, диагностицирани с ревматоиден артрит развиват FS.

Представяме клиничен случай на 42 годишна жена, постъпила в ревматологично отделение по повод болки и скованост предимно в малките стави на ръцете с давност повече от 1 година. Без анамнеза за травми и прекарани инфекции. От прегледа се установяват болезнени и оточни гривнени, метакарпофалангеални и проксимални интерфалангеални стави. Ултрасонографски установени данни за възпалително ставно заболяване. При палпация и УЗ изследване – спленомегалия. Кръвните изследвания показват данни за анемия, левкопения, положителен ревмафактор, повишено CRP и утайка. На базата на проведените клинични и параклинични изследвания беше поставена диагнозата ревматоиден артрит със синдром на Фелти.

Горепосоченият клиничен случай представлява интерес поради ниската честота на изява на FS при болни с ревматоиден артрит, както и разнообразната клинична изява и необходимостта от мултидисциплинарен подход при избора на лечение.

## PP-93 **ИНСУЛИНОВА РЕЗИСТЕНТНОСТ ПРИ ПАЦИЕНТ С АКРОМЕГАЛИЯ – КЛИНИЧЕН СЛУЧАЙ**

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**Въведение:** Акромегалията е ендокринно заболяване, причинено от свръхпродукцията на соматотропин. По отношение на въглехидратната обмяна, хормонът има контраинсуларно действие и в условията на хронично повишени нива се наблюдава инсулинова резистентност с хиперинсулинемия и отклонения в гликемичния контрол до захарен диабет.

**Клиничен случай:**

*Анамнеза*

Представен е клиничен случай на 65 годишна жена с оплаквания от сухота в устата, неутолима жажда, обща отпадналост, прием на по-голямо от обичайното количество течности, често уриниране на обилно количество бистра

урина. Хоспитализирана по повод метаболитна декомпенсация.

Преди 11 години, по повод налична акромегалия от средно тежка степен на базата на соматотропинсекретиращ аденом на хипофизата е осъществена оперативна интервенция на същата.

*Параклиника*

Резултатите от глюкозния тест са положителни. Извършените кръвно-захарния профили показват стойности на кръвната захар над 9-10 mmol/l., HbA1c – 8,2%, ниска стойност на С-пептид, липсва глюкозурия.

*Терапия*

Назначава се терапия с бързодействащ инсулин според актуалната гликемия. Не се наблюдават прояви на хипоглемия.

*Диагноза*

Поставя се диагноза декомпенсиран неинсулинозависим захарен диабет.

**Обсъждане:** Нарушения гликемичен контрол при акромегалия може да се обясни с директния хипергликемизиращ ефект на ексцесивните нива соматотропин. Соматотропинът повишава инсулиновата резистентност и намалява инсулиновото действие, което резултира в супресия едновременно на чернодробния глюкозен метаболизъм и на инсулинозависимата тъканна дистрибуция. Компенсаторно се развива хиперинсулинемия, водеща до β-клетъчна недостатъчност и развитие на диабет.

Клиниката на диабета при акромегалия представлява диабет тип 2.

Етиологичното лечение е оперативно, със или без последваща лъчетерапия, медикаментозно.

При успехи и краткотрайна давност на глюкозния интолеранс, при някои пациенти е възможно пълно излекуване.

При персистиране на захарния диабет, хипергликемията се третира според стандартите за лечение на диабет тип 2.

## PP-94 **ПАЦИЕНТ СЪС СВЕТЛОКЛЕТЪЧЕН САРКОМ НА ГОРНИЯ КРАЙНИК СЪС СЪРДЕЧНИ МЕТАСТАЗИ – КЛИНИЧЕН СЛУЧАЙ**

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**Въведение:** Светлоклетъчният сарком е рядък тип сарком, произхождащ от съединителната тъкан на кости, мускули и сухожилия на долния и горния крайник. Засяга предимно млади хора между 20 и 40 години и е резултат от транслокация в гените.

**Начало и развитие на заболяването:** През 2008 г. бива отстранена туморна формация, без хистология, от основата на дясната китка на пациента. Повторната ексцизия с хистология доказва малигнен тумор на периферен нерв (2011). Три години по-късно (2014) бива премахната нова формация – фиброзен хистиоцитом. В началото на 2015 г. е отстранен и доказан светлоклетъчен сарком. На PET/CT се установява висока фиксация в дясната китка и белодробни метастази. Следва ампутация на дисталната трета на дясната предмишница. Първият курс на лечение включва Epirubicin 60 mg/m2, Ifosfamide 1500 mg/m2. След общо 6 курса, в началото на 2016 г., не се налага по-висока ампутация и хирургично лечение на белодробните метастази. В началото на 2017 г. пациентът постъпва с перикарден излив, като в пункта се доказват малигнени клетки, а на PET/CT и трансторакална ехокардиография – метастатични формации в апекса на дясна камера и прехода към дясно предсърдие. Фракцията на изтласкване на дясна камера е 45%, с епикардни депозити и дифузно ангажиране на миокарда с наличие на хипоехогенни зони.

**Дискусия:** Касае се за рядък тип тумор, труден за диагностициране в ранните стадии. Засегнати са белият дроб, плеврата, ребрата и миокардът. Най-честите неоплазми, 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).

**Заключение:** Въпреки че първичните сърдечни тумори са изключително редки, метастазите в сърцето се срещат често. Ехокардиографията е добър способ за откриване а на сърдечно ангажиране след първични или метастатични тумори.

## PP-95 **УНГ ПРОЯВИ НА PEMPHIGUS VULGARIS**

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**Въведение:** Pemphigus vulgaris 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. Реализира се посредством акантолиза, дължаща се на синтеза на IgG антитела срещу десмозомалния гликопротеин и десмоглеина (Dsg), които се съдържат в кератиноцитите. Има два клинични типа на ПВ в зависимост от вида на доминиращите антитела. Мукозната форма (ПВМ) 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.

Независимо от формата си, може да се изяви от страна и на ларинкса. Той се засяга сравнително често, но въпреки това убягва от ДД план на заболяването.

**Клиничен случай:** Пациент с оплаквания от болки и чувство за чуждо тяло в гърлото в продължение на 1 месец. По този повод е проведено антибиотично лечение, което не довежда до положителен резултат. Проведена е мезофаринго- и риноскопия, които не показват патологични промени. Няма данни за везикули и булозни изменения. На индиректна ларингоскопия са установени налепи в двата recessus piriformis. Изписан е противогъбичен медикамент и пациентът е консултиран с 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. Като придружаващо заболяване се отбелязва тиреоидит на Хашимото.

Актуално състояние: в добро общо състояние

**Заключение:** Препоръчва се УНГ преглед да бъде рутинна част, защото 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.

**Ключови думи:** pemphigus vulgaris, anti-Dsg3, акантолиза, IgG

## PP-96 **СЛУЧАЙ НА ФАМИЛЕН ПСЕВДОХИПОПАРАТИРЕОИДИЗЪМ**

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Псевдохипопаратироидизмът \\PHP\\ представлява група редки заболявания характеризиращи се с хипокалциемия, хиперфосфатемия, нормална алкална фосфатаза и завишен паратхормон. Състоянието възниква в резултат на мутация в рецептора или пострецепторния път на предаване на сигнала . В част от случаите има резистентност и към други хормони чийто рецептори имат същата структура като тази на паратхормона \\използващи сАМФ като втори посредник\\-ТСХ, РХ и гонадотропни хормони.

PHP бива четири типа, които се различават по отговора на Паратхормон стимулационния тест-ниво на фосфатурията и сАМФ в урината, както и по наличието или не на АНО фенотип и резистентност към други тропни хормони.

АНО( Albright hereditary osteodystrophy) фенотип-нисък ръст, кръгло лице, брахидактилия, хлътнала основа на носа, УИ, ектопични калцификати-типично в базални ганглии и зъбни аномалии.

Пациентът-14 годишно момче постъпва по повод на първи гърч. От изследванията е с хипокалциемия-1.68 ммол\\л за общия калции и 0.74 за йонизирания, завишен фосфор 2.6 ммол\\л, нормални алкална фосфатаза и магнезии. Паратхормона е силно завишен-437 pg/ml при норма до 70. Детето е с нормален ръст и наподобяващ АНО фенотип-кръгло лице,хлътнала основа на носа, малки длани и стъпала с къси пръсти и рентгенови данни за хипоплазия на проксимална диафиза и основата на четвърта метакарпална кост двустранно. КАТ данни за калцификати в базални ганглии. Нормална тиреоидна функция.

Майката е с диагностицирана епилепсия на 10 год.възраст за което известно време е провеждала лечение. Има известен когнитивен дефицит. Има регистрирани ниски нива на калции, но допълнителни изследвания с цел уточняване не са правени. Нейните изследвания също са с хипокалциемия, хиперфосфатемия, нормална алкална фосфатаза и завишен паратхормон.

По-малкият брат-на 6 години е с тежка умствена изостаналост и аутистично поведение. От изследванията е с нормални калции ,фосфор, алкална фосфатаза и магнезии. Има силно завишен паратхормон и е без калцификати в базални

ганглии. Суспектен АНО фенотип-затлъстяване, кръгло лице, брахидактилия и хлътнала основа на носа.

Представяме рядък и труден за диагноза случай поради различният спектър на изява в различната възраст и най-вече късна хипокалциемия.

## PP-97 **FAMILY CASE OF INTERACTION OF TRIGGERS, EPIGENETIC MODIFIERS AND GENETIC EVENTS-JAK-TYROSINE KINASE DEFICIENCY (MUTATION V617F OF JAK2 GENE), METHYLENE TETRAHYDROFOLATEREDUCTASE DEFICIENCY (MTHFR 677 T /T) AND PERSISTENT BACTERIAL INFECTION**

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**Introduction:** Rare diseases are manifested in a person at all stages of ontogenesis, the launching of their manifestation is closely related to the presence of triggers, mediators and certain genetic events.

**Aim:** To study the role of interaction of triggers, mediators and mutations by the example of the clinical observation of the V617F mutation of the JAK2 gene in the family associated with the MTHFR677 T/T mutation.

**Results:** Among 99,000 patients at our center, the mutation V617F of the JAK2 gene was detected in two. Mutation leads to the development of myeloproliferative diseases with excessive production of mature cells of erythroid, megakaryocytic and granulocyte sprouts.

We manage a family. In daughter (33) we diagnosed chromosomal abnormality (karyotype 46,XX/46,XX, delX(p21-pter), mutation MTHFR 677 T/T, uterine leiomyoma, kerotoconus, low-growth (150 cm), signs of disembriogenesis, hyperhomocysteinemia, autoimmune thyroiditis; in the early neonatal period suffered staphylococcal sepsis.

The mother (66) suffers from progressing thrombophilic state, hypertensive disease, suffered stroke and heart attack, has stable thrombocytosis (439-518*109) and hyperhemoglobinemia (179-180 g/l), varicose veins, persistent streptococcal infection, fibrocalcinosis of the root and valves of the aorta, fibrosis of the mitral valve flaps, organized thrombosis in the region of the left ventricle apex. Insufficiency of JAK tyrosinekinase is suspected. Molecular diagnostics revealed a homozygous mutation V617 of the JAK2 gene, mutation MTHFR 677 T/T.

The developed strategy of treatment under the control of multiparameter data, allowed to stabilize the process and provide favorable social and medical status.

**Conclusion:** In the present observations clearly observed interaction 3 etiopathogenic factors - triggers (infection, violation of epigenetic status (mutation MTHFR 677 T/T) and genomic disorders (mutation V617F of JAK2 gene in the mother and chromosomal structural aberration in the proband)) has been suggested that the clinical genetics enters the successful path pathogenetic therapy combining circuit events listed in personalizing treatment.

## PP-98 **CLINICAL CASE OF A PATIENT WITH ARNOLD-CHIARI SYNDROME**

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Arnold-Chiari's syndrome is a congenital malformation of the brain. It is characterized by prolongation and protrusion of the cerebellar tonsils through foramen occipitale magnum to the cervical spinal cord. There are four main types of the syndrome.

We present a rare case of a 25 year old patient with Arnold-Chiari syndrome type I. The debut of the disease was at the age of 8 years. The patient reported severe neck pain, often accompanied by headaches and sometimes irritation to the left shoulder. The Arnold-Chiari type 1 diagnosis is based on existing complaints, physical examination and MRI. In 2008, he was targeted for surgery due to the downward displacement of cerebellar tonsils through the large occipital opening and syringomyelia from C3 to C6. A suboccipital craniotomy was performed, including removal of foramen magnum.

This condition is rare and due to common and non-specific complaints, it can simulate other diseases and patients are often misdiagnosed.

## PP-99 **ПЪТЕШЕСТВИЕ В ГАСТРОЕНТЕРОЛОГИЯТА**

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Гастроентерологията е наука, обхващаща дълбините на всеки един проблем, свързан с храносмилателната система или водещ до последствия в нея. Пътешествието в тази област започна със случай на пациента М.С., постъпил в клиника по повод оплакване от подуване на корема. В началото е диагностициран с Полицитемия Вера. След многобройни изследвания е установена основната диагноза – Синдром на Budd – Chiari. Честотата на това заболяване е около 3-8 пациента на 100 000 души. В основата на този процес стои запушването на чернодробните вени, в резултат на което произлизат и придружаващите заболявания на разгледания пациент, а именно портална хипертония, спленомегалия, малки по размер варици на хранопровода, хроничен еритемо – ексудативен пангастрит, портална хипертензивна гастропатия и хронична еритремия.

Преди точното установяване на диагнозата, пациентът М.С. е в силно увредено общо състояние. Наблюдават се и отклонения при физикалния преглед и резултатите от инструменталните изследвания. Основният метод, благодарение на който са установени по-горе посочените отклонения, е фиброгастроскопията. След проведеното болнично лечение в Клиника по Гастроентерология към УМБАЛ „Св. Марина“ – гр. Варна, пациентът е изписан с видимо подобрение и с приложение на терапия в домашни условия, включваща бетаблокер, диуретик, хепатопротектор, антикоагулант и др., прилагащи се по съответна схема.

Прилаганото лечение може да подобри състоянието на пациентите в краткосрочен период. За дългосрочни цели се използват различни видове хирургични техники – балонна дилатация с поставяне на стент в съответния кръвоносен съд, ексцизия на увредения участък, използвайки транскардиален подход, създаване на шънт за трансфер на кръв от чернодробния кръвоток в горна кава с цел намаляване на налягането, предотвратяване на асцит и кървене, трансплантация на черен дроб и др.

## PP-100 **ДИАГНОСТИЧНИ ПРИЙОМИ И ТЕРАПЕВТИЧНИ ПРАКТИКИ ПРИ BCKD ДЕФИЦИТ (БОЛЕСТ НА УРИНА КАТО КЛЕНОВ СИРОП)**

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**Въведение:** Болестта на урината като кленов сироп (BCKD-дефицит) (МКБ Е71.0) е резултат от генетичен дефект в декарбоксилирането на аминокиселините с разклонена верига (левцин, изолевцин, валин). При него се натрупват междинни катаболитни деривати – α-кетокиселини, водещи до метаболитна декомпенсация, неврологични и невродегенеративни изменения. Урината на пациентите има характерна миризма на кленов сироп. Ензимопатията засяга средно 1:200000 раждания, без тенденции за пол или раса, но увеличена заболеваемост сред отделни контингенти.

**Цел:** Относително ниския брой регистрирани случаи годишно, изисква обобщаване на натрупания клиничен опит за заболяването от откриването му през 1954 г. до наши дни. Утвърдените добри диагностични и терапевтични практики са гарант за намаляване смъртността и необратимите усложнения.

**Материали и методи:** Анализирани са редица официални статии и съобщения относно диагностичния път и терапията на заболяването спрямо клиничната форма. Оценена е ролята на съвременните технологични средства за скъсяване времето на диагностичния процес и въвеждане на храни с намалено съдържание или с отсъствие на разклонени аминокиселини.
**Резултати:** Златният стандарт в диагностичната практика е изследването на отношението между есенциалните аминокиселини, верифицираща клиничната суспекция и определяща типа на заболяването – класически, междинен, интермитентен или Е3-дефицитен. Показателно е доказването на алоизолевцин в урината (след 6-тия ден от раждането), както и на α-хидроксиизовалерат, пируват, лактат и α-кетоглутарат чрез газова хроматография с мас-спектрометрия. Етиологичното лечение включва диета със строга редукция на храните, съдържащи разклонени аминокиселини, а патогенетичното – корекция на проявите на метаболитна декомпенсация. Чернодробната трансплантация е успешен ход само при пациенти без неврологични усложнения.

**Обсъждане:** 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 урина 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.

## PP-101 **EXTENDED ANALYSIS OF EXOME SEQUENCING DATA IMPROVES DIAGNOSTIC YIELD IN RARE DISEASES**

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**Background:** Exome sequencing has become an essential mode of diagnostics in rare genetic disorders. Considering that exome sequencing is primarily aimed at detection of single nucleotide and insertion deletion variants in coding regions of genes, it may fail to detect a considerable proportion of causative genetic variation.

Recently, several studies have shown that information content of exome sequencing data allows for the expansion of the scope of detectable variation, which may be interrogated with extended bioinformatic analyses.

**Methods:** To address this issue, we implemented routine use of approaches of extended analysis in our diagnostic exome analysis pipeline, including (1) copy number variation (CNV) detection, (2) genomic breakpoint detection, (3) non-consensus splice defect detection, (4) homozygosity mapping and (5) mitochondrial variant analysis. We retrospectively analysed the results of genetic testing in 1.087 distinct cases referred for exome sequencing to our institution.

**Results:** Combined use of selected extended exome analysis approaches assisted in identification of causative genetic variant in 47 cases. This represented an 4.3% increase in diagnosed cases, raising the overall diagnostic yield from 35.6% to 39.9%. In particular, we observed that integration of data from several extended exome analysis approaches showed benefit in diagnosing most challenging cases, that would otherwise only be resolved using whole genome sequencing. We will present a selection of such cases and our experience in using these approaches in routine diagnostic exome sequencing.

**Conclusions:** In conclusion, we show that routine use of extended exome analysis approaches improves genetic diagnosis of heterogeneous genetic disorders and results in considerable increase of diagnostic yield of exome sequencing. Considering the increasing availability and clinical use of exome sequencing, the use of these approaches may improve and facilitate reaching the final diagnosis in patients with rare disease.

## БЕЛЕЖКИ

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