

# **ABSTRACT BOOK**

## **The 9th Balkan Congress of Medical Genetics**

**September 15 to 17 in Timisoara, Romania**



# Keynote lecture

## L 01. Duchenne/ Becker muscular dystrophy: from bed-side to bench-side and vice versa

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The case of Duchenne and Becker muscular dystrophies (DMD/BMD) epitomizes the tremendous impact of molecular genetics upon medicine. Starting in the early 80's a frantic search for the responsible gene(s) was undertaken, ultimately ending with the discovery of a single gene involved in both diseases: the DMD gene encoding a novel protein, dystrophin. This feat was achieved by positional cloning based on the information derived from the in-depth exploration of pedigrees and individual DNA data. In this « Grail quest » the contribution of patients and their family was crucial to corner the culprit gene and to incriminate it. From the knowledge of the 11 kb coding sequence and ultimately of the entire gigantic 2.3 Mb DMD gene a wealth of information was deduced. Some provided an immediate profit-on-return to the patients : (i) positive and differential diagnosis firmly established on molecular grounds at all levels (protein, gene, RNA messenger ; (ii) genetic counseling, both pre-conceptual (detection of female carriers) and post-conceptual (prenatal diagnosis) ; (iii) prognosis based on the impact of the mutation on the reading frame. Besides these benefits the patients and the families reasonably expected to gain a cure for the disease or at least some therapeutic progress. These expectations have not yet been fulfilled, showing that the identification of a disease gene does not automatically provide a therapy. This is a source of big disappointment among both the patient and the scientific communities. The main reason for this gap is that the scientists have been ingenuously convinced that gene therapy was the unique solution to cure dystrophinopathies. After 20 years of intensive fiddling around with the false concept of "DNA-as-a-drug", it became obvious that this strategy was a dead end at least for muscle dystrophies. Fortunately we now have innovative smart strategies based on targets located downstream the gene, in particular at the transcript level with promising results of clinical trials using antisense exon skipping promoting drugs. In addition besides these efforts to fight directly the primary defect by restoring some production of dystrophin, there are other trails consisting in combating downstream patho-physiological consequences such as inflammation, fibrosis, lack of regeneration etc. There is now a world-wide effort aiming at exploring these new avenues, with powerful tools such as proteomics, high-throughput pharmacogenomics and new integrated cell biology. In addition it is now clear that in each patient multiple targeting strategies will have to be combined. This means that the long awaited return from bench-side to bed-side is en route. One can anticipate that the first significant therapeutic success (such as transforming a DMD phenotype into a BMD one) will be obtained in the next few years.

## **L 02. Clinical, genetic and epidemiological study of prevalent autosomal recessive limb girdle muscular dystrophies (LGMD2) in Croatia**

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Autosomal recessive limb-girdle muscular dystrophies (LGMD2) form a group of muscle diseases presenting great clinical and genetic heterogeneity making an etiologic diagnosis very difficult and clinically in majority of patients impossible. Here we report results concerning LGMD2 obtained during 10-years long prospective study on clinical, genetic and epidemiological aspects of muscular dystrophies (MDs) in Croatia. Specific diagnostic strategy was used to speed genetic study up. Emphasis was on: 1. Clinical assessment with CK, EMG; muscle CT or IRM and ECG; 2. Genealogical study with intensive search for secondary cases discovered through a detailed and systematic examination of parents, children and/ or sibs, when necessary; Search for consanguinity; Geographical origine of parents. From the very beginning selection of families with at least two patients; 3. Indirect or direct molecular analysis. The study showed that calpainopathy (LGMD2A) was the prevalent LGMD2. Analysis of 36 apparently unrelated families with 52 patients discovered eight different CAPN3 mutations: 550delA, R541W, P82L, delFWSAL, R49H, Y537X, 2242C>T and 1696G>A including 95% of CAPN3 chromosomes in the studied population. 550delA was the most frequent mutation found on 53/72 (73, 3%) chromosomes. Other seven mutations ranged from 8, 3% to 1, and 3%. In 33 of 36 families, two CAPN3 alleles were identified. In remaining 3 families with only one known CAPN3 allele, 550delA was present in 2 of 6, and P82L in one of alleles. The second, most common LGMD2 seems to be type 2I caused by mutation in FKRP. Direct analysis of only one mutation (C826A) allowed us to identify six unrelated families. One of six homozygous C826A probands was in addition heterozygote for 550delA. Dysferlinopathy was found in three patients from two unrelated, informative families. Diagnosis was based on clinical features (LGMD2B / MM), haplotype analysis and non-invasive monocyte Western blotting. Molecular analysis finally confirmed diagnosis discovering two novel DYSF mutations. Surprisingly, we haven't identified any sarcoglycanopathy, probably because of sampling bias (small number of children) and limited methodology (lack of muscle biopsy and WB of different proteins). Because of high frequency of healthy 550delA heterozygotes (1 in 133) and C826A heterozygotes (1 in 404) in our general population, we need to know both allele to confirm the diagnosis of LGMD type 2A and 2I. In conclusion knowledge of the mutation spectrum occurring in the CAPN3 should help in the design of efficient mutation-screening strategies for calpainopathies in our country. Concerning study of natural history of any LGMD2, both alleles should be known. Moreover, the follow up of genetically homogenous patients' groups using well defined, but as simple as possible protocols would be advisable. Keywords: LGMD2, clinical characteristics, genetics, epidemiology, Croatia Supported in part by Ministry of Science and Technology Republic of Croatia research grand 108-0000000-3435

### **L 03. Progress and hurdles in the diagnosis of autosomal recessive Limb-Girdle muscular dystrophies (AR-LGMD)**

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AR-LGMD is a group of monogenic muscular diseases characterized by progressive muscle wasting due to a chronic process of dystrophy affecting predominantly proximal limb muscles. This broad label covers a variety of overlapping phenotypes determined by a variety of mutations in at least 15 different genes. This heterogeneity generates diagnostic problems since the molecular characterization of the causative mutation in each patient has become mandatory for adequate genetic care. To solve the uncertainty inherent to each case one has to combine information derived from a series of tests. Clinical thorough examination and muscle imaging are essential but not sufficient. In most cases a muscle biopsy is necessary for full pathology examination and as a source of material for protein analysis (both by immuno-histology and western-blotting), and sometimes for cDNA sequencing. The ultimate step is DNA analysis to uncover the underlying genome defect. Contrary to the X-linked dystrophinopathies (DMD and BMD) which also affect proximal muscles, most of the gene defects involved in AR-LGMD's represent a large spectrum of point mutations requesting sequence analysis. At the present time we have to proceed gene by gene, under the guidance of clinical hints and whenever possible of multiplex western blotting. Great expectations to overcome these molecular diagnostic hurdles are now invested in new high through put technologies such as (i) CGH arrays on microchips, (ii) dedicated resequencing microchips, (iii) full exome sequencing.

### **L 04. Water channel proteins (aquaporins and relatives): from their discovery in 1985 in Cluj-Napoca, Romania, to present genetic aspects**

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Water channels or water channel proteins (WCPs) are transmembrane proteins that have a specific three-dimensional structure with a pore that can be permeated by water molecules. WCPs are a large family (over 450 members) that are present in all kingdoms of life. The first WCP was discovered in the human red blood cell membrane in Cluj-Napoca, Romania, in 1985 by Gheorghe Benga's group and reported in publications in 1986. In addition, we also have a priority in the discovery of the implications of WCPs in human diseases: epilepsy and Duchenne muscular dystrophy (DMD). In 1990's other WCPs were discovered in plants, microorganisms, various animals and humans and became obvious that the WCPs belong to the superfamily of major intrinsic proteins (MIPs, over 800 members), that has two other families: glycerol facilitators (GlpFs) and MIPs without identified channel activity. WCPs include

two subfamilies: aquaporins (AQPs), which are specific water channels, and aquaglyceroporins, which are permeable to water and other small molecules. A synthetic overview on the 13 mammalian MIPs will be discussed, with genetic aspects, physiological roles and implications in various diseases.

#### **L 05. Non-malignant aneuploidization of the human brain is a susceptibility factor for complex neuropsychiatric diseases**

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Recently, it has been suggested that aneuploid cells can populate the normal human brain. The human developing brain has demonstrated extremely high rate of mosaic aneuploidy affecting 30-35% of cells. During following ontogenetic stages the brain has exhibited significant decrease to achieve the rate of 10% in adulthood. Assuming that these processes can be altered, it was hypothesized that aneuploidization of the brain is a susceptibility factor for complex neuropsychiatric diseases (Iourov et al., 2006; Yurov et al., 2010). Here, we provide the support of this hypothesis. High level of mosaic aneuploidy involving specifically chromosomes 1, X and 18 was found in 2-5% of neuronal cells of schizophrenia brain. Additionally, chromosome 1-specific instability was shown to affect the schizophrenia brain. Increased aneuploidy rates affecting exclusively chromosome 21 was observed in aging Alzheimer's disease (AD) brain and chromosome 7-, 14- and X-specific instability was found to affect the ataxia telangiectasia (AT) brain. We have also analyzed aneuploidy rate in different areas of the AD and AT brain differentially affected by neurodegeneration. The incidence of abnormal neural cells affected by aneuploidy was significantly higher in degenerating brain areas (hippocampus, prefrontal cortex -- AD; cerebellum -- AT) as to unaffected areas. This suggests that non-malignant aneuploidization of the brain does contribute to pathogenesis of brain diseases. To this end, it is to conclude that these molecular neurocytogenetic studies provide for a new molecular and cellular mechanism of intercellular neural genome diversity representing a susceptibility factor for brain diseases. Supported by BMBF/DLR (RUS 09/006).

#### **L 06. Signaling complexes at the beta-arrestins: cross-talk between receptor tyrosine kinases and G-protein coupled receptors**

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Cancer is today one of the most important public health problems in Europe: there are 3.2 million new cases and 1.7 million deaths each year. With the ageing of the European population these numbers are predicted to steadily increase. Therefore research must focus on disease etiology, new medicines and therapies as well on identification and validation of drug targets and biomarkers that aid in the

prevention, early diagnosis and more tailored treatment. In the selection leading to cancer, cancer cells make use of the normal extracellular signaling for proliferation, migration and/or antiapoptosis to gain a growth advantage over normal cells. These signals are, in part, generated by plasma membrane receptors. The present report refers to the cross-talk between two major classes: G protein-coupled receptors (GPCR) and growth factors receptors (receptor tyrosine kinases, RTKs). Receptor cross talk is of major importance for cells as they must organize responses to multiple extracellular signals. This cross-talk is even more complicated in a multicellular organism by interactions between cells. The fundamental basis of the present report is that a cancer cell exploits these extracellular signals to gain a growth advantage over normal cells. Each cell surface receptor family possesses unique structural characteristics and each leads to specific signaling outcomes in the cell. On the other hand, different receptor families utilize many common intracellular signaling proteins and activate many common signaling pathways. For example, during the last years we have clearly demonstrated that RTK-mediated activation of p42/44 MAPK, are modulated by beta-arrestins, proteins previously considered to be associated exclusively with GPCR. Because of these similarities, cross talk mechanisms between RTKs and GPCRs are likely to include changes in the levels and functions of these common proteins, and such changes may be exploited by the cancer cells. We are reporting here how we are specifically looking for such changes as well as for modalities to reverse them for therapeutic purposes.

#### **L 07. Molecular genetics of premalignant and malignant lesions of the oral cavity**

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Background: oral squamous cell carcinomas (OSCCs) are associated with poor prognosis, and despite advances in therapy approaches, no major improvement in survival has been achieved in the recent years. Efforts are now directed toward finding new biological markers that could more accurately predict tumor behaviour. OSCCs arise from a progressive accumulation of genetic and epigenetic lesions. Aim: to review some of the results of our analyses directed at understanding the mechanisms underlying OSCC pathogenesis. Methods: by means of dPCR, PCR/RFLP and sequencing, mutational analysis of major cancer genes in premalignant and malignant oral lesions and in surrounding normal mucosa has been performed. The tumour tissue has also been analyzed for the presence of oncogenic HPV. Finally, association studies have been used to determine the potential role of some polymorphisms in the susceptibility to OSCC Results: as expected, the most frequently mutated gene in both malignant and premalignant tissues was TP53 (in 60 and 42 % respectively). Furthermore, normal mucosa surrounding the excised tumours harboured TP53 mutations at high percentage (30%). Alterations in H.ras, c-erb and c-myc genes were also frequently encountered in the three types of tissue and their incidence ranged between 20 and 35%. Interestingly, only 10 % of OSCC had an HPV infection. Polymorphisms in genes involved in the control of detoxification, such as glutathione transferases showed a significant association with OSCC. Conclusions: Molecular analysis of somatic changes and genetic polymorphisms

may enhance, our knowledge of OSCC pathogenesis, progression, recurrence and metastasis risk, but also enable the identification of individuals with predisposition to tumours.

#### **L 08. Single nucleotide polymorphisms at five loci are associated with C-reactive protein levels in a cohort of Filipino young adults**

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C-reactive protein (CRP) is a component of non-specific immune defense and is a reliable marker of low-grade inflammation involved in obesity, type 2 diabetes and cardiovascular disease. Genome-wide association studies (GWAS) in middle-aged and elderly populations, predominantly of European descent, demonstrated associations of CRP levels with SNPs at several loci. To examine whether the variants identified are replicated in Filipino young adults, we applied Tobit regression models to study the association of plasma CRP with 12 SNPs at seven loci in a cohort of 1,691 Filipino young adults (aged  $21.5 \pm 0.3$  years) from the Cebu Longitudinal Health and Nutrition Survey (CLHNS). SNPs in or near CRP ( $P = 3.2 \times 10^{-11}$ ), HNF1A, IL6R, APOE-APOC1 and LEPR showed significant associations ( $P < 0.05$ ) and together explained 4.8% of the total variation in CRP. Modest interactions were observed between LEPR rs1892534 and waist circumference (uncorrected Pinteraction = 0.020) and between APOE rs769449 and pathogen exposure (uncorrected Pinteraction = 0.0073) in models predicting CRP. Our results demonstrated that variants in several loci are significantly associated with plasma CRP in Filipino young adults, suggesting shared genetic influences on circulating CRP across populations and age groups. Key words: C-reactive protein (CRP) / Filipinos / genetic association / interaction / SNP / young adults.

#### **L 09. Overview of the current European Society of Human Genetics activities**

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The European Society of Human Genetics ([www.eshg.org](http://www.eshg.org); ESHG) is a non-profit, non-governmental organization which has two main aims: a/ to promote research in basic and applied human and medical genetics and to b/ to ensure high professional standards in diagnostic and clinical practice. ESHG also facilitates contacts between scientists and professionals who share these aims, in particular those working and/or residing in Europe. ESHG was established in 1967 and is one of the founding members of

the International Federation of Human Genetics Societies. In brief: ESHG has an Executive board who reports to the governing Board, whereby broadest possible representation of the field is assured. ESHG also has several important committees such as the Annual Meetings Committee who selects venues for ESHG conferences (which have over 2000 participants, including an increasing number of exhibitors), the Scientific Programme Committee who prepares attractive scientific programs for these meetings, Educational Committee dealing with pre-graduate and post-graduate education in genetics, or the Public and Professional Policy Committee who issues consensual policy documents and/or position statements on current topics. Activities of its Quality Committee, dealing with coordination of external quality assessment in molecular genetics and cytogenetics stem from the European Commission Network of Excellence project EuroGentest ([www.eurogentest.org](http://www.eurogentest.org)) that aimed at harmonization and standardization of genetic services in Europe. ESHG works closely with European National Human Genetics Societies. One the recent examples of successful collaboration have been joint initiatives leading to the official recognition of clinical-/medical genetics by the European Union (EEA) via inclusion of this specialty into Directive 2005/36 or national endorsements of the ESHG official response to European Commission Public Consultation on the amendment of the "IVD" Directive 98/79/EC.

#### **L.10 Clinical genetics-Present and future in the Timisoara Regional Centre**

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Like other medical centers in Europe, pediatricians were the first and most open to understanding the genetic aspects of child pathology. Our hospital started in the late 70 to provide cytogenetic investigations. Tests were available inconstantly, according to technical possibilities of the time. In the past 20 years, efforts of specialists from the hospital, in association with various medical teams in Timisoara, Romania and Europe have made remarkable progress in investigating the complex genetics of malignant blood disorders in children. Our hospital is the first in Romania to do bone marrow transplant in children. Due to the coordination of national programs targeting rare diseases, such as hemophilia, thalassemia, primary immunodeficiency, cystic fibrosis, Prader Willi syndrome, Duchenne and Becker myodystrophia, our center has improved its human and material resources, gaining experience for the best interest of patients. Through its experts, L. Turcanu Children's Hospital is the most actively involved medical unit in shaping policies for rare diseases and sanitation in Romania. To be more efficient and benefit from the experience of other European medical teams, the hospital is involved in numerous European projects and collaborations that have brought the health status of reference unit. In the near future, our center will run a large cross-border project (Romania-Hungary) for antenatal and neonatal screening, in western part of the country, which will help prevent genetic diseases and improve early diagnosis. All the activities of our center need to be enacted under the umbrella of reference centers, which, in turn, would eliminate gaps and ensure a proper management in rare diseases.



## **L 11. The role of the family and patient organizations in the case of Rare Diseases**

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RPWA/ RONARD/ ARCrare

Introduction: Most of the organisations for RD patients are established by patients or parents. Why?  
Debate: Parents and patients feel alone and scared with their own problems. Having a rare disease can make you or your child feel isolated. You may feel as if you're the only one dealing with this illness because you don't know anyone else who has the same condition. The specialists you go to may have only seen a few people with the same rare condition (or don't know it at all). Trying to explain your health condition to someone else can be difficult. Sometimes, people might have difficulty looking at you or talk down to you. If you look different than most people, some people may treat you in insensitive ways. People may offer you advice about how to relieve your symptoms that they know nothing about. In some RD, your disease is all inside and isn't obvious, people may wonder why you complain or go to the doctor so often because you don't "look sick". This is why people affected are trying to join a rare disease support group or create themselves one group or one organization and then, join their efforts with other similar NGOs at international level. National Alliances for RD and EURORDIS at EU level had joined the efforts At EU level for the implementation of Council Recommendation in the field of RD. Already in the Whereas of the Council Recommendation, it is stated that rare diseases call for a global approach based on special and combined efforts to prevent significant morbidity or avoidable premature mortality, and to improve the quality of life and socio- economic potential of affected persons. Amongst the recommendations addressed to Member States by the Council of the European Union, Member States are invited to elaborate and adopt a plan or strategy (...) within the framework of their health and social systems and to take note of the guidelines and recommendations developed in the framework of the EUROPLAN project, where our alliances and EURORDIS have a very important advocacy role. Conclusion: Rare diseases pose a unique challenge to health care professionals and health systems given the scarcity of resources targeting the specific needs of RD patients and their families.

## **L 12. Molecular profiling of infertile men**

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Impaired fertility of the male partner is causative or contributory in up to two-thirds of all couples unable to conceive spontaneously. In about 15% of infertile males genetic abnormalities could be present. Genomic and proteomic research offer new tools for better understanding of the genetics of male infertility. We have performed an extensive molecular profiling of infertile men, including

screening for the presence of sex chromosomal aneuploidies and AZF deletions, clarification of the role of partial AZFc deletions and duplications; screening for the presence of mutations in the cystic fibrosis (CFTR) and androgen receptor (AR) genes, investigation of single nucleotide polymorphisms (SNPs) in different genes; determination of copy number variations (CNVs) and proteomic profiling of seminal plasma and sperm cells. Our results showed that Klinefelter's syndrome and complete AZFc deletions are the most common genetic causes of azoospermia. Partial AZFc deletions, as well as AZFc duplications were present both among infertile and fertile men. They may represent a risk factor for male infertility when present on certain Y chromosomal backgrounds. Among a number of SNPs studied we identified three that are significantly associated with azoospermia and oligozoospermia (rs5911500 in LOC203413, rs3088232 in BRDT and rs11204546 in OR2W3 gene). Using array CGH analysis we have identified several CNVs that might be associated with male infertility. The initial results of the 2D-DIGE analysis of seminal plasma in males with different spermatogenic defects revealed some differentially expressed proteins.

Key words: male infertility, genomics, proteomics, chromosome aneuploidies, AZF deletions, CNVs, array CGH, 2D-DIGE

### **L 13. Preimplantation genetic diagnosis**

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Preimplantation genetic diagnosis (PGD) is an established procedure of embryo genetic analysis. The technique was introduced in England in 1990 by Handyside and colleagues in two couples known to be at risk of transmitting X-linked disorders. Since then, it has been expanding in scope and applications. In Slovenia, law allows PGD practice and the first baby was born in 2005, also to a couple known to be at risk of transmitting X-linked disorder. Embryos are obtained by in vitro fertilization and are biopsied mostly on day 3 (cleavage stage). For monogenic disorders, genetic analysis is performed by polymerase chain reaction. Most indications for PGD for monogenic disorders are identical as for prenatal diagnosis, but PGD testing for adult-onset disorders (familial predisposition of cancer, Huntington disease, etc) and testing for less severe or less predictable disease (CMT disease) have been reported to be more widespread than is the case for prenatal diagnosis. Fluorescent in situ hybridization (FISH) genetic analysis is used for chromosomal abnormalities and for sex diagnosis in cases of X-linked disorders, when a specific diagnosis is not available. Not all couples where one partner carries a balanced chromosome rearrangement are likely to benefit from PGD. Testing embryos in couples with familial chromosomal rearrangement will be appropriate when there is a history of life born children with genetic imbalances, large number of miscarriages, or fertility problems. Accurate reproductive risk assessment and careful counseling is requested prior to PGD. FISH on cleavage stage embryos enables the investigation of segregation modes in male and female translocation carriers. More than 50% of embryos have been shown to be unbalanced. The type of segregation mode seems to be influenced by type of translocation and by sex of the translocation carrier. PGD using FISH-based technique has also

been used to provide aneuploidy screening (preimplantation genetic screening-PGS), with the aim of replacing euploid embryos and increasing pregnancy rates in certain groups of patients undergoing IVF procedures owing to infertility (advanced maternal age, repeated miscarriage, repeated implantation failure, severe male factor infertility). Lately, several randomized control trials have failed to show, that PGS improved the delivery rate compared with a control group. Many reasons have been given, among them high level of chromosome abnormalities and mosaicism at cleavage stage embryos, and technical limitations of FISH method (limited number of chromosomes tested, variation of hybridization efficiency). New technologies for PGD are now emerging. Array-based technologies allow simultaneous testing of aneuploidy and specific genetic diseases in each embryo. But which conditions should we be looking for and who will decide the selection? Plenty of ethical issues will need to be addressed.

#### **L 14. Congenital developmental defects in twins: etiology, spectrum, genetic counseling**

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Congenital developmental defects (CDD) are 2.5 times more frequent in twins as compared to singletons. This increase is almost entirely due to a higher incidence in monozygotic twins and holds true as well for higher-order twins (triplets, quadruplets,...). If CDD are subdivided into malformations, deformations, monogenic disorders, chromosome aberrations, and polygenic disorders and those of completely unknown aetiology and pathogenesis, it turns out that, while their incidence in twins is not increased in monogenic disorders and chromosome aberrations, the overall increase is mainly due to malformations, deformations and disruptions. Deformations are defined as secondary alterations of structures which initially were correctly formed, due to pressure. It is evident that lack of space in twin pregnancies is the main reason for the latter, and consequently their increased incidence is not confined to monozygotic twins. Vascular disruptions, however, occur in increased incidence in monozygotic twins with a single placenta. The mechanism is based on vascular collaterals between the two segments of such placentas and early prenatal demise of one of the partners. Postmortal blood coagulation and cell necrosis may be followed by embolisation of arteries in the surviving twin following transmission of a thrombus to the vascular net of the co-twin. Mostly, cerebral arteries are involved, and the consequences are necrosis of brain tissue, porencephalic cysts, and the clinical picture of severe brain dysfunction combined with spastic paraplegia. The second-most frequent manifestation, and most often combined with the former, is intestinal obstruction as the consequence of embolisation of a branch of the mesenteric artery. The highest contribution to the increased incidence of CDD in twins is formed by malformations of early embryonic origin. Malformations (if concerning single organs or structures) and malformation complexes (if concerning primary and secondary structures) are, in contrast to deformations and disruptions, primarily incorrectly formed structures. Thus, most malformations of inner organs, e.g. of heart, brain and kidneys, fall into this category. A closer look to malformations in twins shows: the earlier a malformation is formed and the later twin separation occurs, the higher is its incidence in twins. The highest incidence is found in exstrophy of the cloaca, sirenomelia, VATER association and sacro-coccygeal teratoma (the latter is in fact an undifferentiated conjoint twin) and in

conjoined and monochorionic monoamniotic twins. Twin partners of a severely disorganised twins (acardius, acephalus etc.) are, if they feature a CDD, always concordant for the malformation in the former. Apart from this, concordance of such malformations is rare and almost never complete in extent of the defect. Recent studies using molecular markers have shown that in twins with one partner affected with a CDD and the other normal, there is more discordance for genomic variants than in controls. These findings indicate that an early accident, be it genetic or environmental, may cause both, twin separation and a CDD in one or both of the partners. Concordance versus discordance for such a CDD seem not to be valid criteria for genetic versus non-genetic origin of these defects. Monozygotic twinning per se can be considered a defect in humans, thankfully often with favourable outcome.

### **L 15. Hair as a genetic marker - from the psychological to the pathological significance**

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Introduction. Hair, often seen as a simply ornament, sometimes offers clues about personality and lifestyle of an individual, but also about his health whether, in common diseases or as a genetic diseases marker. Objective. Presenting a classification of the main congenital anomalies of the hair and their significance in psychology and genetic semiology. As an easily detectable clinical signs, are presented, successively: quantitative anomalies (hirsutism, hypertrichosis, alopecia); anomalies of anatomical position (implantation, whorls, etc.); abnormal structure of the hair shaft (monilethrix, pili torti, etc.); abnormal pigmentation (albinism, premature gray hair) and anomalies of eyebrows and eyelashes. Materials and methods. There were retrospectively analyzed hair anomalies found in the database of the Department of Medical Genetics in Children's Hospital Oradea in a period of 25 years, observing 3956 patients with various genetic diseases. Results and discussion. 6.8% of the patients with genetic diseases presented isolated (49.1%) or associated (41.9%) hair anomalies, in most cases with significant semiological importance, sometimes even a certain diagnostic key for a genetic disease. The most common types of anomalies are quantitative (48.8%), followed by those of implantation (37.2%), pigmentation (23.1%) and structural (0.8%) anomalies. Conclusions. The frequency of hair anomalies and the fact that they are easily detected, makes them an important clinical sign (sometimes the diagnostic key) in genetic pathology.

Keywords: hair, hirsutism, hypertrichosis, alopecia, albinism, gray hair

### **L 16. Genetics and epigenetics of Gastrointestinal Stromal Tumors (GIST)**

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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms in the gastrointestinal tract. A small minority of GISTs are associated with hereditary syndromes. Large-scale

screening of multiple types of molecular aberrations (e.g., mutations, copy number variations, DNA methylations, gene expressions, microRNA expressions) becomes increasingly important in the prognosis and study of cancer, including GISTs. GIST is primarily defined by activating mutations in the KIT or PDGFRA receptor tyrosine kinases. The mutations cause functional changes in KIT and PDGFRA proteins, usually leading to ligand-independent dimerization and constitutional activation. Whereas oncogenic KIT or PDGFRA mutations seem vital to promoting neoplastic transformation, additional genetic and epigenetic alterations are probably required to explain the wide spectrum of clinical behaviour in GIST. In our retrospective study of 126 patients with GIST we investigated KIT and PDGFRA mutational status and further evaluated novel potential biomarkers found by whole genome methylation studies and microRNA microarray analysis. We identified aberrant methylation status using eleven methylation-sensitive CpG islands in GIST with and without mutations. CIMP (CpG islands methylator phenotype, which is defined as methylation involving more than three gene promoters) was present in almost all GIST without mutation, although there was no statistical difference between GIST with or without gene mutations. On the other hand, CIMP was found in 45 % of GIST with c-kit or PDGFRA gene mutations. In terms of risk categories, CIMP was present in 50% of low-risk GIST and in 60 % of high-risk GIST. Aberrant methylation of multiple gene promoters, as well as the c-kit and PDGFRA genes, therefore plays an important role in the tumorigenesis of GIST. Thirty-five miRNAs were found to be differentially expressed in terms of localization and mutation status. Differential expression was further analysed and confirmed for six miRNAs by qRT-PCR in 39 additional GISTs. Differentially expressed miRNAs were functionally mapped to KIT/PDGFRA signalling and G1/S-phase transition of the cell cycle, revealing several predicted miRNA/mRNA interactions for nine gene targets from KIT/PDGFRA signalling. MicroRNAs and their regulation of gene expression in a tissue-specific manner have also potential application for use as therapeutic targets or disease biomarkers. Keywords: GIST, gastrointestinal stromal tumours; mutations, methylation, tyrosine kinase, prognosis, microRNA

### **L 17. Cytogenetic monitoring in occupational risk groups**

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Cytogenetic analysis is the most widely used approach for biomonitoring human exposure to genotoxic chemicals. To justify an occupational exposure of benzene we performed the present cytogenetic study that was aimed to investigate the relationship between chronic exposure to different benzene levels and induction of cytogenetic damage in human peripheral lymphocytes in relation to confounding factors (age, sex and smoking). Chromosome aberrations (CA) and micronuclei (MN) were examined in 114 petroleum refinery workers chronically exposed to a wide range of benzene levels (3.5 - 77.9 mg.m<sup>-3</sup>) and 98 unexposed controls. Conventional chromosomal analysis and the cytokinesis-block MN assay were performed. The workers were allocated to three groups according to the external exposure to benzene:(high, medium and low).The urinary excretion of phenol and mercapturic acids but not t,t-muconic acid was significantly increased in all exposed groups. Our results showed a higher sensitivity

for the cytokinesis-block micronucleus assay compared to the metaphase analysis. A significant exposure-related increase in MN frequencies, positively correlated with the duration of exposure and non-dependent on sex, age (except the medium exposure group) and smoking was detected. Statistically significant increases in isochromatid breaks and percent of aberrant cells, were found in the low exposure group only.

An increased future carcinogenic risk for benzene-exposed groups of the refinery workers with a substantial chromosome damage has been predicted and lowering of the national MAC of benzene has been proposed.

Key words: cytogenetic monitoring, micronucleus assay, occupational exposure to benzene

## **L 18. Genomics of Schizophrenia**

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Schizophrenia is a severe mental disorder marked by hallucinations, delusions, cognitive deficits and apathy, with a heritability estimated at up to 80%. We performed a candidate genes association studies [SERT (17bp, VNTR, 2nd introne); DAT1 (40bp, VNTR, 3'UTR); DBH -1021C>T polymorphism; COMT - Val108/158Met; pcdh  $\gamma$  a genes; Celsr1] using case/controls and family-based association methods and genome-wide association survey of CNVs in patients with schizophrenia and matched controls. Allele DAT\*10 is associated with the severity and frequency of auditory hallucinations. Associations with schizophrenia were found for rare and common CNVs. Large deletions on chromosome 15q13.3 and 1q21.1 were associated with schizophrenia. CNVs in at least three loci act as strong risk factors for schizophrenia in a minority of individuals. Many common variants of small effect were also identified. The contribution of the variants in the major histocompatibility complex for the molecular pathogenesis of the disorder was established. In conclusion, the results provide a strong proof of different models of schizophrenia (monogenic/polygenic component) that include the effects of multiple rare structural variants across the genome or at specific loci and the role of thousands of common alleles of very small effect. The data could explain at least one-third of the total variation in liability to schizophrenia. Acknowledgments: Many thanks to the members of the International Schizophrenia Consortium, especially to M. Owen, G. Kirov, Y. Nakamura and his team, my PhD students, the patients and families who contributed to these studies.

## **L19. Molecular genetics, regulation and induction of myogenesis**

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The presentation will focus on the molecular genetic activities of our laboratory. We are interested in the genetic epidemiology, identification and characterisation of novel mutations which cause inherited endocrinological diseases. Data will be presented about some of these activities. Moreover, we are also interested in the role of genes in regulating myogenesis. Novel mechanisms which are responsible for controlling the formation of skeletal muscle will be described. Finally, data will be presented which demonstrate the role of genetic manipulation in reversing muscle cell differentiation and reactivating muscle cells.

#### **L 20. Identification of disease-associated O-glycoforms in urine of patients diagnosed with Schindler disease type I by chip-nanoelectrospray multistage mass spectrometry**

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Schindler disease is a rare inherited metabolic disorder which belongs to lysosomal storage diseases [1]. The classical and most severe form of the disorder, known as Schindler disease type I, has an infantile onset. Affected children develop normally until approximately 1 year of age, after which drastic developmental regression, neurological and neuromuscular symptoms, severe mental retardation, hearing and visual impairment, and lack of response to stimuli in the environment occur. The disorder being characterized by a deficiency of alpha-N-acetylgalactosaminidase, in individuals suffering from Schindler disease, the enzymatic defect leads to an abnormal accumulation of sialylated and asialo-glycopeptides and oligosaccharides with alpha-N-acetylgalactosaminyl residues [2,3]. In human urine, complex carbohydrates are catabolic products excreted either as free oligosaccharides or linked to peptides, and their structures and amounts are known to vary under different physiological and pathological conditions. For this reason identification of O-glycosylated aminoacids and peptides extracted from patient urine is of major diagnostic importance. In this study our novel protocol [4] developed for efficient glycoscreening by fully automated chip-based electrospray multistage mass spectrometry was optimized and applied to complex mixtures of O-glycosylated sialylated amino acids and peptides extracted and purified from urine of two siblings diagnosed with Schindler disease. A mixture of O-glycopeptides extracted from urine of a healthy, age-matched individual served as the control. Unlike the case of healthy control, structural elements typical for mucine type O-glycosylation of proteins, like expression of core 1 and 2 type O-GalNAc with different numbers of N-acetylglucosaminyl repeats and different degrees of sialylation could be identified in patients' urine, along with several unusual glycoforms elongated by fucosylation and/or extended chains with higher degree of sialylation. The particular advantage of the method in comparison with other techniques was the capability to detect and de novo identify minor species present in the complex mixture, with chain lengths ranging from tri- to dodecasaccharides bearing up to three sialic acid moieties, which were not detected and accessible to structural characterization before. Present data confirmed that in the case of Schindler

disease, the deficient N-acetylhexosaminidase is causing a much higher concentration and diversity of O-glycans in urine than in healthy controls. De novo identification and structural characterization of unknown O-glycoforms in urine of patients suffering from Schindler disease type I achieved in this study is of high importance for determination of disease biomarkers, useful in early diagnosis and possibly in designing an appropriate therapeutic scheme.

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## **L 21. Genetic Investigations of Membranous Nephropathy**

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Background Membranous Nephropathy (MN) is a rare autoimmune glomerulopathy, the etiopathology of which is not fully understood. MN is one of the most important causes of nephrotic syndrome, leading in approximately one-third of the cases to renal failure. Methods Genome wide mapping performed on biopsy proven MN cases compared with ethnically matched controls from three Caucasoid populations (British, Dutch and French) led to the identification of two loci which reached genome wide significance for association with the trait. Sequencing of both loci was performed in order to ascertain the basis for the GWAS findings. Results The two loci are located on chromosome 2 and on chromosome 6. The chromosome 2 locus includes the PLA2R gene, the product of which was previously identified (using an immunological approach) as a potential autoantigen in idiopathic MN. The highest significance for association on chromosome 6 was reached by an allele of HLA-DQA1, within an extended association signal located in the Human Leukocyte Antigen (HLA) system region on chromosome 6p21. Conclusions A possible immunopathogenetic mechanism is suggested: susceptibility to membranous nephropathy may be associated with an autoimmune response in which the synthesis of autoantibodies oriented against targets (like PLA2R1 variants) is triggered by the presentation of epitopes by HLA type II HLA-DQ dimers to immune-effector cells. Keywords Nephrotic syndrome, membranous nephropathy, genome-wide association study, immunogenetics, variation detection, sequencing, causality, HLA-DQA1, PLA2R1, autoimmune response, autoantibodies

## **L 22. Syndromes presenting adducted thumb with/without clubfoot and "Ehlers-Danlos Syndrome, Musculocontractural Type; Dundar Syndrome**

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Congenital adducted thumb can be associated with genetic disorders, or it is usually a sporadic finding. The syndromes that include adducted thumb and club foot as a cardinal feature are few in the literature and one of them is "Ehlers-Danlos musculocontractural type which is also known as Dunder Syndrome (OMIM 601776). This syndrome is an autosomal-recessive rare disorder that is characterized by typical facial appearance with facial clefting, wasted build, thin and translucent skin, severe congenital contractures of thumbs, club feet, joint instability. Besides dermatan-4-sulfotransferase 1-deficient Ehlers-Danlos Musculocontractural type, the most common syndromes that include adducted thumb with/without club foot as a cardinal feature are Christian Adducted Thumbs Syndrome (CATS), Escobar or Multiple Pterygia Syndrome (MPS), MASA Syndrome, Arthrogryposis Multiplex Congenita Type I, Arthrogryposis Type 2a. While CATS is consisting of adducted thumbs, craniosynostosis and severe neurological abnormalities, MPS's characteristic features are; pterygia, congenital contractures, scoliosis and different kinds of finger and foot deformities. MASA is an acronym of mental retardation, aphasia, shuffling gait, and adducted thumbs, and Arthrogryposis types are characterized by the anomalies of the distal parts of the limbs especially hands and feet with different abnormalities. These syndromes must be discussed at the differential diagnosis of the Dermatan Sulfate-Deficient Adducted Thumb Club Foot Syndrome. Although these syndromes are rare, early diagnose of them gives us to choose the correct molecular investigations besides making correct management of the clinical problems and also it is so important for giving well informed genetic counseling to the families.

Key Words: Dermatan Sulfate Deficient, Adducted Thumb-Clubfoot Syndrome, Dunder Syndrome, Arthrogryposis

### **L 23. Prader-Willi syndrome – an imprinting disease: a conceptual and technical approach for the establishment of practical diagnosis guidelines**

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Introduction. Prader Willi syndrome (PWS) has been commonly described as a genetic disorder caused, like its sister, but phenotypical distinct, Angelman syndrome (AS), by deletions in the critical 15q11-q13 chromosomal region. The epigenetic factors explained the basic cause that results in two different pathological phenotypes: altered parental contribution to the expression of particular genes, named imprinted genes. Imprinting process epigenetically marks alleles according to their parental origin during gametogenesis and these heritable modifications regulate their monoallelic activity resulting in their

functional differences during development. PWS is therefore a complex disorder whose diagnosis may be difficult to establish on clinical grounds, because of the individual phenotype variations that develops with age. A diversity of techniques were described, each of them tackling either the genetic or epigenetic causes. Thus a need for consensus testing, reporting guidelines and also the involvement of cost efficiency aspect imposed the approach of new, more sensible and relevant techniques.

**Materials and methods.** A cohort of 36 clinically PWS diagnosed cases were tested by molecular cytogenetic (FISH), the epigenetic (MSPCR) and DNA sequencing (MLPA and MS-MLPA) methods. The resulted DNA biobanck is presently tested for the validation of these methods and also for the estimation of the real incidence of PWS in Romanian population.

**Results and discussions.** The laboratory study confirmed only 11 cases showing a relative correlation between clinical score and molecular approaches of suspected PWS cases. FISH method detected only the large deletions; methylation method covers both deletional and non-deletional cases, however it does not precisely indicate for these specific causes and leaves unconfirmed the suspected UPD involvement in nondeletional cases. Therefore, we have developed a combined MS-MLPA technique that concomitantly detected the genetic and epigenetic defects in representative methylation and deletional cases. This technique is currently tested for the already established DNA PWS biobanck in order to be implemented in routine diagnosis.

**Conclusions.** So far, the most sensitive (covering 99% of cases) single approach for both PWS and AS diagnosis is the molecular methylation assay through MSPCR technique. Also, the MS-MLPA approach is under validation process in order to reveal concomitantly genetic and epigenetic causes for the counselling and for improving knowledge of the molecular mechanisms that underly this disorder. Clinical behavioral pattern can be of assistance in guiding the investigation; for the final diagnosis, further study and experience gathered by the project team will allow a refinement of techniques and a more accurate explanation of the causes and type of their transmittance.

**Key words:** Prader-Willi syndrome, imprinting disease, diagnosis guidelines

#### **L 24. Following the best practice guidelines for Duchenne/Becker muscular dystrophies genetic diagnosis in Romania - a brief report regarding our experience as reference laboratory in this project**

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**Background** The genetic diagnosis is a basic requirement for the Duchenne/Becker muscular dystrophies (DMD/BMD) medical management. Although molecular testing is now available in public or private Romanian laboratories, it does not take into account, with some exceptions, the neurological disorders. For DMD/BMD, the genetic investigations are supported by the Ministry of Health, yet they started for a research study. **Aim** We report our experience as a reference laboratory performing genetic testing for all the subjects included by the time in the research and national program. **Methods.** Our laboratory developed a complex and high standard testing protocol intended to detect all possible mutations in the dystrophin gene. It comprises several molecular methods (MLPA, HRM analysis and sequencing) for which the laboratory passed since 2007 the European Molecular Quality Network proficiency schemes.

Thus, mutations are accepted in the Leiden DMD Database. Results. A total of approximately 700 subjects (patients, relatives and 27 pregnant women) were investigated between 2005-present. We noticed that the molecular result was "maximized" when genetic counseling was accomplished. The genetic data together with the clinical data are recorded in the national dedicated registry belonging to the Ministry of Health, and when patients consent the information is forwarded to the international registry. Conclusions. Following the best practice guidelines a complex algorithm of genetic diagnosis was set up for DMD/BMD in Romania in a reference genetic laboratory. Based on the results, a significant progress has been achieved by means of a sustained engagement in the work of neuropaediatricians and geneticists.

## **L25. Clinical features, genetics and outcome of Romanian patients with Lysosomal Storage Disorders**

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Introduction: Lysosomal storage disorders (>50) are monogenic diseases, caused by deficiency of some specific hydroxylases activity and characterized by pathologic accumulation of unmetabolized substrate in lysosomes with multisystemic involvement and severe outcome. Specific diagnosis of these diseases has become available in Romania since 1997. Aim: To present clinical and genetic characteristics and outcome of Romanian patients with lysosomal diseases. Patients, methods: The study group included 288 patients with a clinical picture suggestive for a lysosomal disease. The assay of lysosomal enzymes activity in leucocytes established the diagnosis of lysosomal diseases in 111 patients. Clinical status, biochemical and imagistic assessment were performed in accordance with each disease. Results: The lysosomal diseases diagnosed were: 1) sphingolipidosis (88 patients): Gaucher disease; Fabry disease; GM1 gangliosidosis and others (GM2 gangliosidosis, Nieman Pick disease and methacromatic leucodystrophy) in 61;12;11 and 4 patients, 2) mucopolysaccharidosis (type I, II, IIIB, IVB, and VII) in 18 patients; mucopolipidosis in 3 patients and ? mannosidosis and Pompe disease in one patient. Regarding patients with Gaucher disease, there are 59 patients with type 1 and 2 with type 3; F/M=1,44/1; age (x) 15 ys at clinical onset and 28 ys at specific diagnosis; they represent only 27,7% of the expected total number of Gaucher patients in Romania; 1/3 of patients underwent splenectomy before ERT; the main clinical features were anemia, thrombocytopenia, splenomegaly, hepatomegaly and bone disease. The severe genotyp N370S/L444P is frequent (35,9%). Forty-five patients are receiving ERT with a favorable outcome; 4 patients died. The author presents also the clinical characteristics of 23 patients with other lysosomal diseases; one patient (MPS II) died. Nine patients (2 with MPS I, 6 with MPS II and 1 with M. Pompe) are receiving ERT. Conclusions: Seventy-nine percent of Romanian patients with treatable lysosomal disease are receiving specific treatment. Although specific diagnosis of lysosomal disease is now available in our country, these diseases are underdiagnosed.

# ORAL PRESENTATIONS

## **O 01. Role of genetic factors in a multifactorial disease - osteoporosis**

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Osteoporosis is a multifactorial disorder with a complex pathophysiology, characterized by a decrease in bone mass and deterioration of bone architecture. There is a genetic contribution to the etiology of osteoporosis, genetic factors determining peak bone mass and influencing age-related decreases of bone mass. The magnitude of individual genetic effects differs in different population subsets and in different environments. Family studies as well as twin studies have shown that genetic factors play an important role in bone mass regulation. The heritability of bone density, measured by several methods in twin studies and family inter-generational studies, has been shown to be very high. This study included a group of 82 patients investigated by DXA for detecting osteoporosis, admitted in the Rehabilitation Clinical Hospital of Baile Felix between July 2009 - May 2010, with a mean age of  $62.6 \pm 8.5$  years, ranging between 44 and 81 years. 51 % of the cases with vertebral osteoporosis had affected parents. From the patients with generalized osteoporosis, 29% had at least one affected parent and when considering the subjects with osteopenia, 27.5% of them had a parent with osteoporosis. Familial predisposition is considered to be an important risk factor, together with early menopause, kyphosis, vitamin D deficiency, body mass index, etc. Quantification of the risk has clinical implications and influences therapeutic decision.

## **O 02. ErbB3 leukocytes mRNA levels as biomarker of major depression**

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Background: Major depressive disorder (MDD) is a psychiatric disorder characterized by the interaction between genetic and environmental risk factors. The etiology is still poor understood and peripheral

biomarkers have not been identified yet. Leukocytes are an interesting peripheral model to study gene altered in the central nervous system of depressed patients. A recent candidate is ErbB3, which codifies for a neuregulin receptor. ErbB3 mRNA levels have been found altered in temporal cortex of MDD patients and in leukocytes of patients affected by bipolar disorder in a depression state. Aim: We conducted a study to assess whether ErbB3 levels could be altered in MDD patients. Methods: ErbB3 mRNA levels were analyzed by Real Time PCR in leukocytes obtained from a sample of 27 MDD patients and 19 controls. Results and Conclusions: Results show that ErbB3 mRNA levels are reduced in patients (CTRL=1.19±0.74; Patients=0.66±0.35; p=0.003). To evaluate whether the ErbB3 deficit could be due to a previous antidepressant treatment, we stratified the patients sample in two sub groups: "drug free" (13 patients who had a "washout" period of two weeks following to a previous antidepressant treatment) and "drug naïve" (14 patients who have never received antidepressants). The analyses show no difference in ErbB3 mRNA levels between the two groups (Drug free= 0.69±0.44 Drug Naïve=0.64±0.26 p=0.75). These results support the usefulness of leukocytes as peripheral system to identify biomarkers associated with MDD and suggest that ErbB3 may be considered as specific biomarker of depressive episode.

Keywords: Major depression, Peripheral biomarker, leukocytes, ErbB3

### **O 03. Six-mercaptopurine influences thiopurine S-methyltransferase gene transcription: another evidence of pharmacogenetic potential of the promoter variable number of tandem repeats**

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Six-mercaptopurine (6-MP) is a broadly used cytotoxic drug whose efficacy and toxicity is dependent on thiopurine S-methyltransferase (TPMT) activity. TPMT gene promoter contains a variable number of 3 GC-rich tandem repeats, namely A, B and C, ranging from 3 to 9 in length in a AnBmC architecture. We have previously shown that variable tandem repeat architecture affects TPMT gene transcription, mediated most likely by Sp1 and Sp3 transcription factors' binding to the VNTR repeats. We investigated the role of 6-MP on influencing human TPMT gene transcription, mediated by the different VNTR (variable number of tandem repeats) architecture in the TPMT gene promoter region. Transient transfections of 6-MP pretreated cells were performed with constructs containing TPMT gene promoter variants with different VNTR alleles. Electrophoretic mobility shift and South-Western blot assays with probes containing VNTR motifs were performed using nuclear extracts from 6-MP treated and untreated K562 cells. Also, 6-MP toxicity was correlated with the VNTR genotype of pediatric ALL (acute lymphoblastic leukemia) patients. Using reporter assays we demonstrated that 6-MP treatment results in a VNTR architecture-dependent decrease of the TPMT gene transcription. The level of decrease of TPMT gene transcription is dependent on the binding of newly-recruited protein complexes to the TPMT gene promoter, upon 6-MP treatment. ALL patients that undergo 6-MP treatment, bearing different

VNTR genotypes, display a VNTR-dependent response to 6-MP. Our findings highlight a potential role of the TPMT gene promoter VNTR region as a potentially useful pharmacogenomic marker to individualize thiopurine treatment in ALL patients. TPMT, VNTRs, 6-mercaptopurine, pharmacogenetics

#### **O 04. The frequency of NPM1 mutations and their prognostic significance in Acute Myeloid Leukemia patients**

Tosic N.<sup>1</sup>, Spasovski V.<sup>1</sup>, Kostic T.<sup>1</sup>, Karan-Djurasevic T.<sup>1</sup>, Nikcevic G.<sup>1</sup>, Suvajdzic N.<sup>2</sup>, Vidovic A.<sup>2</sup>, Dunic I.<sup>2</sup>, Tomin D.<sup>2</sup>, Elezovic I.<sup>2</sup>, Colovic N.<sup>2</sup>, Colovic M.<sup>2</sup>, Pavlovic S.<sup>1</sup>

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Background: Mutations in nucleophosmin (NPM1) gene are the most frequent abnormalities found in adult AML. Current findings show that the presence of NPM1 mutations is a favorable prognostic marker. Aims: To evaluate the incidence and the prognostic relevance of NPM1 mutations, their association with FLT3 mutations and other clinical characteristics. Methods: Bone marrow samples from 124 adult de novo AML patients were studied. NPM1 mutations were detected by PCR method, followed by direct sequencing. Results: NPM1 mutations were detected in 26/124 patients (21%). Three types of mutation were detected; type A in 24/26 patients (92.3%) while the remaining two patients were carriers of type D and type K mutations. NPM1 mutations were associated with increased leukocyte count ( $p=0.02$ ), normal karyotype (77%), CD34+ status ( $p=0.003$ ) and FLT3 gene mutations (57.7%). Complete remission (CR) was achieved in 10/26 NPM1+ patients. CR rate in NPM1+ patients was significantly lower than in NPM1-/ FLT3- patients ( $p=0.01$ ). Surprisingly, median duration of disease-free-survival and overall survival among NPM1+ patients was significantly lower compared to NPM1- patients; 10 vs. 36 months,  $p=0.05$  and 6 vs. 24 months,  $p=0.015$ , respectively. Conclusions: In our study NPM1 mutations had an unfavorable prognostic impact. Association of NPM1 mutations with FLT3/ITD mutations, which have dominant adverse prognostic effect, may explain this unfavorable impact of NPM1 mutations. Another explanation may lie in the fact that some other secondary genetic lesions may cooperate with NPM1 mutations influencing the prognosis.

#### **O 05. Genetic polymorphism of TLR4 in 15 Iranian subpopulations**

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Infectious diseases exert a constant evolutionary pressure on the innate immunity genes. TLR4, an important member of the Toll-like receptors family, specifically recognizes conserved structures of various infectious pathogens. Two functional TLR4 polymorphisms, Asp299Gly and Thr399Ile, modulate innate host defense against infections, and their prevalence between various populations has been proposed to be influenced by local infectious pressures. If this assumption is true, strong local infectious pressures would lead to a homogeneous pattern of these ancient TLR4 polymorphisms in geographically close populations, while a weak selection or genetic drift may result in a diverse pattern. We evaluated TLR4 polymorphisms in 15 ethnic groups of Iran, to assess whether infections exerted selective pressures on different haplotypes containing these variants. The Iranian subpopulations displayed a heterogeneous pattern of TLR4 polymorphisms, comprising various percentages of Asp299Gly and Thr399Ile alone or in combination. The Iranian sample as a whole showed an intermediate mixed pattern when compared with commonly found patterns in Africa, Europe, Eastern Asia and Americas. These findings suggest a weak or absent selection pressure on TLR4 polymorphisms in the Middle-East, that does not support the assumption of an important role of these polymorphisms in the host defence against local pathogens. ACKNOWLEDGEMENTS: M.I. was supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109.

#### **O 06. The role of FASR/FASL system in pathogenesis of myeloproliferative neoplasms**

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**Background.** Myeloproliferative neoplasms (MPN) are hematological malignancies characterized by uncontrolled cell proliferation and impaired apoptosis of different cell types. FasR/FasL system is involved in control of apoptosis in different cell types, and its role in immune system is very well characterized. The role of Fas system in apoptosis of granulocytes and erythrocytes is not clear, although it is known that granulocytes and erythrocytes express Fas receptor on their surface during different phases of development. **Aim.** We have investigated the role of FasR/FasL system in pathogenesis of MPNs. We have also compared the expression of Fas receptor and Fas ligand in MPN patients harboring JAK2 V617F mutation and patients negative for this mutation. **Methods.** Using quantitative PCR method we analyzed expression of Fas receptor and Fas ligand in 24 MPN patients and 6 healthy controls. We also analyzed the presence of JAK2 V617F mutation using allele specific PCR in MPN patients. **Results and Conclusions.** We analyzed 24 MPN patients and found statistically significant increase of FasR

expression compared to healthy controls. No significant difference was detected in FasL expression in this cohort. Mutation V617F in the JAK2 gene, a hallmark of MPN, was detected in 13 of 24 patients. We found that neither FasR nor FasL expression were related to the presence of JAK2 V617F mutation. Therefore, we show here that Fas system is involved in the pathogenesis of MPN and that its role is unrelated to JAK2 V617F mutation.

Key words: myeloproliferative neoplasms, apoptosis, FasR-FasL, V617F JAK2

### **O 07. The role of C-kit and PDGFR-alpha genotyping in the management of patients with gastrointestinal stromal tumors**

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Gastrointestinal stromal tumors (GISTs) are the most frequent mesenchymal tumors of the gastrointestinal tract. Most GISTs are associated with molecular anomalies of two target genes: c-KIT (85-90%; >70% in exon 11, 13% in exon 9) and PDGFRalpha (35% of the KIT "wild-type" GISTs). Mutations in c-KIT or PDGFR alpha result in ligand-independent tyrosine kinase activity, autophosphorylation of KIT, uncontrolled cell proliferation and stimulation of downstream signaling pathways. The presence or absence of a mutation and its type are correlated with the tumor localization and biology, its malignant potential, treatment response and primary and secondary resistance to kinase inhibitors, progression-free period and survival. Two different kinase inhibitors (Imatinib and Sunitinib) have been approved for treatment of GISTs and numerous other inhibitors are being tested for treatment of resistant tumors. Peculiar situations appear in case of familial, syndromic and pediatric GISTs. The aim of this study is to prove the usefulness of the genetic analyses in establishing GISTs response to treatment, their evolution and prognosis and the diagnosis of CD117 negative GISTs, C-KIT mutations in exons 11 and 9 were analysed by PCR and DNA sequencing. Point mutations and deletions were identified in c-KIT gene, exon 11, causing different tumor behaviors. The introduction of c-KIT and PDGFR alpha genotyping in the management of patients with GIST contributes to the estimation of prognosis of primary disease and offers a tool to identify patients with tumors who would benefit from molecular-targeted therapy and patients in need for more intensive follow-up.

Key words GISTs, c-KIT, PDGFR alpha, kinase inhibitors, DNA sequencing.

### **O 08. Quantitative analysis of chimerism after allogeneic stem cell transplantation by multiplex fluorescent short tandem repeat analysis - the NILM experience**

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Background. Following allogeneic haematopoietic stem cell transplantation (allo-HSCT), it is of importance to perform systematically the chimerism analysis (quantification of the donor derived cells in patients' blood). This process plays an increasingly important role in predicting outcome for engraftment, rejection or residual disease. Aim. We report our results regarding monitoring of chimeric status for more 100 allo-HSCT cases during 2006-2011. Methods Peripheral blood cells from donor and receptor have been used in the short tandem repeat (STR) assay to serially characterize the haematologic course. More than a hundred patients from a single centre were followed serially for periods ranging between 14 days and 6 months. For each donor-recipient pair, was selected and analyzed the most informative STR loci. The results obtained were expressed as a percentage of the donor profile. Results. More than 80% of patients presented with complete chimerism. The rest of them had different proportions of mixed chimerism. The genetic data correlated with the clinical follow-up and the informativity of a STR marker was related to the genetic difference between the donor and receptor. Conclusions. The STR-based analysis of chimeric status is sensitive and specific, detecting down to about 5% chimerism. The sequential follow-up employed in our study provides relevant clinical information but differ according to the underlying disease and type of transplantation.

#### **O 09. Cytokine gene polymorphisms and risk of gastric adenocarcinoma**

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Gastric cancer remains the second leading cause of cancer death in both sexes worldwide. Interactions of three major factors including bacterial pathogenicity, host susceptibility and environmental factors play a crucial role in gastric carcinogenesis. *H.pylori* infection induces a predominant host inflammatory Th1 response. Therefore, genetic variations in inflammation-related genes especially cytokines and their receptors could influence gastric carcinogenesis. The aim of our study was to assess the potential association of 8 cytokine gene polymorphisms (IL-1B-511C>T, IL-1B-31T>C, IL-1B+3954C>T, IL1-RN+2018T>C, IL-4R-3223C>T, IL-8-251T>A, IL-10-1082A>G and TNF-308G>A) with gastric cancer susceptibility in an Eastern Europe population (Romanian population), a region where the association between gastric cancer and these polymorphisms has not been previously studied. 105 gastric cancer patients and 242 controls were genotyped by allelic discrimination TaqManPCR assay. Allelic distributions were examined for deviation from their corresponding Hardy-Weinberg equilibrium. The association between polymorphisms and gastric cancer risk was estimated by odds ratios and 95% confidence intervals. A significant association was observed for IL-1RN+2018T>C and IL-4R-3223C>T, the subjects carrying IL-1RN+2018CC respectively, IL-4R-3223TT genotype were at a 2.5 fold elevated risk for gastric cancer. Association of these polymorphisms with tumor site and histologic type were examined separately. Also, we found a positive association only for IL-1RN+2018T>C and IL4R-3223T>C, limited to increase the risk of non-cardia adenocarcinoma and intestinal type. None of the remaining cytokine

polymorphisms were associated with increased gastric cancer risk. In conclusion, IL1-RN+2018T>C and IL-4R-3223T>C polymorphisms could contribute to gastric cancer susceptibility, mainly for non-cardia and intestinal types of gastric adenocarcinoma.

#### **O 10. The fetal RHD genotyping and gender determination from cell-free fetal DNA circulating in maternal blood - the non-invasive prenatal tests available in Romania**

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Background: Although the prenatal diagnosis procedures currently employed in Romania are based on invasive sampling procedures of fetal cells, the detection of cell-free fetal DNA in maternal circulation raised the challenge of finding safer methods for prenatal diagnosis. Aim: Our aim was to develop a protocol for non-invasive fetal RHD genotyping in pregnancies where the mother is RhD negative and the partner is RhD positive to avoid the unnecessary administration of anti-D immunoglobulin to mothers that does not undergo amniocentesis. Methods: We developed a three-steps method for fetal RHD genotyping and we tested 75 pregnant RhD negative women with RhD positive partners. The cell-free total DNA extraction was performed from 1ml maternal plasma using the QIAamp® DSP Virus kit with some adjustments to the standard QIAGEN protocol. We performed the fetal RHD genotyping by a multiplex PCR reaction with specific primers for two sequences in the exon 5 and respectively exon 7 of the RHD gene. We also included the GAPDH and DYS14 sequences detection as internal controls. The PCR products were automated analyzed by high-resolution capillary electrophoresis. Results: We detected 52 fetuses with RHD positive genotype and 23 fetuses with RHD negative genotype. All cases were analyzed in duplicate. Conclusion: Our results confirm that this non-invasive approach is feasible and accurate and will improve the management of mother-fetus RhD incompatibility by eliminating the invasive procedures for fetal RHD genotyping. Regarding the fetal gender determination, this approach can be used as a screening procedure in cases at risk for inheriting an X-linked recessive disorder.

#### **O 11. Limb defects associated in syndromic intellectual disability**

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Intellectual disability (ID) is a common consultation in Medical Genetics services. It affects 3% of the general population and may be classified according to severity in mild/ moderate/ severe and according to associated features in nonspecific/ specific (syndromic) ID. Limb defects (LD) represent another problem frequently encountered in genetic disorders. They are produced by abnormal or incomplete

differentiation and they could be very suggestive for the diagnosis in patients with syndromic ID. We present a comprehensive discussion on limb defects - classification and patterns, embryologic development, networks of genes involved, as well as their association with intellectual disability. The theory is illustrated with cases from our experience, underlying the aspects suggestive for specific diagnosis frequently encountered in practice. Evaluation and investigation protocol used in Iasi Medical Genetics Center for patients with LD and ID will be presented, as well as genetic counseling directions. In conclusion, our presentation on limb defects associated with intellectual disability in patients with genetic disorders shows illustrative cases and examples of good practice, aiming to ease patient diagnosis and management in daily practice.

## **O 12. Wilson's disease in Romanian children: clinical and genetic profile**

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**Background.** Wilson's disease (WD) is an autosomal recessive disease of copper metabolism, involving the liver and/or nervous system. The genetic analysis may confirm the diagnosis, especially in controversial cases. **Aim:** assessing the clinical and genetic profile of WD in Romanian children. **Methods.** The children with WD were assessed regarding clinical manifestations, Kayser-Fleischer ring, serum ceruloplasmin, 24h-urine copper excretion, genetic mutations (PCR and sequential analysis). Diagnostic probability was assessed using the Ferenci score. **Results.** We have studied 26 children with WD (M/F=14/12, mean age 13.44 years), with hepatic disease (65.38%), hemolytic anemia (23.08%) and neurologic disease (11.54%). Five children presented Kayser-Fleischer ring (19.23%). 25 patients had low serum ceruloplasmin and also increased urinary copper. The genetic analysis revealed homozygous status in 11 patients (7 patients G1341D mutation, 4 patients W939C mutation) and compound heterozygous status in 9 patients. In 6 patients no known mutations were detected. Ferenci score was: 2 (unlikely diagnosis) in one case, 3 (possible diagnosis) in 2 cases, 4 (certain diagnosis) in 23 cases. Zinc therapy was introduced in 25 children. D-penicillamine (alone or associated to zinc) was introduced in 12 children. One patient, with hemolytic anemia and fulminant liver failure, deceased one day after admission. **Conclusions.** WD in children is mainly a hepatic disease, but the hemolytic anemia with liver failure is possible a very severe on-set. The genetic analysis is important for confirmation of diagnosis in controversial cases. H1069Q mutation, the most frequent in Central Europe patients, was rarer than G1341D and W939C in our patients.

**Key-words:** Wilson's disease, children, genetic analysis

## **O 13. The importance of chromosomal classical analysis in genomic era considerations on 10 years analysis**

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The last decades improved the genomic diagnosis by implementation of new techniques. The karyotype remains a good method, because it is simple, easy and cheap. In our 10 years study (2001-2010) we have analyzed 2268 karyotypes with 71 failures (3.13%). In 552 cases with Down syndrome we have identified 21 trisomy: 483 homogenous, 28 mosaics, 23 Robertsonian translocations (96.73% confirmed cases). In 56 cases with male gonadal dysgenesis we have identified: homogenous XXY trisomy (27 cases), mosaic XXY trisomy (4 cases) XXXY tetrasomy (2 cases), other anomalies (2 cases) (58.92% confirmed cases). In 130 patients with female gonadal dysgenesis we have identified: homogenous X monosomy (31 cases), gonosomal mosaics (13 cases), structural X chromosome abnormalities (21 cases) 46,XY (4 cases) (57.69% confirmed cases). In 51 cases with intersexuality we had 11 abnormal results. In 529 cases with plurimalformative syndromes we had normal results in 426 patients and we have discovered different abnormalities: trisomy 13 (14 cases), trisomy 18 (16 cases), deletions (26 cases), add (11 cases), der (7 cases), inversion on chromosome 9 (7 cases), insertion (5 cases), translocations (4 cases), ring chromosomes (2 cases), fraX (2 cases), gonosomal anomalies (4 cases), other numerical anomalies (5 cases) (19.47% confirmed cases). Karyotype performed for reproductive failure in 262 couples revealed the following anomalies: inversions (18 cases), gonosomal anomalies (10 cases), robertsonian translocations (9 cases), reciprocal translocations (6 cases), insertions (5 cases) and chromosomal polymorphisms (4 cases) (19.84% confirmed cases). These results show the importance of karyotype, but only in concordance with clinical examination.

#### **O 14. Chromosome abnormalities found in 7400 cases of pre and post natal cytogenetic analysis**

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There are over 100 chromosomal syndromes which have been reported until now. Chromosomal abnormalities are a major cause of spontaneous abortions, recurrent pregnancy loss and infertility. Cytogenetic studies performed in new-borns have shown a high rate of chromosomal anomalies in this group. Some of these genetic anomalies appear de novo or they are derived from a genetic anomaly present in one of the parents. The classic cytogenetic analysis can identify chromosomal abnormalities and it can offer important information for genetic counselling. In our laboratory, over the past decade, we analyzed over 7400 pre and post natal karyotypes. A total of 814 aberrant karyotypes were diagnosed. The chromosomal abnormalities found were comprised of numerical aberrations, such as trisomies of the chromosomes 13, 18, 21, sex chromosome aneuploidies, and also supranumerary marker chromosomes. The autosomal aberrations found included translocations, deletions, duplications and inversions. Low-level mosaicism for numerical sex chromosome aberrations were found as well. A total of 235 patients showed single cell aberrations, but the significance of these cells is still unclear at the moment. A high number of chromosomal anomalies were found in the fetuses of many women

under the age of 30. Also, our data confirms previous studies that a high number of infertile couples have a chromosome aberration which occur in both sexes. In conclusion it is suggested that a chromosomal analysis should be performed on both partners with reproductive problems and in all pregnant women, regardless of their age. Key words: karyotype, chromosome aberrations, infertility, prenatal diagnosis

#### **O 15. Genetic markers associated with nonsyndromic hearing loss in Romanian children**

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Mutations in the GJB2 gene encoding for connexin 26 are considered to be a major contributor to prelingual autosomal recessive nonsyndromic hearing loss. 35delG GJB2 has been reported to be the most common mutation among Caucasians. It has been shown that two deletions in the GJB6 gene, which encode connexin 30, are another common cause of hearing loss (HL). Our goal was to use molecular testing at deaf children for identifying the contribution of the genetic markers to HL; these markers are known to be of high prevalence in European populations. This study was carried out on 253 children with prelingual HL. In a first step we investigated the three most common mutations. The 35delG GJB2 mutation was detected by ARMS-PCR and analysis of del(GJB6-D13S1830) and del(GJB6-D13S1854) was performed by multiplex-PCR. We started to test the children with negative results by MLPA. We also developed the GJB2 and GJB6 genes sequencing to search for the other point mutations. 35delG homozygous GJB2 mutation was detected in 33% of patients. Only 3% of children presented 35delG mutation in a heterozygous form. None of them was found to have the GJB6 mutations. We tested by MLPA ten deaf children found negatives for three above mentioned mutations. One of them had IVS1+1G>A mutation, confirmed also by GJB2 exon1 sequencing. The identification of the most frequent causal mutation of prelingual HL in Romanian population will contribute to develop both prenatal and postnatal molecular diagnosis protocols well suited for our population.

#### **O 16. Genetic testing for Friedreich's ataxia: first experience from clinic of neurology, clinical center of Serbia**

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Friedreich's ataxia (FRDA), the most common autosomal recessively inherited ataxia, with an estimated prevalence of 2–4:100000 in European populations is characterized by progressive gait and limb ataxia, dysarthria, areflexia, loss of vibratory and position sense. This condition is due to a homozygous GAA triplet repeat expansion in the first intron of the FXN gene in about 98% of patients. Approximately 2%

of FRDA patients are compound heterozygotes with a GAA repeat expansion in one allele and a point mutation in the coding region of the second allele. Routine diagnostic procedure for FRDA implies estimation of the GAA repeat length by PCR. Less often laboratories perform sequencing of coding exons to detect inactivating mutations outside of the GAA repeat region. Molecular genetic testing for FRDA, has been established in our clinic two years ago and since then we tested 36 patients with clinical symptoms of FRDA, and 94 with clinical symptoms of Spinocerebellar ataxia (SCA). In all patients heterozygous for expansion, coding exons were sequenced. Twelve FRDA patients and four SCA patients with homozygous expansion were detected. Also two heterozygotes for GAA repeat expansions were detected in each group of patients. Subsequent sequencing showed missense substitution in exon 3 (L106S) in one patient. We successfully introduced genetic testing for FRDA including expansion detection and sequencing of coding exons. Our results emphasize the importance of introducing both coding regions sequencing in routine FRDA testing and FRDA testing in SCA patients. key words: Friedreich's ataxia; repeat expansion; point mutation; genetic testing.

### **O 17. Tumor Necrosis Factor alpha polymorphism and susceptibility to multiple sclerosis: an Iranian study**

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Background and aims: Multiple sclerosis (MS) is a heterogeneous disease, which results in different clinical manifestations. One of the genetic factor is polymorphism of human leukocyte antigen (HLA) region microsatellite (Msat) polymorphism such as tumor necrosis factor alpha(TNFa) .TNF genes are located within the HLA III region. TNF is an important mediator in the inflammatory response. The aim of our study is to confirm if there is any association between Msat polymorphism & predisposing to MS. Subject & methods: polymerase chain reaction by specialized primers was carried out on 40 relapsing remitting MS patients(30female &10male;) affected with MS according to Poser criteria(Poser et al.,1983) and 39age- and sex-matched healthy controls of Hormozgan province in Iran. The results detected on 8% non-denaturing polyacrylamid gel electrophoresis (PAGE) and subsequence comparison with standard (allelic) ladder. Computational analysis & test were performed using GenePop1.31 .Exact test of Hardy-Weinberg equilibrium were performed for TNFa Msat. Result: The high frequency of TNFa\*11 Msat(25%, p=0.05) in cases in compare to controls(8% ,p=0.05) is remarkable & this characteristic is the same as recent Europeans studies and also TNFa\*2 exhibited very low(2%,p=0.05) signal intensities and also the most common manifestation of the disease was fatigue(47.7%). Conclusion: Allele frequency comparison of TNFa Msat shows significant association between patients compared with healthy controls. Our finding suggests a potentially important role of TNFa gene in susceptibility to MS. Key words: Multiple sclerosis (MS), relapsing remitting MS (RRMS), tumor necrosis factor alpha (TNFa) Msat polymorphism, susceptibility.

### **O 18. An unexpectedly high incidence and complexity of subtelomeric genomic rearrangements in children with mental retardation and congenital malformations uncovered by array CGH**

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Subtelomeric microdeletions and microduplications are known to be relatively frequent among children with mental retardation. Introducing of array CGH have recently shown that such subtelomeric genomic rearrangements (SGRs) might be significantly more complex than previously recognized. Here, we have attempted at evaluation of incidence and complexity of SGRs studying 43 children with mental retardation and congenital malformations by array CGH. Microdeletions and microduplications were detected in 14 (33%) out of 43 patients. Large terminal deletions (size: 4.7–6.1Mb) was identified in 4 cases (del4pter/dup8pter; del7qter; del10qter; dup19pter). Three cases were associated with a gain (1 male) and losses (2 female) of Xq28 (including MECP2 gene). A case exhibited a subtle 2qter deletion (<1Mb). Remaining SGRs were referred to more complex rearrangements presenting as multiple subtle deletions or duplications (<0.3Mb) located more distally from telomeres at a distance of 0.5-1.5Mb. Among these, two cases were recurrent co-occurrence of deletions in 9qter and 21qter. Four cases exhibited following SGRs (microdeletions and microduplications sized from 0.1 to 0.3Mb): del1pter/del5pter; del4qter/del5qter; dup16pter/del6pter; del1pter/del4pter/del20qter. Finally, a case exhibited constitutional genomic instability manifested as multiple deletions and duplications (size: from 1Mb to 2.5Mb) involving several subtelomeric regions (1pter; 16pter; 20pter; 22pter). These data suggests that subtle (<1Mb) highly complex SGRs (manifesting as interchromosomal DNA sequence exchange or translocations) do frequently exist in children with mental retardation and congenital malformations.

### **O 19. Molecular analysis-usefull tool for diagnosis and new syndrome discovery**

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One of the karyotype limitations is the resolution level which is 5Mb for conventional cytogenetics. In time it was shown that there are inframicroscopic genomic disorders associated with mental retardation with or without other phenotypic alterations. In this context, there were developed many diagnosis techniques like FISH, MLPA, qPCR, QM-PSF and array CGH which allowed the diagnosis of very small imbalances, the discovery of new anomalies and a pangenic analysis with a high level of resolution. In

some cases these techniques have showed genomic imbalances in genetic syndromes but frequently new chromosomal rearrangements and micro reshuffling were found associated to a phenotype more or less clinically evident. In this context we present 2 clinically situation. The first case is a newborn, with radial aplasia and without cytogenetic anomalies evidenced after GTG banding. Genomic DNA evaluation using qPCR identified a 200 kb microdeletion including a region with 11 genes on 1q21.1 chromosome. Even it is described this microdeletion is rare among patients and it is prerequisite for phenotype. Another case, it is a female with compartmental disorder and obesity. She was investigated using conventional karyotype and FISH analysis for Prader Willi due to an overlapping phenotype with Prader Willi syndrome. Because it was possible to be performed, array CGH was done. In conclusion, we consider that it is harder for clinicians to have significant conclusion based on clinical feature and to establish a diagnosis without the support of molecular techniques.

## **O 20. Could tetrahydrobiopterin be a promising treatment for phenylketonuria patients in Serbia?**

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Phenylketonuria (PKU) is caused by mutations in the phenylalanine hydroxylase gene (PAH). Several studies showed that a number of PKU patients respond to tetrahydrobiopterin (BH4) supplementation treatment. Apparently, the effect of BH4 therapy is dependent on a specific PAH mutation. Therefore, we present the update on PAH mutations detected in Serbian PKU patients and the calculated frequency of patients who could benefit from BH4 use. In total, we performed genotype analysis of 44 unrelated patients by PCR-RFLP and 'broad range' DGGE/DNA sequencing analysis. Altogether, we identified 20 different mutations and only one, R243X, was not previously found in Serbian population. The most frequent mutations, L48S (27.3%), R408W (17%), P281L (8%) and E390G (7%), accounted for 59.3% of all mutant alleles, while remaining ones occurred at frequency less then 5%. The L48S mutation as well as 4 other mutations (E390G, R261Q, R158Q and R413P) were characterized as BH4-responsive ones in previous European studies. Accordingly, the total frequency of BH4-responsive mutations for Serbian population is 43.2%. Number of Serbian patients carrying at least one BH4-responsive mutation (including four L48S homozygotes) is 24. Thus, the calculated frequency of BH4-responsiveness in Serbian PKU population is 54.5%, quite close to the predicted average value for European populations. Tetrahydrobiopterin supplementation treatment is not yet available in Serbia. However, our genetic assessment implies that this treatment could be promising for the half of Serbian PKU patients.

Key words: mutation detection, phenylalanine hydroxylase gene, phenylketonuria, supplementation treatment, tetrahydrobiopterin



## **O 21. Detection of Cystic Fibrosis mutations by Multiplex Snapshot Analysis**

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Based on the frequency of the most common CFTR mutations worldwide and those in the populations of the Republic of Macedonia and neighboring countries we have developed a new diagnostic assay based on multiplex SNaPshot analysis. It contains two multiplex PCR and SNaPshot mixes for simultaneous detection of 10 common CFTR mutations (DF508, G542X, N1303K, 621+1G-T, R117H, R553X, G551D, W1282X, R1162X, 2184insA, 1717-1G-A and IVS8 polyT alleles). Using this method as a first step in the detection of CFTR mutations we have performed 170 analyses of suspected CF patients, CF carriers and their families. Twenty eight of them were for prenatal diagnosis of CF. Four different mutations were detected in 80 individuals. In five patients with one detected mutation and in two patients with no CF mutation, but clear clinical evidence of CF, further analyses using reverse line-hybridization tests Inno Lipa CFTR19/17+Tn update (Innogenetics, Belgium) and multiplex ligation probe amplification (MLPA) P091-CFTR kit (MRC Holland) were performed. Homozygosity for R347P and 2798+5G-A was detected in the two patients with no CF mutation detected by SNaPshot analysis. In one of the patients the deletion involving exon 11 suspected by the SNaPshot analysis IVS -8 poly T results in the family, was confirmed by MLPA. No CF mutation was detected in the remaining four patients. In conclusion, we have developed a rapid, simple, reliable and inexpensive method for determination of the most common mutations in the CFTR gene.

KW: CF, SNaPshot, mutation

## **O 22. Association of PAI-1 and TNF-alpha gene polymorphisms in Familial Mediterranean Fever (FMF) patients**

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Familial Mediterranean fever (FMF) is a hereditary recurrent fever associated with mutations in the gene MEFV encoding pyrin. The imbalance between pro- and anti-inflammatory cytokines may play a role in its etiology. In this study we aimed to investigate whether cytokine gene polymorphism is

associated with FMF, and to evaluate the relationship between these polymorphisms and genotypic manifestation of FMF. We investigated single nucleotide polymorphisms of tumor necrosis factor (TNF-alpha) promoter at positions -308 G/A and PAI-1 4G/5G genes in DNA from the peripheral blood leukocytes of 177 cases with different genotype combinations. Polymorphisms of TNF-alpha promoter at positions -308 G/A and PAI-1 4G/5G were detected by using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). There was no association between TNF-alpha/308 genotypes and mutations in FMF. In contrast to TNF-alpha/308 polymorphisms, PAI 4G/5G gene polymorphism has a more significant effect in FMF disease. Screening with PAI polymorphism tests may be beneficial for tracing future FMF patients. Nevertheless, further investigations are needed to reach a conclusion about the association between PAI polymorphisms and FMF.

### **O 23. Prenatal genetic diagnosis in Mucoviscidosis (Cystic Fibrosis)**

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Our goal was to detect CFTR mutations in fetal genomic DNA isolated from amniotic fluid collected by classic amniocentesis and early amniocentesis. Ten couples were selected for performing prenatal diagnosis. Molecular diagnostic was performed on Genomic DNA isolated from venous blood samples collected on EDTA from both parents and on amniotic fluid samples collected by classic amniocentesis (16th week of pregnancy) and by early amniocentesis (13th week of pregnancy). For detection of CFTR mutations we used the Elucigene CF29 kit. The results showed: 6 couples with negative genetic test, 3 with heterozygote for one of next mutation (DF508, G542X, 621+1 G-T), and one of them affected DF508/621+1 G-T). Conclusion. Prenatal diagnosis can be performed on samples of amniotic fluid collected by normal amniocentesis or by early amniocentesis however, due to the greater risks for pregnancy and fetus of this early procedure and because the volume of collected amniotic fluid is reduced. Also, for an accurate prenatal diagnosis in mucoviscidosis it is required to have a good sampling technique for the amniotic fluid. Mutation detection by ARMS-PCR with Elucigene CF29 is applicable only to those couples in which at least one of the parents is a carrier of the DeltaF508 mutation, or when both parents carry different CFTR mutations, other than Delta F508, because the kit can differentiate between the condition of heterozygote (carrier of a mutation) and homozygote (diseased) only in the case of DeltaF508 mutation.

### **O 24. Congenital lung malformations and their implication in life's quality**

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Background: Congenital lung malformations are rare, often discovered on routine prenatal sonography and most asymptomatic at birth. Complications can occur in all patients: pneumonia poorly responding to medical treatment, malignancies, hemoptysis, pneumothorax and hemothorax. The lesions may spontaneously regress (asymptomatic congenital lobar emphysema) or even disappear (congenital cystic adenomatoid malformation). Management of asymptomatic lesions is controversial: conservative approach with CT scan surveillance or surgical excision. Early surgery is required for symptomatic neonates and infants. The postoperative outcome is good, with normal respiratory function. Assessment of associated anomalies is required. Aim: To determine the outcome and life's quality in pediatric patients with congenital lung malformations according to medical and/or surgical management. Methods: Antenatal ultrasonography, history, physical examination, postnatal imaging studies (chest X ray; abdominal, cardiac and transfontanelar ultrasound; thoracic CT), laboratory and interdisciplinary evaluations have to be made. Results: Prenatal identified lung malformations directs perinatal management and completes diagnostic work-up. Congenital cystic adenomatoid malformation (CCAM), bronchogenic cyst and congenital lobar emphysema may be asymptomatic at birth or at the time of discovery. The outcome is favorable for CCAM after partial, early performed lung resection. Bronchogenic cyst and lobar emphysema have a poor quality of life (numerous lower respiratory infections and multiple extended hospitalizations; noncompliant parents who refuse surgery). Conclusions: The natural history of congenital lung malformations is not fully known. Neonatal presentation doesn't allow a reliable prognosis. In symptomatic patients, surgery before 6 months of age is recommended to prevent the complications. Genetic evaluation, interdisciplinary management and long-term follow up are required.

## **O 25. Hepato-splenomegaly syndrome in inborn metabolic diseases**

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Hypothesis. Inborn storage diseases are monogenic disorders caused by intracellular excessive accumulation of unchanged substances, secondary to the genetic deficiency of enzymes involved in different metabolic pathways. The clinical feature is complex, reflecting the multisystemic nature of these diseases. Objective: The authors aim to establish frequency of hepato-splenomegaly in lysosomal storage diseases and glycogenoses. The material was represented by a group of 120 patients aged between 6 months and 58 years with clinical signs suggestive of an inborn metabolic diseases

Study methods: included: 1) nonspecific examination: clinical examination, anthropometric measurements, biochemical tests, imaging exams, cardiological, neurological, psychological, ophthalmological assessment and 2) specific examination: determination of enzymes involved in metabolism of sphingolipids, mucopolysaccharides, glycoproteins, mucolipids and glycogen .

Results. Complete diagnostic evaluation revealed various degrees of hepato-splenomegaly in 9/9 patients with glycogenoses and 96/111 patients respectively with lysosomal diseases. The types of glycogenoses diagnosed were the following: type III, type IX, type I and type IIB (5,2,1 and 1 patients). Hepato-splenomegaly was revealed in all patients (100%) with mucopolysaccharidosis, mucopolipidosis,

and glycoproteinosis (18, 3 and 1 cases) and in 96 patients (86,4 %) with sphingolipidosis (Gaucher disease, GM1, GM2- gangliosidosis and Niemann-Pick disease 61, 11, 1 and 2 cases).

Conclusions. The differential diagnosis of hepato-splenomegaly syndrome should include a possible inborn metabolic disease not only in children but also in adult patients.

## **O 26. Biochemical and molecular genetic diagnosis of Lysosomal Storage Disorders in Romanian patients**

Cristina Drugan

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**Background** As an significant group among inherited metabolic disorders, lysosomal storage disorders may represent a major healthcare concern. This presentation reports the experience of the laboratory for the diagnosis and follow-up of romanian patients with lysosomal storage disorders.

**Aims** This study aims to provide an insight into the distribution of lysosomal storage disorders in our population. A second objective was the analysis of the most prevalent mutations in romanian Gaucher disease patients. **Methods:** 293 suspects originating from all regions of our country were referred to our laboratory during the last fifteen years. Lysosomal enzyme assays performed in plasma and peripheral blood leukocytes indicated specific deficiencies in 105 patients (35% of the investigated suspects).

**Results** Gaucher disease was the most prevalent in our population, as it was confirmed in 62 patients (59%), belonging to 53 unrelated families. Other less prevalent sphingolipidoses were GM1 gangliosidosis (13 patients), Fabry disease (12 patients), GM2 gangliosidosis (one patient) and metachromatic leukodystrophy (one patient). Mucopolysaccharidoses and mucolipidoses were identified in 15 patients. Mutation analysis in the acid beta-glucosidase gene indicated a high degree of genetic homogeneity. Genotype-phenotype correlations in Gaucher disease patients were similar to those reported in other Caucasian populations, but also indicated specific characteristics.

**Conclusions** Lysosomal storage disorders represent an important pathology in our population. Many such patients may still await for the confirmation of a yet unidentified disorder. Specific diagnosis and follow-up is the key step in the accurate management and treatment of these patients.

**Keywords:** Romanian population, lysosomal storage disorders, mutation analysis

## **O 25. Prader-Willi Syndrome in a 30 year-old patient - a case report**

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**Background:** Prader-Willi Syndrome (PWS) is a complex genetic disorder with a prevalence of 1:12000 to 15000 in all races and both sexes and is caused by a lack of expression of paternally inherited genes on chromosome 15q11-q13. **Case presentation** We report the case of a 29 years and 10 months old female patient, 149 cm in height and weighing 119 kg. The patient was born to healthy biological parents. At the age of 24 the patient was confirmed through FISH testing with PWS. The particularities of this case

are the low level of mental retardation of the patient and the threshold of pain feeling, also much lower than in most cases with PWS. The difficulties of the case derive from these particularities. She refuses blood testing, injections and some medication. Her actual status is very severe. Diabetes mellitus was detected but due to lack of compliance to treatment and diet is getting critical. The patient has a hyperglycemia of 550 mg/dl (and refusing insulin injections), cardiac failure, generalized pruritus. Her legs are extremely swollen with edema and cellulitis and present a number of infected ulcerations. A series of allergic reactions to most classes of antibiotics were also reported. Conclusions: We report this case because of its particularities which worsen the treatment possibilities. The patient is in desperate need of further investigations and therapy, psychologic counseling and medical care. This presentation also emphasizes the importance of the continuous follow-up of the psychological state of patients with PWS. The difficulties of this case derive from the behaviour problems and from the poor compliance of the patient to treatment and diet. Keywords: Prader-Willi syndrome, particularities, lack of compliance to treatment, hyperglycemia

# Poster

## **P01. Cellular DNA damage and lipid peroxidation after whole body gamma irradiation and treatment of total extract of *Haberlea Rhodopensis***

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*Haberlea Rhodopensis* (HR) is a naturally occurring endemite in the Balkan region and is a world record-holder in desiccation tolerance. In vivo studies have shown that total extract of HR protected DNA against ionizing radiation. New Zealand white rabbits were exposed to 2.0 Gy of gamma radiation in presence and absence of HR. Extract of HR was implemented at a dose of 200 mg/kg b.wt. to rabbits prior and after to whole body radiation exposure. Whole body irradiation resulted in damage to cellular DNA (as measured by alkaline comet assay) in peripheral blood lymphocytes and increase in peroxidation of lipids (as measured by the level of MDA) in blood plasma. Administration of HR at a dose of 200 mg/kg b.wt. to rabbits prior to whole body radiation exposure reduced the peroxidation of lipids and the damage to the cellular DNA indicating in vivo radiation protection of membranes and DNA by extract of HR.

Key words: comet assay, *Haberlea Rhodopensis*, lipid peroxidation, radioprotector

## **P02. Genetic investigation in acute leukemias**

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Background: Acute leukemias represent heterogeneous disorders characterized by accumulation of leukemic stem/progenitor cells. Cytogenetics plays an essential role in diagnosis, prognosis and treatment outcome prediction in acute leukemias. Aim: We report on the results of a genetic study of myeloid and lymphocytic acute leukemias. Methods: Chromosomal studies - GTG banding and fluorescence in situ hybridization - were performed on bone marrow cytogenetic slides. Oligonucleotide arrayCGH (Agilent 44k) were applied on few selected cases with complex quantitative genomic changes. Results: Both simple and complex cytogenetic anomalies were detected in our study group. Eight

patients presented reciprocal translocation as sole anomaly: t(8;14), t(8;21), t(9;11), t(11;19), t(15 ;17), and t(16 ;16). Complex karyotypes changes were detected in 6 cases, comprising both balanced and unbalanced chromosomal abnormalities. Conclusions: Some of the genetic anomalies detected in our patient group are highly specific for certain leukemia subtypes and have a well defined prognostic value. For rare/new chromosomal abnormalities the prognostic impact is difficult to be established. Even for the same genetic change, differences in the disease evolution/treatment outcome may appear due to the presence of submicroscopic, unrecognized genomic/genic defects. Therefore, larger patient groups, well characterized at molecular level, are needed in order to further the knowledge into acute leukemia biology.

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Keywords: acute leukemias, chromosomal abnormalities, reciprocal translocation, complex karyotype

### **P03. Identification by microarray technology of candidate genes maintaining interstitial Cajal Cell and interstitial Cajal-like Cell phenotypes in C-kit mutant mice**

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Interstitial Cajal Cells (ICCs) are involved in digestive motility and neurotransmission, but also in gastrointestinal tumors pathogenesis. Other extra-digestive organs (such as: gall bladder, pancreas, heart, uterus) have been shown to contain interstitial Cajal-like cells (ICLCs), whose function is yet unknown. Both ICCs and ICLCs express the c-kit protein, a tyrosine-kinase receptor with essential role in differentiation and maintain their phenotype. In order to contribute in understanding the physiology of ICC and ICLC we performed comparative investigation of gene expression in normal and mutant mice by DNA microarray. Total RNA isolated from different organs of control and mutant mice (WBB6F1/J-KitW/KitW-v/J strain) by AllPrep DNA/RNA Mini Kit (Qiagen) was analyzed by Bioanalyzer and RNA 6000 Nano assay Kit (Agilent Technologies). Microarray slides of Whole Mouse Genome Microarray Kit (Agilent Technologies) were hybridized and scanned by Agilent DNA Microarray Scanner. The data analysis was performed by Feature Extraction and GeneSpringGX10 Software (Agilent Technologies). More than 2000 genes demonstrated differential expression by >2 fold in mutant versus control mice. Some genes found to be up-regulated, such as: Dmbx1, Eif4a1, Zfp593 and Zfp69 (involved in transcription regulation), Abi2, CaCng8, Hbb-b1 (cellular transport and cell junctions) and Calcr, Cyp3a44, Mcpt1 (signal transduction and metabolic process regulation). Other genes showed to be down-regulated, such as Wnk1, Sema5a, Nf1, Tnfrsf21 (involved in apoptosis, intra-cellular transport and inter-cellular communication). Some of these genes, validated by RT-qPCR, may become candidate biomarkers for studying ICC and associated pathology in humans.

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#### **P04. Chromosomal Changes in Acute Leukemia**

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Acute myelocytic leukemia (AML) and Acute lymphoblastic leukemia (ALL) is characterized by a variety of numerical and structural chromosome aberrations. 156 cytogenetic analyses of patients with leukemia with ages between 1 month –73 years have been performed in the Cytogenetic laboratory of the Emergency Children Hospital "Louis Turcanu" Timisoara and University of Medicine and Pharmacy,,Victor Babes" Timisoara during 2004-2011. The cytogenetics diagnosis is a prognostic factor with the capacity to predict the remission rate, the length of the remission and the survivorship period, regardless of the haematological, immunological and clinics parameters. For the confirmation of previous data, CGH may provide useful information regarding the nature of genomic aberrations that take place in cases with complex karyotypes.

#### **P05. GSTP1 gene hypermethylation as a molecular biomarker in noninvasive prostate cancer diagnostic**

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Background: Promoter hypermethylation of GSTP1 on chromosome 11q.13 is the most frequent DNA alteration in prostatic carcinoma, being specifically detectable in more than 90%, including early stages. Curative treatment is feasible provided when the disease is diagnosed in its earliest stages, but current screening methodologies are characterized by low specificity. Aim: In our study we determined the diagnostic significance of detecting aberrant promoter hypermethylation of GSTP1 gene in serum DNA for early noninvasive detection of PCa. Material and method: For our study we collected tissue and serum samples from 64 patients with the histologically confirmed cases of prostate adenocarcinoma, Gleason score of 3 to 9, and 44 cases with BPH. Patients with BPH were used as control subjects. Methylation-specific PCR (MSP) method was used to evaluate the methylation status of GSTP1 promoter gene. Results: By MSP method the GSTP1 promoter hypermethylation was detected in 62 from 64



prostate cancer samples (96.87%), but none of the BPH showed aberrant methylation. A receiver operating curve (ROC) that included clinicopathologic parameters (PSA levels, pathological stage, Gleason score) and hypermethylation status of the GSTP1 gene, gave a predictive accuracy of 96% with sensitivity and specificity of 98% and 90%, respectively. Conclusion: GSTP1 promoter hypermethylation distinguishes between PCa and BPH lesions, and could be used as a biomarker for PCa screening and early molecular detection of this disease. Key words: prostate cancer (PCa); benign prostatic hyperplasia (BPH); glutathione S-transferase P1 (GSTP1); methylation-specific PCR (MSP)

#### **P06. Genetic Polymorphism of DNA repair gene ERCC2/XPD (Arg 156 Arg) (A22541C) and Lung Cancer Risk**

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Background. Polymorphisms for genes encoding proteins that act like DNA repair factors might contribute to the variability in individual susceptibility to lung cancer. The role of ERCC2/XPD in lung carcinogenesis might be very important in exposure to carcinogens. Objectives. This is a cross-sectional, randomized, case control study for the evaluation of the frequency of ERCC2/XPD (A22541C) alleles among patients with lung cancer. Subjects The study included 80 cases of lung cancer diagnosed patients (histopathological examination), recruited from the Pneumology Hospital Leon Daniello Cluj and 125 healthy unrelated controls, selected among patients observed in the Internal Medicine Department. Methods. ERCC2/XPD genotyping was carried out using PCR amplification of relevant gene segment was followed by restriction enzyme digestion. Detection of ERCC1/XPD (A22541C) alleles was determined through analysis of resulting restriction fragment length polymorphism (RFLP) followed by gel electrophoresis. Results. Molecular analysis revealed increased frequency of ERCC2/XPD Arg 156Arg (A22541C) mutant allele in the study group compared to the control group (p=0,03). The variant allele of XPD Arg 156Arg was also associated with an increase risk of lung adenocarcinoma. Conclusions. The results of our study reached statistical significance, so we consider that ERCC1/XPD null allele carriers is related with lung cancer risk. Our findings suggest that heritable XPD Arg156Arg polymorphic status may influence the risk of lung cancer development.

#### **P07. Detection of p53 gene mutations and mitochondrial DNA mutations in bladder tumors**

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**Background:** The effect of p53 tumor suppressor gene mutations and mitochondrial DNA (mtDNA) mutations have been studied separately in various tumors; however, both of these factors did not frequently investigated. It has been suggested that analysis of p53 gene mutations and mtDNA mutations might be useful to monitor the prognosis of bladder cancer and its response to certain therapies. **Aim:** Therefore, to understand the significance of p53 gene mutations and mtDNA mutations in bladder cancer development, we investigated the mutations in p53 gene and mtDNA in bladder cancer patients. **Methods:** 30 patients and 27 controls were recruited to the study. Bladder cancer tissues were obtained by radical cystectomy or transurethral resection. Genomic DNA was extracted from peripheral blood. Exon 5,6,7 and 8 regions of p53 gene as well as ATPase6, Cytb, ND1, and D310 regions of mtDNA were amplified by PCR and then sequenced. The relationship between p53 gene mutations, mtDNA mutations and development of bladder tumors were evaluated statistically. **Results:** In patients 40 mtDNA gene mutations were found in which 7 of them were defined as novel mutation. 4 mutations were found statistically significant in bladder cancers. Investigation of p53 gene mutations proceed. By this time, totally 8 mutations in p53 gene were detected. **Conclusions:** In conclusion, the high incidence of mtDNA mutations and p53 gene mutations in bladder cancer suggests that mitochondria and p53 gene could play an important role in carcinogenesis

#### **P08. Cytogenetic characterisation of variant Philadelphia translocations in patients with chronic myeloid leukemia**

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**Background:** The t(9;22)(q34;q11) translocation is found in about 90% of chronic myeloid leukemia (CML) patients. The minority of cases presents a variant type of Ph translocation. The prognostic impact of variant translocations has not been fully elucidated. **Aim:** The purpose of the study we conducted was establishing the significance and frequency of complex chromosomal rearrangements developing during progression of CML. **Methods:** The study was conducted between January 2000 and May 2011. The study group included 92 patients: 50 males and 42 females. The average age was 41,11 years. The cytogenetic studies were made on hematopoietic bone marrow using culture for 24-48 hours on a culture medium dedicated for hematopoietic cells, followed by standard cytogenetic exam, GTG banding and karyotyping. The hematologic and cytogenetic evaluations of patients were monitored during the treatment. **Results:** Out of 92 patients with chronic myeloid leukemia cytogenetic analyzed, 8 patients had Philadelphia (Ph) negative, 79 patients had Ph positive, of which 5 patients carried a variant Ph translocation. Four patients had complex translocations involving 9q34, 22q11, and a third

chromosome: t(9;10;22)(q34;q24;q11), t(6;9;22)(p21.3;q34;q11), t(7;9;22)(p22;q34;q11) and (9;11;22)(q34;p11;q11). One patient had a complex variant Philadelphia (Ph) translocations, t(8;19;22), with no obvious involvement of chromosome 9. Two patients, had after treatment the unusual secondary changes t(1;4)(q32.1;q13.2) and t(14;20)(q24.2;13.1), which were transient. Conclusions: The present findings strongly suggest that variant Ph translocations of CML occur as primary cytogenetic changes similar to the classical Ph1 translocations. Identification of complex chromosomal aberrations may provide information of genetic mechanisms playng role in disease progression.

#### **P09. MOLECULAR GENETIC SERVICE OF COMMON AND RARE DISEASE IN BULGARIA**

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During the last 5 years the molecular genetic service in Bulgaria exit the frame of the most common genetic disorders and spread over the rare disease. The aim was to answer the need of each family in regard to adequate genetic counseling and prenatal diagnosis. Our recent achievements in the field of molecular diagnostics of rare disorders are discussed in the presentation. The groups of disorders covered are: neurological and neuromuscular disorders, ataxias and neurodegenerative disorders, mental retardation, autism and epilepsy - syndromic and non-syndromic forms, mitochondrial disorders, skeletal dysplasia, deafness, metabolic disorders and cancers. In the field of common genetic treats we study molecular bases of cystic fibrosis, beta-thalassemia, haemophilia A and B, congenital adrenal hyperplasia due to 21-hydroxylase deficiency, familial mediterranean fever, the most common aneuploidies, DNA analysis of infectious disease. A number of genetic predispositions were recently introduced in our laboratory panel: genetic predispositions to early and late spontaneous abortions, to cardiac problems and periodontitis. Pharmacogenetics and drug metabolizers are investigated on molecular level. Biomarkers (KRAS, BRAF and microsatellite instability) in cancer research are applied in our routine programs for genetic testing. Some interesting newly diagnosed cases are presented: Gorlin syndrome (two cases); neurofibromatosis type 1; SCA2; MDC1A (LAMA2 deficiency); LGMD2B (dysferlinopathy); Aicardi-Goutieres syndrome; von Willebrand disease; 21-hydroxylase deficiency, familial Mediterranean fever, multiple endocrine neoplasia type 2A (MEN2A). The molecular genetic service in Bulgaria moved to a higher level. Each genetic test is performed in a short time frame, suitable for prenatal performance.

#### **P10. A child with Killian-Pallister Syndrome detected with FISH on buccal smear**

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Killian-Pallister syndrome is a dysmorphic chromosomal syndrome that affects most organ systems. It is characterized by existence of tetrasomy of 12p chromosome in mosaic state and is tissue- limited mostly in fibroblasts. The extra metacentric chromosome is an isochromosome for part of the short arm of chromosome 12: i(12)(p10). The syndrome is often difficult to diagnose despite characteristic facial appearance. It is characterized with moderate mental retardation, short stature, seizures and specific facial dysmorphism. A girl approached our clinic due to moderate motor and mental developmental delay, seizures and specific dysmorphism. It was a second child of young and unrelated parents. The pregnancy was normal; the baby was a full-term neonate, with birth weight and length within normal limits. The child had failure to thrive and was below 3rd percentile for the height and had dysmorphic features - sparse hair bitemporaly, prominent forehead, upslanting and narrow palpebral fissures, short nose with anteverted nares, protruding lips. Epileptic spasms started late at 4 years of age and were hard to cope. The karyotype of blood leukocytes was normal. The diagnosis was confirmed using FISH probe for chromosome 12 on buccal smear in 10% of the cells. The diagnosis of Killian-Pallister syndrome is difficult to establish, since the karyotype of the leukocytes is normal, and skin fibroblasts are not routinely evaluated in all cases with developmental delay especially when seizures develop frequently. FISH for 12p probe on the buccal smear cells can be efficient diagnostic tool if a suspicion for this syndrome exists.

#### **P11. Direct detection of DMD/BMD carriers by quantitative real-time PCR**

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Introduction: Duchenne and Becker muscular dystrophy (DMD and BMD) are X-linked neuromuscular disorders caused by mutations in the dystrophin gene located at the Xp21 region. As an effective treatment still remains unavailable, determination of carrier status is very important for genetic counselling and prevention of these diseases. Aim: The aim of our study was to optimize quantitative real time PCR assay based on SYBR Green I chemistry so that it can be easily used for routine diagnostics of DMD/BMD deletion carriers. Material and methods: Twenty female relatives of DMD/BMD patients with deletions in exons 6, 47 and 52 were studied. Relative quantity of the target exons was calculated by comparative threshold cycle method. Normal female samples were used as calibrator and non-deleted exons as reference exons. Results: Using this assay carrier status of all subjects was successfully determined. Results previously obtained by indirect carrier detection were confirmed. The gene dosage ratio for non-carriers was  $1.07 \pm 0.20$  and for carriers  $0.56 \pm 0.11$ . The ratio ranges between carriers and non-carriers of the deletions did not overlap allowing the accurate discrimination of deletion carriers and normal individuals. Conclusion: This assay proved to be simple, rapid, reliable, and cost-effective. It may be used for direct determination of deletions in female relatives of DMD patients with known

deletions. Also, it may be easily adapted for other genetic conditions involving deletions and duplications.

### **P12. Splicing mutation in intron 2 (nt. 656) of the CYP21A2 gene in Macedonian patients with Congenital Adrenal Hyperplasia**

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Background: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder. In 90-95% of cases it results from mutations in the gene for 21-hydroxylase (CYP21A2). It can present as a classic salt-loosing (SL) and simple virilizing form (SV), and nonclassic late onset form (LO). The IVS2 (nt656) mutation allows for 2% enzyme activity, and comprises 25% of the classic 21-hydroxylase deficiency alleles and 51% of the SL alleles. Aim: To perform direct molecular analysis of the IVS2 mutation in 53 patients from Republic of Macedonia, with different ethnical origin and clinical presentation of CAH, and 75 their healthy relatives, from 49 unrelated families. Method: We performed direct molecular diagnosis using the differential Polymerase Chain Reaction (PCR), ACRS (Amplification Created Restriction Site) followed by a restrictional endonuclease digestion. Results: The IVS2 mutation was detected in 41.5% of the patients, 34% homozygotes with severe classical CAH phenotype and 7.5% heterozygotes. Different ethnical distribution of the IVS2 mutation (100% in Gypsies, 57.1% in Albanians and 20.6% in Macedonian patients) was observed. IVS2 mutation was detected in 71.7% of the SL alleles, 25% SV and 2.8% of the LO alleles. The IVS2 mutation was also found in 29.3% of the healthy relatives. Conclusion: The observed frequency of IVS2 mutation was comparable to other reports. Genotype-phenotype correlation was detected in all patients with IVS2 mutation. These results support a role of the IVS2 mutation in severe classical CAH phenotype and it showed that there are ethnic specific differences in the IVS2 distribution.

Key words: Congenital adrenal hyperplasia, CYP21A2 gene, IVS2 (nt655) mutation.

### **P13. Central core disease with a new mutation of RYR1 gene in a Romanian family**

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Central core myopathy is a rare disease, with a wide spectrum of clinical findings, resulting from the ryanodine receptor type 1 (RYR1) gene mutation on chromosome 19q13.1. Prevalence is unknown but the condition is probably more common than other congenital myopathies. More phenotypes associated with RYR1 mutations have been identified. We report a case of a 30 years-old pregnant woman, 10 weeks of gestational age, who was referred to our Genetics Department for genetic counseling. She was known to have skeletal muscle weakness since birth and had a history of delayed motor milestones as a child. She had also dysmorphic facies with ptosis, downslanting palpebral fissures and facial weakness, congenital hypotonia, exercise intolerance. Her daughter, 3 years old, presented delayed motor development, but at this age the phenotype is that of her mother. The electromyography showed normal result, serum creatine kinase concentration was normal, but muscle biopsy revealed histological diagnosis of congenital fiber type disproportion. After molecular genetic testing of the mother and child were found to possess the same novel mutation in the RYR1 gene. We did also prenatal diagnosis for pregnant mother, but the male fetus was normal. Most RYR1 mutations that result in Central Core Disease (CCD) are inherited in an autosomal dominant manner. Management is mainly supportive and has to anticipate susceptibility to potentially life-threatening reactions to general anaesthesia. In this report, we discuss also the classes of RYR1 mutations which have been associated with CCD, Multiminicore Disease and related neuromuscular phenotypes. KEYWORDS: Central Core Disease, congenital myopathy, RYR1 mutation

#### **P14. Balanced translocation t(4;8) in father causing chromosome abnormalities in three consecutive offsprings**

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Background: Parental balanced translocations is a common reason for chromosome abnormalities in their offsprings. All autosomal and sex chromosomes can be involved and clinical presentation is variable. Aim: Chromosomal analysis in a family with a child who has partial 4q trisomy . Method: We performed standard G banded karyotype from peripheral blood lymphocytes. Results: A 2 year old girl was referred because of motor and mental delay and dysmorphic features (hypertelorism, bulbous nose, retrognathia, low slanted, displastic ears, antimongoloid slanted eyes, broad forehead). It was a second child of young and unrelated parents. Chromosome analysis showed extra material on the short arm of 8th chromosome. Chromosomal finding in her parents revealed balanced translocations t(4;8)(q28;pter) in the father. The mother was normal. The older sister of the patient was also normal. This finding showed that girl inherited aberrant 8th chromosome from her father. Following two pregnancies were terminated due to identical chromosome abnormalities in the fetus. Conclusions: Our results show that carriers of balanced translocation have a high risk for chromosomal aberration in their children. Genetic counseling and prenatal diagnosis is necessary in these families. Key words: Balanced translocation, karyotype, chromosome aberration.

### **P15. Laboratory approach for diagnosis of lysosomal storage disorders**

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Summary: Lysosomal storage disorders (LSD) are a group of more than 50 rare genetic (monogenic) diseases due to disturbed functions of lysosomes and subsequent storage of abnormal metabolites in cells of different tissues and organs. There is expression and progressive evolution of clinical signs varying from coarse face, skeletal and skin changes and organomegaly to neurological symptoms and severe developmental delay. LSD as single disorders are rare with total average frequency 1 in 5000 to 7000 newborns. They are inherited in autosomal recessive manner with exception of the X-linked Fabry and Dannon disease and mucopolysaccharidosis type II (Hunter disease), therefore the recurrence risk is 25%. Recently LSD attain significance for the pediatricians because of the possibility for enzyme replacement therapy and testing of enzyme activity in dried blood sample. At the National genetic laboratory about 4500 patients have been tested for LSD up to now. In 227 (5%) out of them a final diagnosis of LSD is reached: 66 patients with mucopolysaccharidosis. 33 with glycoproteinosis, 7 with mucopolipidosis; 40 patients with Gaucher disease, 30 with Niemann Pick B and 3 with Niemann Pick C disease, one with cholesterol esters storage disease; 2 patients with Tay-Sachs disease, 4 with Sandhoff disease and 2 patients with late infantile form of GM1; 27 patients with metachromatic leukodystrophy and 12 patients with Krabbe disease. In 68 patients the diagnosis was confirmed by DNA analysis. A prenatal diagnosis of 59 risk pregnancies and 15 (25.42%) affected fetuses were detected. The reliable biochemical diagnosis allowed the therapy of 15 patients - 13 with Gaucher disease and 2 with Fabry disease.

### **P16. Chromosome 1p36 deletion in two patients with MR / MCA syndrome detected by MLPA**

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The advent of new technologies for molecular cytogenetics such as FISH, MLPA, array CGH has dramatically increased the number of novel microdeletions/ microduplications associated with wide range of clinical phenotypes of a relative stable and distinctive MCA/MR syndromes. 1p36 deletion syndrome is characterized by typical craniofacial features consisting of straight eyebrows, deep-set eyes, mid-face hypoplasia, broad and flat nasal root/bridge, long philtrum, pointed chin, large, late-closing anterior fontanelle, microbrachycephaly, epicanthal folds, and posteriorly rotated, low-set, abnormal ears. Other characteristic findings include brachy/camptodactyly and short feet. Developmental delay/intellectual disability of variable degree are present in all, and hypotonia in 95%. Seizures occur in 44% to 58% of affected individuals. Other findings include structural brain abnormalities, congenital heart defects, eye/vision problems, hearing loss, skeletal anomalies, abnormalities of the external

genitalia and renal abnormalities. We report two girls (9 and 2 years old) with detected 1p36 deletion. Both of them presented with developmental delay, typical facial dysmorphic features and hypotonia. Other findings included pubertas praecox in the first patient and autistic behavior and seizures in the second patient. Routine cytogenetic studies (400 band level) were normal. MLPA, performed using kit P245 (MRC, Holland), detected deletion of the loci GABRD, GNB1 and TNFRSF4. Follow-up studies by arrayCGH were performed. Although presenting a very rare disorder, the 1p36 microdeletion syndrome is already well defined MCA/MR syndrome despite its wide phenotypic variability. Expanding professionals' knowledge about clinical and molecular features of the disease will help to diagnose suffering individuals and provide genetic counseling of their families.

### **P17. A case with the novel 4q21 microdeletion syndrome**

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Background: Here we report on one case with 4q21 microdeletion syndrome - 5,5 years old boy with severe growth retardation, delayed bone age, severe psychomotor retardation (IQ=44), absence of speech ability, bilateral sensorial deafness. There are also other abnormalities: dismorphic features - dolichocephaly, retrognathia, up slanting palpebral fissures, small mouth with thin vermilion of the upper lip, teeth anomalies, brachidactylia, kiphosis. Aim: Molecular karyotyping was applied in order to reveal the underlying etiology. Methods: We have used BlueGnome CytoChip oligo 2X105K microarray, v1.1, with 35kbp backbone resolution. The pathogenic result was confirmed with fluorescence in situ hybridization (FISH). Results: The deleted region was 1,700,916 bp in size and harbors 15 HGNC and 7 OMIM genes from 82,048,224 to

83,182,488 bp. The FISH analysis with BAC - RP11-45I20/Spectrum green confirmed the deletion.

Conclusion: After analysis of the smallest region of overlap between the reported cases with 4q21 microdeletion syndrome, we narrowed the common region to a 1.134 Mb which contained the PRKG2 and ASGEF1B genes.

Key words: molecular karyotyping, 4q21 microdeletion, PRKG2, ASGEF1B. Acknowledgements: Grant 02/76-21.12.2009, National Science Fund, Bulgaria.

### **P18. Familial pattern of Marfanoid habitus**

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Marfanoid habitus is a clinical feature found in a number of syndromes including Marfan syndrome, Spritzen-Goldberg syndrome, Lujan-Fryns syndrome and Congenital Contractural Arachnodactyly. We reported a female and her newborn with marfanoid phenotype. The mother was evaluated for the first time when she gave birth. She had a marfanoid habitus, measuring 169 cm in height and 43 kg in weight). Her face was long and narrow with down-slanting palpebral fissures, long and low-set ears but without the characteristic "crumpled" appearance of CCA. The oral cavity was narrow with very high-arched palate, crowded teeth with ectopic eruption and enamel hypoplasia. She had arachnodactyly of the fingers with fusiform aspect, bilateral flexion contractures of both elbows to approximately 15° but no limitation of movement in any of the other major joints. The feet presented minor arachnodactyly and the fingers II, III, IV and V were with camptodactyly. Echocardiogram showed aortic regurgitation and dilatation. Ophthalmologic examination revealed no evidence of subluxation or dislocation of lens. The newborn weighed 2820 g, had a head circumference of 36 cm and length of 48 cm. He had similar phenotypic features as his mother, with down-slanting palpebral fissures and appearance of "crumpled" low set ears. He presented long hands, camptodactyly with ulnar deviation of hands, plantar aspect longer than the toes, joint contractures of knee and elbows. Eye examination showed normal lens. Echocardiogram revealed slightly ectopic aortic valve, tricuspid valve with regurgitation. Our patients' features were compared with the cardinal signs on the above mentioned syndromes.

#### **P19. Minor dysmorphic features in a child with balanced reciprocal translocation (9;14)**

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Translocations are considered the most frequent chromosomal rearrangements in the human. Balanced translocation are usually harmless to carriers, individuals who are carriers for balanced translocation usually appear completely normal and healthy but minor dysmorphism and other anomalies associated were reported. We report on a 5-month-old boy with dysmorphic features and severe anemia. The patient was the first born from an uncomplicated pregnancy of a nonconsanguineous couple. Facial anomalies included hypertelorism, epicanthus, depressed nasal bridge, low set ears, tongue protrusion, long philtrum, macrostomia and short neck. We performed a cytogenetic analysis using G-banding protocol and we found an apparently balanced translocation between chromosomes 9 and 14, constitutional karyotype was 46,XY,t(9;14)(q34.1;q24.3). Blood samples were taken from both parents and chromosome analysis had been carried out on peripheral lymphocytes. Father had normal karyotype.

Mother was found to be a carrier of the same 9;14 translocation as the child, the karyotype was 46,XX,t(9;14)(q34.1;q24.3). We performed FISH analysis using telomeric probes TelVysion 14q SpectrumOrange and TelVysion 9p SpectrumGreen, Abbott Vysis. FISH analysis confirmed the presence of the translocation for the child and his mother. It still remains a question of discussion if the balanced translocation could be associated with dysmorphic features and severe anemia in child. We took into consideration the possibility of certain genes loss in breakpoint regions and array CGH will be performed for both, the child and his mother. We sustain the importance of molecular cytogenetic characterization of structural rearrangements to assist in genetic counseling and for reproductive possibilities.

## **P20. 35 years neonatal selective screening for inborn problems of metabolism In Bulgaria - past, current and perspectives**

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National genetic laboratory

The National genetic laboratory performs a selective screening program for diagnosis of 75 inborn errors of metabolism from 35 years. Patients are referred by one specialized clinic, 5 university genetic laboratories more than 70 pediatric and 119 neonatology departments in Bulgaria. The analytic platform of the program includes new technologies such as HPLC (detects over 30 aminoacids), gas chromatography with mass spectrometry (for detecting of 129 metabolites), 32 enzymatic and DNA methods for more than 60 diseases. In 2010 a method for simultaneous detection of 11 aminoacids and 29 acylcarnitines in dried blood sample was introduced and validated, based on the newest and effective metabolic screening technology - tandem mass spectrometry (MS/MS). Based on clinical signs, an original and effective algorithm for diagnosis of more than 76 rare genetic diseases, mainly with neonatal presentation, was developed. During 35 years period 9794 patients are investigated. In 35.3% of the cases the clinical presentation was in the neonatal age. In 666 (6.8%) of the tested patients 75 rare genetic metabolic diseases were diagnosed: lysosomal storage disorders - 236 (2.4%) patients, aminoacidopathias - 150 (1.53%) patients, disorders of carbohydrate metabolism - 125 (1.28%), organic acidurias - 94 (0.96%), hyperamonemia - 22 (0.22%), fatty acid beta oxidation defects - 20 (0.2%) and peroxysomal disorders - 19 (0.19%) patients. Using the developed MS/MS technique 2443 newborns aged 3-5 days have been investigated. The cut offs for 40 metabolic markers have been defined. In 2011 the National genetic laboratory will investigate more than 5000 newborns from neonatology departments for intensive cares by MS/MS method.

## **P21. Wheezing in children with Cystic Fibrosis**

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Background: Wheezing is a common symptom in asthma, allergic bronchopulmonary aspergillosis (ABPA), cystic fibrosis (CF), even in tuberculosis (TB). Sometimes these pathological conditions are difficult if not impossible to be differentiated. The aim of the paper was to evaluate the frequency of co morbidities like asthma, ABPA or TB in children with CF. Methods: One hundred and twenty-four children with CF , aged between 5-21 years, with median age at diagnosis = 11.34 years were evaluated. Study design: observational, retrospective for ten years period. For the retrospective analysis on the frequency of associated TB, asthma, ABPA, we used data records of CF Centre. The aim of the paper was to evaluate the frequency of co-morbidities like asthma, ABPA and TB in children with CF. Results: A small percent of these children 7.25% were diagnosed with associated asthma. Thirteen children (10.4%) were diagnosed with sensitization to ABPA, 3 of them had aspergilosis, with rapid decline of lung function. Regarding the tuberculosis, only one patient (<1%) with CF had criteria for TB diagnosis. Interestingly, before being diagnosed with CF, almost 13% of patients were considered and treated as TB cases, most of them with predominant respiratory symptoms. Conclusion: ABPA is significant co morbidity in CF patients, while asthma occurs rarely. Although TB is a quit common condition in our area, CF children seemed to be protected against it. Further studies need to be done to evaluate this hypothesis.

## **P22. Syndrome Pfeiffer - case report**

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OBJECTIVE: To report on a case of Pfeiffer Syndrome type 1, with a discussion of the diagnostic characteristics, diagnostic classification and the differential diagnosis. DESCRIPTION: The authors present a case of a 2 month old admitted in our clinic for microcephaly, exorbitism, hypertelorism, micrognathia and a bigger first toe. Pfeiffer Syndrome was suspected on the clinical features and was confirmed by the genetic test. COMMENTS: Pfeiffer Syndrome is a genetic disorder that results from mutations in the FGFR1 or FGFR2 gene, characterized by the premature fusion of certain skull bones (craniosynostosis). The head is unable to grow normally, leading to proptosis and hypertelorism, an underdeveloped upper jaw, and beaked nose. About 50% of children with Pfeiffer Syndrome have hearing loss, dental problems, broad thumbs and toes. Pfeiffer Syndrome is divided into three subtypes: type 1 or "classic" Pfeiffer Syndrome has symptoms as described above, most individuals with type 1 have normal intelligence and a normal life span. Types 2 and 3 are more severe forms, often involving problems with the nervous system. Type 2 is distinguished from type 3 by more extensive fusion of bones in the skull.

Key words: Pfeiffer Syndrome, hypertelorism, micrognathia.

## **P23. Low level mosaicism identification by FISH technique in patients with Turner Syndrome**

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Background: Turner syndrome is commonly due to X monosomy, but is also seen in patients with mosaicism or X chromosome abnormalities. The phenotype of Turner syndrome patients is highly variable, and depends on the karyotype. It was observed at 5% of patients some mosaicisms with Y chromosome, his identification being important due of the high risk of gonadoblastoma in these patients. The identification of minor cell populations is clinically important and a challenge to laboratories. In cases of hidden mosaicism or structural chromosomal aberrations, conventional cytogenetic techniques can be ineffective and molecular investigation is indicated. Aim: Mosaicism identification by Fluorescence in Situ Hybridization (FISH) in patients with Turner syndrome who had a 45, X homogeneous karyotype in conventional cytogenetics. Material and method: In study it were included 231 patients diagnosed with 45,X homogeneous karyotype. It was done the FISH analysis of peripheral blood lymphocytes, using centromeric probes for X and Y chromosomes. Sex chromosomes mosaicism identification was done by the analysis, in each patient, of 200 cells in interphases and 50 metaphases. Results: It was identified 23 patients (9,9%) with sex chromosomes mosaicisms, the second cell line being observed at 19 (8,2%) patients (45,X/46,XX; 45,X/47,XXX) and the third cellular line at 4 patients (1,7%) (45,X/46,XX/47,XXX). The level of mosaicism was between 2 and 32%. None of the patients studied by FISH had the Y chromosome. Conclusions: FISH analysis of sex chromosomes may be an useful instrument, associated to the techniques of conventional cytogenetics, the identification of a mosaicism of X and Y chromosome being important for the clinical management.

#### **P24. Monozygotic twins with Marfanoid Habitus possible due to disrupted MED 12 gene**

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Lujan (Lujan-Fryns) syndrome is an X linked inherited disorder. The genetic defect and its prevalence in the general population are not known. The Lujan-Fryns syndrome affects predominantly males. The major clinical criteria for the diagnosis of Lujan-Fryns syndrome were established by Fryns and include: mild to moderate mental retardation, Marfanoid habitus, general hypotonia and hypernasal voice, normal secondary sexual development and characteristic craniofacial appearance. We report on two siblings 25 years old, with mild mental retardation (IQ =70, IQ=65), Marfanoid habitus and similar craniofacial anomalies (long and narrow face, long nose, high-arched palate, abnormal dental eruption, crowded teeth, hypernasal voice). The marfanoid features include a tall stature, long thin hyperextensible fingers and toes, but no true arachnodactyly. Joint hyperextensibility and pectus excavatum is present. Secondary sexual development is normal. The patient had an aortic dilatation and prolapsed

mitral valve. Their height is tall (181 cm), but still in the normal range. Both patients showed similar behavior and psychiatric disorders (depression). Cytogenetic analysis was performed and an inverted X(q12;q25) chromosome was found for both of them. Knowing that the MED 12 gene is located on Xq13 it is possible that the inversion determined the disruption of the gene. It remains that further studies by BAC FISH to confirm or infirme this suposition.

### **P25. Cytogenetic and molecular cytogenetic study of Turner's Syndrome: a single institution experience**

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Background: Turner's syndrome is the most common sex chromosomal abnormality in females, characterized by the presence of a single normal functioning X chromosome. The other X chromosome may be missing (according to recent reports, only about half of all Turner's syndrome patients are really monosomic) or abnormal (different abnormalities of the X chromosome may be observed). Patients also may have a mosaic karyotype with the second cell lines carrying numerical or structural sex chromosome anomalies. Aim: The aim of this study was to analyze, at cytogenetic and molecular cytogenetic levels, patients with diagnosed Turner's syndrome. Methods: From 1990 up to 2011, patients with Turner's syndrome diagnosed in Clinical Center of Serbia were analyzed in Laboratory of Cytogenetics and Molecular Genetics, Institute of Hematology, Clinical Center of Belgrade. For chromosomal analyses, peripheral blood lymphocyte culture was carried out. In some patients, conventional cytogenetics was supplemented by fluorescence in situ hibridization (FISH), according to specific protocols. Results: The most common was monosomic karyotype, being observed in almost 50% of patients. In about 20% patients, abberation i(Xq) was observed, followed by about 10% of patients which showed a loss of X in mosaic form, and 10% of patients in which r(X) was found. Few other abnormalities were observed in 1% - 3% of patients: they will be briefly described and discussed. Conclusions: In Turner's syndrome, the combined application of conventional cytogenetics and molecular cytogenetics can provide adequate chromosomal analysis in patient with different chromosomal complement.

### **P26. Congenital dyerythropoietic anemia associated with a familial translocation, t(2;16)(p11;p13.3)**

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**Background.** Congenital dyserythropoietic anemias (CDAs) are a group of very rare genetic disorders (0.1-3 cases/ 1 million births) characterized by ineffective erythropoiesis and distinct morphological abnormalities of erythroblasts in the bone marrow. **Aims.** Presentation of a CDA case associated with a familial genetic abnormality, t(2;16)(p11;p13.3). **Methods.** A female patient from gemellar pregnancy, prematurely born at 7 months (detachment of the placenta), diagnosed with severe congenital hemolytic anemia after birth, and requiring repeated transfusions, was first suspected of CDA at the age of 2 years (hyperplastic bone marrow, macrocytosis, 16% chromatin bridge, 1% binucleated erythroblasts). **Results.** Type I or variant CDA complicated with secondary hemosiderosis and billiary lithiasis was confirmed at the admission in our service; the patient also had short stature and facial dysmorphic features (micrognathia, hypertelorism, epicanthus, severe dental dystrophy). The cytogenetic examination revealed a reciprocal balanced t(2.16)(p11;p13.3) in the patient, patient's father and paternal grandmother; translocations involving the16p13.3 region were described in an incomplete form of the Rubinstein-Taybi Syndrome. No relationship between CDA and t(2;16)(p11;p13.3) was described. Most balanced translocation carriers are healthy and do not have any symptoms but about 6% of them have a range of symptoms which may include autism, intellectual disability, or congenital anomalies. A gene disrupted or dysregulated at the breakpoint of the translocation carrier is likely the cause of these symptoms. **Conclusions.** Taking into account the rarity of CDA (about 540 reported cases), finding of any genetic abnormalities in these patients justifies further discussions.

**Key words:** CDA, t(2;16)(p11;p13.3)

## **P27. Hallopeau-Siemens disease - case presentation**

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Epidermolysis bullosa (EB) is a heterogeneous group of inherited rare diseases, characterized by trauma-induced blister formation of the skin and mucosa. The underlying cause is a functional deficiency of structural proteins of the epidermis or the dermis. Depending on the level of the blister formation, EB is divided into EB simplex (intra-epidermal), junctional EB (within the lamina lucida), dystrophic EB (below the lamina lucida). Besides different distinct blister formation and pain symptoms secondary problems like anaemia, oesophageal stenosis, cardiomyopathy could be present. We presented a very rare case of dystrophic EB. It is a boy, 2 years old, diagnosed at birth as epidermolysis bullosa, associated in evolution, at 9 months, with Diabetes mellitus type I.

## **P28. Nijmegen breakage syndrome in siblings with atypical spleen angiomas and particular cardiac involvement**

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Background: Nijmegen breakage syndrome (NBS) is a rare autosomal recessive congenital disorder, caused by a mutation in NBS gene, located on chromosome 8q21.

Aim: Our aim is to present two siblings of Slavic origin, with NBS, a 9 year boy and a 4 year girl, detected with microcephaly and recurrent pulmonary infections. The boy associated severe cyanotic congenital heart defect (CHD), polyglobuly, multiple cerebral infarctions and recurrent seizures.

Material and methods: Both patients performed clinic, cardiology, neurology, ultrasound and genetic examination, followed by complex laboratory investigations. Microscopy was done for boy.

Results: Both patients present distinct facial appearance, micro-cephaly convergent strabismus, short stature, recurrent infections and multiple spleen tumors. Sister also has right palpebral ptosis, skin hypopigmentation areas, and severe IgA immunodeficiency. She was treated with intravenous immunoglobulin. The boy have CHD: double outlet right ventricle, VSD, inefficient pulmonary artery banding, severe pulmonary artery hypertension, NYHA III cardiac failure, cashexy, severe thrombocytopenia, severe motor delay, low mental retardation, and cellular immunodeficiency. A paravertebral T8-T9 tumor was detected in boy. He was treated for heart failure. Thrombocytopenia was treated with IGIV and thrombocyte supplementation, but represented contraindication for PAH treatment. Spleen and paravetebral tumors were angiomas, detected after death in boy. Genetic tests confirmed NBS in both.

Conclusions: NBS is rare in siblings and need to be diagnosed because of immunodeficiency that has to be treated, because of radiation sensitivity, which needs to avoid radiation exposure, and a strong predisposition to lymphoid malignancy, which have to be searched. Spleen angiomas in both are not a NBS characteristic. CHD and severe thrombocytopenia is a particular association that concurred to death in boy.

## **P29. Clinical and cytogenetic correlation in primary amenorrhea: retrospective study on 493 patients**

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Primary amenorrhea (PA) has many causes, including hypothalamic and pituitary disorders, gonadal dysgenesis and utero-vaginal malformations. We performed a retrospective study, with the purpose of establishing the frequency and the type of chromosomal abnormalities, in 493 patients with PA who were clinically and cytogenetically evaluated (1985-2009) in Iasi Medical Genetics Center. The X chromatin test, used as a screening test, was abnormal in 201 cases (40.8%) and normal in 292 cases (59.2%). The karyotype was normal in 224 cases (45.43%) and abnormal in 269 (54.56%) patients; the most frequent abnormality detected was X chromosome monosomy, homogeneous (137 cases - 27.78%) or mosaic (80 cases - 16.22%). Other 22 cases (4.46%) had X chromosome structural unbalanced

abnormalities (homogeneous or in mosaic). One particular group, represented by 23 patients with PA, had a Y chromosome cell line and the final diagnosis was: pure gonadal dysgenesis (8 cases), CAIS (6 cases), mixed gonadal dysgenesis (4 cases) and true hermaphroditism (5 cases). Other 7 patients presented X trisomy (4 cases) and structural chromosomal abnormalities (3 cases). Our results were similar with other reported studies and attest the importance of cytogenetic investigations in the etiologic diagnosis of amenorrhea. Keywords: amenorrhea, karyotype, X chromatin test.

### **P30. Clinical findings in a family with Gorlin syndrome**

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Gorlin syndrome (GS) is an autosomal dominant genetic disorder with variable expressivity and complete penetrance. GS is characterized by dysmorphic face, skeletal abnormalities (kyphoscoliosis, tall stature), basal cell nevi and carcinomas, odontogenic keratocysts of the jaw, hyperkeratosis of the palms and soles and mild mental retardation (5% of patients). GS is caused by mutations in the PTCH gene (9q22.3) or PTCH2 gene (1p32) - a tumor suppressor gene. 60% of cases are new mutations. We present a family (mother and son) with GS. The primary clinical consultation was made for keratocysts of the jaw. The son (21 years old) presented macrocephaly, coarse face, palmar hyperkeratosis with pits, skin tumors, dorsal kyphosis, normal intellect. Investigations revealed: mandibular cysts, intracranial calcifications, mild neurosensory deafness, retinal dysplasia (right eye). The mother presented mental retardation and her investigations revealed mandibular cysts and dilated cerebral ventricles. Because of the extreme variation in clinical expression, GS diagnosis may be difficult sometimes. To avoid errors, diagnosis criteria have been proposed in the medical literature (Kimonis et al, 1997). Gene mutation analysis may be helpful for presymptomatic diagnosis. The medical management of the patient involves a multidisciplinary team. Genetic counseling is indispensable. In conclusion, we present two cases diagnosed with Gorlin syndrome to illustrate a rare disorder that is sometimes misdiagnosed and to discuss diagnostic criteria, management and importance of genetic counseling.

### **P31. Congenital anomalies in association with dextrocardia and situs inversus totalis**

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Dextrocardia is a congenital anomaly in which the heart is positioned abnormally within the right side of the chest with the apex pointing to the right. Sometimes it is associated with situs inversus totalis or heterotaxy. The prevalence and the type of associated anomalies (including heart defects) are variable. We have analysed the prevalence and types of associated congenital anomalies in 11 children with dextrocardia and situs inversus totalis recorded in the files of Iasi Medical Genetics Center. There were 5 girls and 6 boys. Congenital anomalies were present in 9 children - heart defects (81.81%), duodenal atresia (9.09), hydrocephalus (9.09), renal agenesis (9.09), vertebral anomalies (9.09%), lipodystrophy (9.09%), polysplenia (9.09%). Types of associated heart defects were: septal defect (50%), tricuspid insufficiency (25%), Fallot tetralogy (12.5%), transposition of great arteries (12.5%), left ventricle hypoplasia (12.5%), hypertrophic cardiomyopathy (12.5%) and mitral valve prolapse (12.5%). Complex heart defects were present in 3 cases (37.5%), only in males. Some particular features of our cases will be presented in detail. In conclusion we present a study of 11 cases with dextrocardia and situs inversus totalis, 81.81% of them having associated congenital anomalies. Cardiac defects are commonly associated with dextrocardia and situs inversus totalis and males are more severely affected than females. Patient's prognosis depends on the presence/ absence and severity of associated congenital anomalies.

Keyword: dextrocardia, congenital anomalies, heart defects, situs inversus totalis

### **P32. Van der Woude syndrome - familial cases**

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IRF6 (interferon regulatory factor 6) - related disorders are a spectrum extended from isolated cleft lip/palate (CL/P) and Van der Woude syndrome (VWS) to popliteal pterygium syndrome. Van der Woude syndrome (VWS) is an autosomal dominant genetic disorder characterized by the association of lower lip pits with CL/P. VWS represents the most frequent form of syndromic CL/P (2% of all individuals with CL/P), with a prevalence in the general population of around 1/60 000. We describe a 3 months old patient with complete left CLP and labial pits. After a careful family history we have diagnosed the mother with the same syndrome (right lip cleft, lip pits, multiple cavities and abnormal tooth shape). Clinical manifestations of VWS are highly variable within the same family (lower lip pits alone, isolated CL/P or both). For a correct clinical diagnosis and genetic counseling we recommend the examination of lower lip for all children with CL/P and careful evaluation of first-degree relatives. Treatment involves surgical repairing of the CL/P and lip pits for cosmetic reasons.

### **P33. Autosomal recessive distal myopathies - the experience of institut of myology**

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Background: Distal myopathies belong to a clinically and genetically heterogeneous group of rare disorders, characterized by progressive muscular weakness and atrophy beginning in lower legs or feet and/or hand, forearm. The increasing knowledge of the genetic background now allows a more accurate classification of will know in entities as well as new disorders Aim: We report the Institut of Myology experience about autosomal recessive distal myopathies focusing on diagnostic strategy. Methods: we retrospectively reviewed the cases of genetically proven autosomal recessive distal myopathies including 8 GNE myopathies, 30 Miyoshi myopathies and 3 anoctamin 5 myopathies and assessed their clinical, imaging, electromyography, pathological and genetic characteristics. Results: the comparison of the different forms has shown an important phenotypic and genotypic variability, in terms of age of onset and pattern, differential involvement of the limbs muscles, clinical course, pathology and genotype-phenotype correlation. Conclusion: Miyoshi myopathy appear as the most frequent form of autosomal recessive distal myopathies. In case of negative dyspherline staining on the biopsy, anoctamin 5 myopathy should be considered as its presentation does not differ for dyspherlinopathies. GNE mutation should be searched for in case of a quadriceps sparing, vacuolar myopathy predominantly in the anterior compartment of the legs.

Key words: Miyoshi myopathy, GNE myopathy, anoctamin 5 myopathy

#### **P34. Genetic counseling in Autism Spectrum Disorders**

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Introduction. Autism is a disorder that includes together impairment of neurobehavioral deficits in social interaction and language development and communication, stereotyped and repetitive behaviors .

The literature often refers to Autism spectrum disorders (ASD) as to disorders that occur in approximately 1:150 live births in all ethnic groups and social classes. Therefore can be considered a public health problem. It is discuss a real increase in the prevalence of these diseases in the population and the implication of different risk factors. However, the increase appears to result from disseminating information leading to the identification of more cases, from the fact that the diagnosis is made earlier and that because of education, both parents and medical personnel are aware of the symptoms.

Although there were numerous studies in the last years, the classification of phenotypes in autism aetiology remains difficult and much more. Knowledge about the aetiology of ASD is increasing, but the causes remain elusive for most cases. ASD associated with a known cause is called autism syndrome.

There is an extensive list of medical conditions in the literature associated with autistic manifestations, ranging from environmental intervention agencies, different gene mutations that define the known genetic syndromes, chromosomal abnormalities and metabolic diseases. In the cases where the cause is identified, autism is considered secondary manifestation. However, the biological mechanisms involved in the determinism of ASD are still studied. ASD is clinical heterogeneity probably reflects the complexity of the genetic profile. Heritability is estimated at 90% and the rate of concordance in monozygotic twins is 95%. The situation is complicated by significant interindividual heterogeneity, involvement of many loci and gene-environment interactions. Materials and methods. Our study has proposed establishing the exact aetiology of phenotypes as many autistics and tracking genetic anticipation phenomenon. Identification of minor variants of familial aggregation suggests that some genes confer susceptibility to varying degrees in ASD and assessing the susceptibility is useful in assessing risk in early disease diagnosis and early and appropriate therapeutic intervention. Conclusions. ASD has become a public health issue, but there are many misunderstandings about the heritability of these disorders. Detection of genetic changes may contribute to diagnosis, to understand the biological mechanisms involved in pathogenesis, the genetic counselling of families, prevention and educational planning

### **P35. Pierre-Robin sequence - case report**

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Pierre-Robin syndrome is a congenital medical condition characterized by micrognathia, glossoptosis with upper airway obstruction and cleft palate. Upper airway obstruction is present in the majority of patient and is the main concern in the neonatal period. We present a patient with classic features of Pierre-Robin sequence: micrognathia, complete "U" shape cleft palate, glossoptosis with upper airway obstruction. The child was borne preterm at 35 week of gestation, birth weight of 2580g from nonconsanguineous parents. The patient suffered immediately after birth from severe upper airway obstruction with signs of respiratory distress and infection. Prolonged tracheal intubation and ventilator assistance were necessary soon after birth. Glossopexy was performed at 30 days of life but with no significant improvement to the airway obstruction. Two weeks later a second intervention was scheduled and a tracheostomy was performed. Further course of the case was favorable. In infants with Pierre-Robin, glossopexy is a helpful surgical intervention but not always sufficient to release the airway obstruction, in certain cases alternative measures like tracheostomy being necessary.

### **P36. Scoliosis in patients with Prader Willi Syndrome - a 22 patients series**

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Background: Prader-Willi (PWS) syndrome is a genetic disorder characterized by severe hypotonia and feeding difficulties in early infancy, followed later by excessive eating and gradual development of morbid obesity. The management of patients with PWS requires the attention of many professionals. Musculoskeletal disorders like scoliosis, hip dysplasia, and lower limb alignment, are universal features of PWS. Scoliosis is a major concern for the orthopedist, affecting up to 80% of the PWS patients. Choosing the proper treatment for these patients is a challenging job. Aim: This study analyzes the musculoskeletal issues of a series of 22 patients with PWS. Results: A total of 22 patients, 15 girls and 7 boys, were included in the study, age between 2 and 28 years, mean 13.2 years. Scoliosis was diagnosed in 14 cases (63%), 4 boys and 10 girls, age 5 to 28 years. The Cobb angle was  $< 30^\circ$  in 11 of the 14 patients. Severe scoliosis was present in 3 of the patients but with Cobb angle less than  $50^\circ$ . All 14 patients with confirmed scoliosis underwent physical therapy and regular surveillance. In addition to physical therapy the 3 patients with  $> 30^\circ$  scoliosis had indication for spinal bracing but failed because of low patient compliance. Conclusions: Scoliosis is an important medical concern for the PWS patients. Usually the degree of the spinal curvature is low or medium and the disease may be treated by nonsurgical means, physical exercises and physiotherapy. Spinal bracing is not a valid option for PWS patients because of low patient compliance.

### **P37. Complex malformative association - case presentation**

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The OEIS complex refers to a combination of defects consisting in omphalocele, cloaca or bladder exstrophy, imperforate anus and spinal defects and represents a rare nosologic entity with an incidence of 1:2000000 live births. There is no obvious cause for the disease, most cases being sporadic. We present a case of a baby who was born at 40 weeks of gestational age, weighing 2380g with OEIS complex. The complex malformation association included ano-rectal malformation with cloaca, omphalocele, absence of the vertebral column beneath T12, malformation of the rib cage, paralysis of lower limbs, ear and nose malformations. Till now the patient underwent early repair of the abdominal wall. She will need multiple surgical interventions for the ano-rectal, cloaca and the other malformations, with many potential complications. The patient will require the care of a multidisciplinary team of neonatologists, pediatric surgeons, pediatric urologists, pediatric neurosurgeons and pediatric orthopedic surgeons. Long time prognosis is shadowed by possible life threatening complication and sequelae including renal impairment, sexual dysfunction and impaired reproduction, impaired ambulation in the presence of damage to the spinal cord and malformations of the pelvis and extremities.

### **P38. Joubert syndrome: clinical variability in a family**

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Joubert syndrome is a rare genetic disorder, inherited in an autosomal recessive manner, characterized by the underdevelopment or even the absence of vermis cerebelli and a brain malformation (molar sign). Most common manifestations are ataxia, hyperpnea, sleep apnea, ocular anomalies, hipotonia. Four genes causing Joubert syndrome have been identified: AHI1, CEP290 (NPHP6), TMEM67 (MKS3), and NPHP1. At conception, each sib of an affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Presentation: Non-consanguine couple with 6 children of whom 4 with typical manifestations of Joubert syndrome. Results: Family history reveals one child that died 1 day after birth and one spontaneous abortion at months 2-3 of pregnancy. The affected children have different severity degrees of disease in terms of motor coordination, ocular troubles, intellectual impairment, and respiratory problems. The mother presents with retinitis pigmentosa with no other symptoms. Laboratory testing and interdisciplinary consults were used to evaluate the disease. MRI in all affected siblings revealed the specific "molar tooth sign". Genetic testing that we had available did not show abnormalities. Conclusions: The syndrome is managed differently in the four children. Genetic counseling is challenging because of the variable clinical picture as well as the different progression and prognosis of the disease. The children benefit from the adequate genetic and psychological counseling of the family in order to offer the best support for each particular case.

### **P39. Distributions of p53 codon 72 polymorphism in primary open angle glaucoma in a Romanian cohort (Alba County)**

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Primary open angle glaucoma (POAG) is the most common type of glaucoma. The p53 codon 72 Arg-Pro (CGC to CCC) polymorphism of exon 4 affects various biological properties and it has been proposed that genomic instability of p53 gene may play a role in glaucomatous neuropathy. We examined the distribution of this polymorphism in 13 unrelated POAG patients and in 44 normal healthy individuals without history of POAG registered at the County Emergency Hospital of Alba Iulia, Romania. The controls were recruited among individuals undergoing ophthalmological examination. Their genomic DNA was analyzed for p53 gene codon 72 polymorphism by polymerase chain reaction. We found a significant difference in the distribution of the codon 72 polymorphism between groups ( $p = 0.017$ ). The genotype distribution in the POAG group was 61.5% Arg homozygote, 7.7% heterozygote, and 30.8% Pro homozygote, while in the control group it was 45.5% Arg homozygote, 45.5% heterozygote, and 9.1%

Pro homozygote. We also found a significant difference in the ratio homo-/heterozygote genotypes between the two groups in that the homozygotes represented 92.3% of the POAG group and only 54.5% of the control one. We concluded that the p53 codon 72 Arg/Pro polymorphism is associated with glaucoma in Romanian patients and the homozygotes are at higher risk for POAG. Key words p53, POAG, PCR, Pro, Arg, Romania

#### **P40. Myeloperoxidase as risk factor for ischemic stroke**

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MPO. has a role in pathogenesis of atherosclerosis. The aim of the study was to evaluate the time course of MPO plasma levels in the early stage of ischemic stroke. The study included 78 patients with acute ischemic stroke, 46 females and 32 males, mean age 74.3±6.8 years. Blood samples for MPO measurement were taken within 24 hours after the onset of ischemic stroke. Seventy-two patients served as matched controls 43 females and 29 males, mean age 71.3±6.4 years. MPO was measured in plasma using the Abbott Architect platform (Abbott Diagnostics Inc., Abbott Parck IL). Comparisons between patients and controls and patients group were expressed as relative risk with its 95% confidence interval (RR [95% CI]). All p values were determined by Fischer's exact test. A value of p<0.05 was considered statistically significant. Mean plasma MPO level was in patients with acute ischemic stroke 583±48 pmol/L. Seventy-one patients out of seventy-eight patients with ischemic stroke presented mean plasma MPO levels greater than the upper of normal (425±36 pmol/L, p<0.0001, (RR 8.188, [95% CI 4.038 to 16.600])). Twelve controls presented mean plasma MPO level greater than the upper of normal. In conclusion, plasma MPO levels were statistically significantly higher in patients with ischemic stroke as compared to controls. MPO has been associated with acute ischemic stroke but its direct role in its pathogenesis has not been established. MPO could be proposed as a risk factor for ischemic stroke. MPO is a new biomarker and a possible future therapeutic target. Key words: myeloperoxidase, acute ischemic stroke, risk factor

#### **P41. Considerations upon a case of intrahepatic biliary atresia**

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Biliary atresia (BA) is a progressive obliteration of intra or extrahepatic biliary system occurring in neonatal period. During the perinatal period an exogen factor influences the innate immune system of a genetically predisposed individual inducing an uncontrollable immune response with consecutive atresia of the intra/extrahepatic bile ducts. Genetic factors that could account for the disease are assessed by

recent studies. GWAS identified a susceptibility locus for BA on 10q24.2, while other authors suggested region of potential disease susceptibility on 2q37.3. The authors present a case of a 3 months female admitted for sclero-tegumentary jaundice and growth retardation. The family medical history was insignificant. Clinical examination showed cutaneous trophic disturbances and hepatosplenomegaly. The infant didn't associated phenotypic particularities or others malformations. Biological assessment showed increased conjugated bilirubin, increased colestasis enzymes, hipertransaminasemia, hipercholesterolemia and negative serology for maternofetal infections. The abdominal ultrasound combined with biliary scintigraphy and liver biopsy confirmed the diagnosis of intrahepatic BA. The differential diagnosis was made with Alagille syndrome - an autosomal dominant disorder that associates intrahepatic BA with heart, skeleton, eyes malformations and characteristic facial appearance. Besides medical treatment ursodeoxycholic acid, bile acid sequestrants and parenteral liposoluble vitamins, the patient was proposed for liver transplantation. The particularity of this case was the late stage of BA diagnosis associating chronic cholestatic hepatitis (Knodell 14, Fibrosis 3). The diagnosis should be established in the first weeks of life at every infant with prolonged colestatic jaundice by increasing the awareness about this condition to primary care physicians. Keywords: jaundice, biliary atresia

#### **P42. Estrogen receptor alpha gene polymorphisms in Bulgarian women with Systemic Lupus Erythematosus**

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Systemic lupus erythematosus (SLE) is an autoimmune disease that affects predominantly females. Estrogens are known modulators of the immune system functions and are involved in the lupus disease process. The aim of the study was to investigate whether XbaI/A/G and PvuII/T/C polymorphisms in estrogen receptor-alpha gene are related to the age of onset and clinical manifestations of SLE as was reported in a previous study. A total of 162 women (112 with SLE and 50 healthy controls) were genotyped by RFLP analysis. The absence of PvuII and XbaI restriction sites were indicated by "P" and "X" and their presence - by "p" and "x", respectively. No differences in the allele distribution between SLE-patients and controls were found. The most common genotypes in both groups were PpXx (43.8%), ppxx (36.4%) and PPXX (8.6%); ppXX and PpXX were not detected. The patients with ppxx genotypes did not differ from PPXX subjects according to the age of the disease onset ( $33.76 \pm 12.22$  vs.  $36.40 \pm 13.72$  years,  $p > 0.05$ ) and duration of the disease ( $9.39 \pm 8.31$  vs.  $7.40 \pm 6.13$  years,  $p > 0.05$ ). None of the patients with PPXX genotype had a neurological involvement, while 36.6% from the ppxx women were affected ( $p = 0.024$ ). Photosensitivity tended to be more frequent in PPXX-patients - 90% vs. 58.5% in ppxx,

p=0.077. Our preliminary results showed that subjects with the PPXX genotype had more often a milder form of SLE with a more frequent skin involvement and less neurological symptoms. The study was financially supported by grant 26/2010 from MU - Sofia, Bulgaria.

#### **P43. Parental origin of X chromosome in Turner Syndrome**

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Background: X chromosome imprinting is presumed to explain the phenotypic variability in Turner syndrome and also some phenotypic features, specific for each sex. The patients with Turner syndrome have only one X, maternal (XM) or paternal (XP), thus representing a unique clinical model in which may be observed the phenotypic influence of X genes, related to the parental origin. Aim: Determination of the parental origin of X chromosome in patients with homogeneous X monosomy, with the purpose to correlate this with the phenotype. Patients and method: 42 patients (and their mothers), diagnosed with homogeneous 45,X karyotype, were included in the study. By FISH analysis, the patients with undetected mosaics by classical karyotype have been excluded from the group. The parental origin of X was determined by the study of 9 microsatellites, by comparing the length of the mother-daughter alleles. Results: From 42 couples mother-daughter analyzed, the parental origin of X was established in 38 couples, thus: 26 (68%) patients presented XM and 12 (32%) XP. Conclusions: In this study it was used the microsatellites analysis to establish the parental origin of the X chromosome. The 2:1 distribution of the genotypes which have maternal or paternal X is presumed to be that the mother has two X chromosomes to contribute in chromosomal constitution of the descendent and the father has only one X chromosome. From this observation, it will be further analyzed the correlation with the phenotype, thus trying to explain a clinical signification for the X chromosome imprinting. Keywords: Turner syndrome, X chromosome, parental imprinting, microsatellites

#### **P44. Cardio-vascular disease in ageing - role of epigenetic factors**

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Background Genetic and epigenetic mechanisms involved in cardiovascular disease (CVD) are not entirely understood although this is still a major cause of mortality worldwide. One of the most important conditions in CVD is atherosclerosis which often leads to ischemic heart disease and stroke. Many studies linked the evolution of atherosclerosis with genetic factors, a small number being well characterised. The aim of our study was to correlate the polymorphisms of MTHFR (5,10-methylenetetrahydrofolate reductase) gene with TIMP-1 (tissue inhibitors of metalloproteinases) and ESR1 (estrogen receptor 1) genes methylation. Materials and methods Two groups were studied: case group - 37 old patients with CVD and a control group - 25 young/normal subjects. We used MS-PCR (Methylation Specific Polymerase Chained Reaction) for TIMP-1 and ESR1 genes methylation and PCR-RFLP (restriction fragment length polymorphism) for MTHFR gene polymorphisms. Results and conclusions ESR1 and TIMP1 presented a statistically extremely significant frequency of hypermethylation in case versus control group (P <0.001 for each gene).

We found MTHFR C677T polymorphism in 59.45% case group patients, only 32% control group presenting this mutation. A1298C mutation was found in 62.16% of case group and in 40% of control group. A good association was found between these conditions and CVD/ageing.

Mutant alleles of MTHFR gene were found to be susceptibility factors for CVD and also associated with ageing. Hypermethylation of the two studied genes (TIMP-1 and ESR1) can be considered as a risk factor for cardiovascular diseases.

Keywords: MTHFR, TIMP-1, ESR1, cardiovascular disease

Acknowledgements - Study supported by Romanian Research Authority PN2-62057/2008

#### **P45. Pulmonary fibrosis in infant - case report**

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Background: Interstitial lung disease in children is rare and severe, predominantly affecting boys and infants. Its incidence in european children is 3.6 /1 million. Common features are represented by aberrant healing, structural remodeling of the distal alveolar spaces, fibrosis, impaired gas exchange and restrictive respiratory dysfunction. Genetic factors and various injuries are involved in the mechanism of pulmonary fibrosis in children. In 59-67% of patients with familial idiopathic or sporadic pulmonary fibrosis a gene variant of the regulatory protein implied in mucus formation was identified in the chromosome 11. Alveolar epithelial phenotype alterations in the presence of genetic predisposition for remodeling leads to diffuse parenchymal lung disease and fibrosis. Aim: Presenting a 9 months old male infant with interstitial lung disease and fibrosis. Methods: History, clinical exam, laboratory, functional, imagistic and interdisciplinary assessments were done. Results: He is the first child of a young healthy couple, born at term, with normal weight and length. Exposure to second-hand smoking, brochiolitis and right middle lobar pneumonia were noted. Atopy, infections (TORCH, HIV and tuberculosis), LES, cystic

fibrosis, gastroesophageal reflux and heart diseases were ruled out. Thoracic CT revealed bilateral ground glass aspect, sub pleural atelectasis and thickened bronchial walls. During hospitalization the evolution was favorable. Parents refused conventional therapy. Pulmonary physical changes recurred and persisted. Conclusions. Early diagnosis requires a comprehensive approach and provides stabilization of the lung changes with optimal therapy. Difficult management (exploratory and therapeutic limitations; noncompliant caregivers; accessing of alternative therapies) may worsen the prognosis. Genetic counseling is mandatory.

#### **P46. Discrepant findings for trisomy 13 by QF-PCR and karyotyping in a female newborn**

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Quantitative Fluorescent-Polymerase Chain Reaction (QF-PCR) is used as a rapid and successful pre- and postnatal diagnostic method, for the detection of aneuploidies of chromosomes 21, 18, 13, X and Y. We report on a female newborn that was born at term after an uneventful pregnancy and presented postpartum with hypotrophy, retrognathia, strabism and nasal septum deviation. DNA extracted from cord blood was analysed for the most common aneuploidies by QF-PCR. The result showed an unambiguous allele distribution for trisomy 13 and was confirmed in a second blood sample. Long-term cultured lymphocytes, however, showed a normal female karyotype. In addition, Fluorescence-in-situ-Hybridisation (FISH) studies on 200 uncultured and 200 cultured interphase nuclei revealed no evidence for trisomy 13. At the age of 7 weeks the girl presented with adequate development without any clinical signs of Patau syndrome. QF-PCR on blood sample of both parents showed normal allele distribution for chromosome 13. To evaluate a possible tissue mosaicism, DNA from buccal cells of the child was analysed by QF-PCR, and a normal allele distribution for chromosome 13 was found. In a further blood sample of the index patient we confirmed again trisomy 13 by QF-PCR and identified the supernumerary alleles as to be of parental origin. Our case shows a striking discrepancy between the results of QF-PCR and karyotype in a female newborn. The aberrant QF-PCR result is reproducible, but not in agreement with the patient's phenotype. So far we do not have any explanation for this inconsistency.

#### **P47. Teaching genetics, a quality educational task**

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Universitatea de Medicina si Farmacie " Carol Davila"

"In many countries teachers are actively engaged in redefining curricula and learning materials". During the Unesco's General Conference in 2003 it was also stated that teachers as purveyors of knowledge are

responsible for the future of the young and that one should support them and learn from them. In this context quality of education has become a dynamic concept. In my previous studies I have shown that students can have better results when the information from the lectures is repeated during the practicals. The purpose of this investigation is to prove whether through repetition there is a significant increase in the marks the students obtain. 132 students were tested twice. The middle term test consisted of two open questions from the practicals' and lectures' content. After receiving their corrected papers and marks, all questions from the lectures were discussed, so students learned what was expected as answers. These answers were briefly repeated with students' participation at the beginning of the following practical. At the final examination 88 students answered exactly the same two open questions, while 32 were used as comparison receiving other open questions than in the first test, but from the same question pool. Analyzing the significance of improvement in the students' marks and comparing these two groups new questions arose, that can be the origin of future investigations. Discussing results, achievements and limits of my study I conclude that there is a need of new parameters in proving the quality of teaching genetics to medical students.

Key words: teaching genetics

#### **P48. Generating awareness towards nutrigenomics in medical practitioners**

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Advances in the knowledge of gene - nutrient interactions, create the necessity of educating health care providers in this domain. Thus, especially general medical practitioners must become aware that their nutritional recommendations have to be based on the possible associations between biological variation and nutrient induced diseases. From Hippocrates' advice to let food be the medicine, we have come to matching diets according to the individual's genetically determined ability to digest, absorb, and use the nutrients within those foods. A new concept, personalized nutrition, developed based on nutrigenetics and nutrigenomics. The core idea is that nutrients interact with genes in ways that are benign, but can also be deleterious in other circumstances. Epigenetics can link the role of early life exposure to specific nutrients and the risk of developing metabolic diseases in adulthood, while genomics technologies can help predict if dietary intervention effects will occur early at a molecular, cellular or tissue level. Medical practitioners in specialties like pediatrics, obstetrics, internal medicine could find such information useful for their patients. The quest for an optimal human diet includes a nutritional profiling, the challenge being multilayered, from determining what the best matched nutrients for the human species and ultimately, for each individual would be. Therefore, feeding genes right means avoiding foods that are not an appropriate match and focusing on those with a positive impact on health. Different medical specialties could benefit from courses or lectures on nutrigenomics if introduced in the present curricula.

Key words: medical education, nutrigenomics, epigenetics

#### **P49. Molecular cytogenetic diagnosis in children with mental retardation: molecular karyotyping (array CGH) and high-resolution CGH**

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Molecular karyotyping is one of the most popular techniques for molecular cytogenetic diagnosis (MCD). However, due to the high cost and difficulties of interpretation of subtle genome imbalances (i.e. CNVs), its application encounters problems of introduction to diagnostic practice, especially in low- and medium-budget laboratories. Here, we propose the use of high-resolution metaphase CGH (HRCGH) before application of array CGH to reduce MCD costs. HRCGH (resolution: about 1.5 Mb) was tested by analysis of 100 children with idiopathic mental retardation and congenital malformations non-randomly selected according to clinical and cytogenetic data and detected rearrangements in 47% of cases. These were deletions (28 cases; 60%), duplications (15 cases; 15%), subtelomeric rearrangements (12 cases; 25%). Five cases were confirmed by molecular karyotyping. In addition, 25 independent cases were analyzed by array CGH. Genomic/chromosomal rearrangements were detected in 14 out of 30 cases (47%). 1.2Mb to 36.3Mb. One case exhibited constitutional genomic instability manifested as multiple deletions and duplications (size: from 1Mb to 2.5Mb). Clinically relevant CNVs were detected in 9 cases (30%). Additionally, we have elaborated a bioinformatic approach towards evaluation of CNV pathogenicity using gene expression and proteome (interactome/reactome) meta-analysis by Gene Expression Omnibus, BioGPS, Cytoscape 2.8.0 and Pathway Commons. Our data demonstrate HRCGH to be applicable for prescreening before array CGH as well as propose a new way of processing of MCD results obtained by molecular karyotyping. To our knowledge, this is the first array CGH and high-resolution CGH study of children with idiopathic mental retardation in Russia.

#### **P50. Genetic approaches of chromosomal disorders associating intellectual disabilities**

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Backgrounds: Intellectual disabilities (ID) affect 2-3% of the general population, standing as the most common developmental disability. The underlying causes include environmental factors, genic and chromosomal abnormalities. The increase in the ability to investigate the human genome has led to the detection of new syndromes, besides a better understanding of mechanisms that generate genomic defects. Methods: We report the results of a study that included 100 children with clinical diagnosis of

nonsyndromic ID, investigated by GTG-banding, FISH, and - in 20% of the patients - oligonucleotide array comparative genomic hybridization - aCGH - (Agilent, 44K Platform). Results: The genetic approaches allowed us to refine the diagnosis in 5 patients. Both recurrent and non-recurrent genomic rearrangements were detected, presumably generated through different mechanisms. 4p deletion, 8p deletion, 22q deletion, 12p duplication, Xp and Xq duplications, and unbalanced t(3;20) translocation were among the detected genetic defects. Conclusions: Genetic abnormalities are an important cause of ID in children. While clinical diagnosis and cytogenetic investigations are essential in the management of these patients, aCGH proves to be an important diagnostic tool for nonsyndromic ID.

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### **P51. Approach of congenital lymphedema in Turner Syndrome**

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Congenital lymphedema (early edema in the hands and feet) is among physical features commonly seen in girls with Turner syndrome. We present a case of a newborn ( 3 weeks female infant) which had edema of the upper and lower limbs. Karyogram in this case shows mosaicism (XX/X0). According to the literature the karyograma in the Turner syndrome with lymphedema is more often X0 (38%) than mosaicism (12%). In this last situation it is associated with a variable number of Turner anomalies , whose frequency depends in mosaicism by the presents of haploid cells. Key words: newborn, congenital lymphedema, Turner syndrome, karyotype XX/X0

### **P52. Variable phenotypes associated with two new frameshift mutations in the DMD gene**

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Background. Frameshift mutations of the DMD gene are usually associated with altered protein structure and the Duchenne form of muscular dystrophy. However, additional rearrangements may induce different phenotypes than expected based on mutation analysis. Aim. We aim to present two new frameshift mutations in the DMD gene, resulting in premature stop of translation of the dystrophin and in different forms of progressive muscular dystrophy. Methods. The two patients of 9 and respectively 3 years old were clinically, biochemically and genetically evaluated in order to confirm the diagnosis of dystrophinopathy and to assess disease severity. Genetic testing was performed both by MLPA (Multiplex Ligation Dependent Probe Amplification) and Sanger sequencing of all 79 exons and adjacent intronic regions. Results. Both patients presented with highly elevated muscular enzymes but

the clinical evaluation showed different severity: autistic features and little muscular involvement, compatible with Becker muscular dystrophy (BMD) and respectively a severe form of disease in the second child, compatible with Duchenne muscular dystrophy. MLPA showed in both cases absence of large mutations in the DMD gene. DNA sequencing allowed us to detect two new frameshift mutations, predicted to induce premature stop of translation, located in the exon 43 of the gene: c.6271delA in the BMD patient and c.6261delA in the DMD patient. Conclusions. In some cases, the predicted severity of dystrophinopathies does not correlate with the detected mutation. In these cases more complex investigations are needed, including gene expression analysis both at mRNA and protein level, particularly in those cases with incomplete clinical picture.

### **P53. Comparative molecular approaches in Prader-Willi/ Angelman Syndrome diagnosis**

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Background Prader-Willi (PWS) and Angelman (AS) syndromes are distinct neurogenetic disorders, caused by chromosomal deletions, uniparental disomy or loss of imprinted gene expression in 15q11-13 region. While PWS occurs from a lack of paternally expressed gene contribution, AS originates from a lack of maternally expressed gene expression in the region, specifically UBE3A. The aim of the study was to evaluate the clinical diagnosis using different comparative molecular methods. Materials and methods The study group consisted in 14 children (2-10 years) presumptively diagnosed with PWS, one case with AS at UMF Timisoara- Pediatric Hospital and 10 normal patients (control). DNA and RNA samples were isolated from white cell blood. For MS-PCR, 700ng of each DNA was sodium-bisulfite treated. RNA samples were reverstranscribed with Access Quick Kit (Promega). Epigenetic changes at SNRPN gene locus were evaluated with MS-PCR technique. Two non-imprinted genes [removed]NIPA1 and OCA2) was evaluated by qReal-Time PCR for identification of deletions type 1,2,3. SALSA MS-MLPA kit ME028 was used to detect copy number changes and to analyze CpG islands methylation of the 15q11 region. Results and conclusions 4/14 children presented deletions type 1,2,3 and 6/14 display imprinting defect (all PWS). In children with 15q11-13 deletions, no or poor NIPA1 or OCA2 gene expression was found, depending on deletion type. The deletions were confirmed in FISH and MLPA analysis, thus recommending NIPA1 and OCA2 gene expression as an alternate method for deletions investigation. Keywords: Prader-Willi/ Angelman syndrome, MLPA analysis, genomic imprinting Acknowledgements - Study supported by Romanian Research Authority PN2-42113/2008

### **P54. The association between obesity and polymorphisms in FTO and NAIP genes**

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The relationship between increasing body mass, obesity and mutations in different genes has been tested in numerous studies. However, molecular substrate of these observations is not completely understood. Aim. To evaluate the association of FTO and NAIP mutations with obesity. Material and methods. We selected Caucasian patients with obesity (n=120) and healthy controls (n=150) from two medical centers from Bucharest. The FTO polymorphism (rs17817449) and NAIP exon 5 deletion were genotyped by PCR based method. Indirect methods (SSPC, HA and HRM) were used to test the existence of mutations in the FTO and NAIP genes. Results. An association between obesity and FTO was detected ( $p < 0.05$ ). Homozygous mutation of FTO increases the risk for obesity in our lots. A different distribution of homozygous deletion of NAIP exon 5 in patients and control was observed, although it is not statistical significant. Indirect methods did not reveal new mutations in the FTO and NAIP loci. Conclusions. The rs17817449 in FTO gene is associated with obesity. Given the number of patients with homozygous deletion of NAIP exon 5 in patients and control lots it is necessary to extend this study. Acknowledgements. This work was supported by the Romanian Ministry of Education and Research - Project PNII- 42-161/2008 Key words: obesity, FTO, NAIP, SSPC, HA, PCR RFLP.

#### **P55. Two siblings with Nephronophthisis and preemptive renal transplantation - a success**

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Introduction Nephronophthisis is chronic tubulointerstitial nephritis with autosomal recessive inheritance that progress to end-stage renal failure during adolescence. The first signs appear after years with a urine concentration defect along with polyuria and polydipsia, and deterioration of renal function without signs of glomerular disease. Renal ultrasonography reveals normal-sized kidneys and, just in advanced stages, medullar cysts. Aim Our case presentation want to focus on importance of examination in children with signs of renal function deterioration, for obtaining in proper time a correct diagnosis and to present a fortunately preemptive simultaneous transplantation in twins. Material We present two girls, univitelin twins, which were diagnosed in our department with nephronophthisis. They were without failure to thrive and no other involvements - eyes, cerebellum, liver, or bones (dental). Results The first symptoms developed in our twins after the age of 10 and they consist of polyuria with polydipsia and than anemia. Renal ultrasonography revealed normal-sized kidneys. We performed (in US) NPH1 detection and it was negative. We did not perform bioptic exploration in these two girls. At age of 12 they were in stage 4 of renal insufficiency and both with preemptive transplantation with cadaver kidney. Conclusion There is no specific therapy other than correction of

water and electrolyte imbalances so the transplantation is the saving life procedure. Despite the large amount of NPH1 cases with median onset of ESRD at this age, our girls tested were negative for NPH1. We emphasize the identical progression of the disease and the probability that is a form with NPH2 or 3 gene mutations.

**P56. ADIPOQ 45T>G adiponectin is not predictive for plasma adiponectin levels, type 2 diabetes or diabetic kidney disease in a Romanian cohort of patients**

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Background: Single nucleotide polymorphisms at the gene encoding adiponectin have been associated to plasma adiponectin levels, insulin resistance, diabetes and its complications. The aim of the present study was to evaluate the prevalence of this polymorphism in an Eastern European Caucasian population from Romania and to determine whether it influences adiponectinemia; relationship to diabetes and diabetic nephropathy was also evaluated. Materials and methods: One hundred and sixty-eight subjects were genotyped for the ADIPOQ 45 T>G polymorphism: 115 unrelated type 2 diabetes patients and 53 nondiabetic volunteering health-employees. Medical history, standard laboratory evaluation including lipid profile, total plasma adiponectin, and in diabetic patients glycated hemoglobin urinary albumin/creatinine ratio were assessed. Results: The TT genotype occurred in 146 subjects (86.95%), TG genotype in 20 subjects (11.9%) whereas GG genotype was observed in only 2 subjects (1.2%) of subjects. Genotype was not associated to plasma adiponectin levels. When comparing diabetic to nondiabetic patients genotype was not significantly different; logistic regression found as significant factor for prediction of type 2 diabetes age ( $p=0.00001$ ; OR 1.086; CI 1.048/1.13), abdominal circumference ( $p=0.0003$ ; OR 1.074; CI 1.033/1.11) but not genotype. If diabetic patients were divided according to genotype, again no significant differences are found. Patients with albuminuria didn't have different genotype distribution as compared to normoalbuminuric diabetic subjects; logistic regression showed as factors that predict presence or absence of kidney disease systolic blood pressure  $p=0.044$  (OR 1.04; CI 1.01/1.08) and plasma adiponectin  $p=0.03$  (OR 1.07; CI 1.00/1.14).

Conclusion: The 45T>G adiponectin polymorphism does not influence plasma adiponectin levels; in our cohort this polymorphism is not predictive neither for type 2 diabetes, nor for diabetic kidney disease.

**P57. ADIPOQ 276G>T polymorphism in relation to plasma adiponectin levels, prevalence of type 2 diabetes and diabetic nephropathy in a Romanian population group**

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Background: The 276G>T polymorphism of the ADIPOQ gene has been studied in association to features of insulin resistance and diabetes across different ethnic groups with conflicting results. The aim of our study was to observe whether this polymorphism has an influence on plasma adiponectin levels in Eastern European Caucasians; prevalence of type 2 diabetes and diabetic nephropathy in relation to genotype was secondarily assessed. Materials and methods: One hundred and fifty-three subjects were genotyped for the ADIPOQ 276G>T polymorphism: 105 unrelated type 2 diabetes patients and 48 nondiabetic volunteering health-employees. Results: GG genotype was present in 72 subjects (47.05%), GT genotype in 67 subjects (43.79%) whereas TT genotype was observed in 14 (9.51%) subjects. Subjects with TT genotype had higher adiponectin levels ( $20.28 \pm 5.58$  ug/ml) than GT ( $11.95 \pm 1.82$  ug/ml) or GG ( $11.36 \pm 1.75$  ug/ml) subjects, the difference failed to reach significance ( $p=0.08$ ); in diabetics TT genotype carriers had significantly higher adiponectin levels ( $19.03 \pm 3.46$  ug/ml) than GT ( $9.96 \pm 1.76$  ug/ml) or GG patients ( $8.71 \pm 1.60$  ug/ml)  $p=0.003$ . Genotype distribution was not significantly different between nondiabetic and diabetic patients; in logistic regression the only variables predictive for the odds of being diabetic were age  $p=0.0001$  (OR 1.086; CI 1.041/1.133); abdominal circumference  $p=0.00049$  (OR 1.084; CI 1.036/1.135) and plasma adiponectin levels  $p=0.036$  (OR 0.963; CI 0.929/0.998) but not genotype. Diabetic patients with albuminuria had higher adiponectin levels ( $13.97 \pm 2.07$  ug/ml) than normoalbuminuric patients ( $6.91 \pm 0.88$  ug/ml),  $p=0.003$ , however T allele tended to be more frequent in patients without nephropathy ( $p=0.02$ ). In logistic regression the factors that predict presence or absence of kidney disease are adiponectin ( $p=0.034$ ; OR 1.09; CI 1.01/1.18), age ( $p=0.027$ ; OR 1.08; CI 1.01/1.15) and systolic blood pressure ( $p=0.029$ ; OR 1.04; CI 1.00/1.08). Conclusion: The 276G>T adiponectin polymorphism is related to plasma adiponectin levels, at least in diabetic patients. The influence of genotype for the prevalence of type 2 diabetes or diabetic nephropathy was not significant in our cohort.

### **P58. Complex genetic predisposition in rrms and TNF-a relationship**

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Background and aims: Multiple sclerosis (MS) is a heterogeneous disease, which results in different clinical manifestations. One of the genetic factors is polymorphism of human leukocyte antigen (HLA) region microsatellite (Msat) polymorphism such as tumor necrosis factor alpha (TNF $\alpha$ ). TNF genes are located within the HLA III region. TNF is an important mediator in the inflammatory response. The aim of our study is to confirm if there is any association between Msat polymorphism & predisposing to MS. Subject & methods: polymerase chain reaction by specialized primers was carried out on 40 relapsing remitting MS (RRMS) patients (30 female & 10 male) affected with MS according to Poser criteria (Poser et al., 1983) and 39 age- and sex- matched healthy controls of Hormozgan province in Iran. The results detected on 8% non-denaturing polyacrylamid gel electrophoresis (PAGE) and subsequence comparison with standard (allelic) ladder. Computational analysis & test were performed using GenePop1.31. Exact test of Hardy-Weinberg equilibrium were performed for TNF $\alpha$  Msat. Result: The high frequency of TNF $\alpha$ \*11 Msat (0.25,  $p<0.05$ ) in cases in compare to controls (0.09,  $p<0.05$ ) is remarkable & this

characteristic is the same as recent Europeans studies and also TNFa\*2 exhibited very low(0.2,p<0.05) signal intensities. Conclusion: Allele frequency comparison of TNFa Msat shows significant association between patients compared with healthy controls. Our finding suggests a potentially important role of TNFa gene in susceptibility to MS.

Key words: Multiple sclerosis (MS), relapsing remitting MS (RRMS), tumor necrosis factor alpha (TNFa) Msat polymorphism, susceptibility.

#### **P59. Aneuploidy in the autistic brain: the first molecular cytogenetic study**

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Autism is common childhood psychiatric disorder characterized by impaired social interaction and communication, repetitive and stereotypic behavior. Numerous genetic and environmental factors are suggested to play a role in etiology and pathogenesis of autism, indicating genetic and epigenetic heterogeneity. Previous molecular cytogenetic study of chromosomal variation in peripheral blood cells of 120 children with idiopathic autism indicated that 16% of them have low-level mosaic aneuploidy (Yurov et al., 2007). However, the role of subtle genomic imbalances and mosaic aneuploidy in the autistic brain has not been addressed. For the first time, we evaluated the incidence of aneuploidy in postmortem brain of 12 autistic patients and 7 age- and sex- matched controls (the cerebral cortex and the cerebellum) provided by the Brain and Tissue Bank for Developmental disorders, University of Maryland. In the male autistic brain, we observed a 2-to-3-fold increase of chromosome X aneuploidy (additional chromosome X) in the cerebral cortex (2–5% versus 0.8-1.6% in controls), while no differences in the cerebellum of autistic patients and controls were found. We conclude somatic mosaic aneuploidy to contribute differentially to intercellular genomic variation in different brain areas in autism. Our findings support hypothesis that somatic genome (epigenome) instability and aneuploidy could affects neuronal homeostasis and functioning in the autistic brain, playing, therefore, a critical role in the pathogenesis of the disorder. These data provide for explanation of cooperative interaction between both environmental and genetic factors in autism. Supported by BMBF/DLR (RUS 09/006).

#### **P60. Four case reports of generalized epilepsy**

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The disease-caused mutations in the SCN1A gene show different phenotype with several epilepsy syndromes sharing some common clinical features with different severity and in rare cases of familial migraine and autism. We report two patients which phenotypes being consistent with Dravet syndrome (DS) and myoclonic astatic epilepsy (MAE, or Doose syndrome), respectively. The genetic analysis of SCN1A revealed a heterozygous de novo frameshift mutation (c.4205\_4208delGAAA) in DS patient, and a recurrent missense mutation (c.3521C>G) in MAE patient. The missense mutation has been reported in patients with neuronal diseases of various manifestations, which suggests influence of additional factors. To date, truncation mutations have been found exclusively in patients with DS, while mainly missense mutations and very few nonsense mutations have been found in the milder GEFS+ phenotypes. We also report on two patients with different phenotype of idiopathic generalized epilepsy (IGE) bearing microduplication and microdeletion of the 15q13.3 region, respectively, both inherited from their unaffected mothers. A 13 years old girl suffering IGE with generalized tonic-clonic seizures carried a microduplication involving only the CHRNA7 gene, inherited from her healthy mother. The second patient is a boy with idiopathic childhood absence epilepsy and mild mental retardation who carried 15q13.3 deletion, covering [15q13.2 - MTMR15, TRPM1, KLF13, CHRNA7 - 15q13.4] gene probes. The patient inherited this deletion from his asymptomatic mother. Acknowledgements: The study was supported by the grant No29/2010, Sofia Medical University, Bulgaria

#### **P61. MECP2 gene mutations in Bulgarian and Romanian Rett syndrome patients**

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Mental retardation in its syndromic form is highly heterogeneous in clinical and genetic point of view. Rett syndrome (RTT) is typically characterized by caused by severe mental retardation, absent speech, progressive neurodevelopmental deficits, seizures and ataxia. This complex phenotype is mainly caused by mutations in the methyl-CpG-binding protein 2 (MECP2) gene. Here we report 6 types of mutations in the MECP2 gene, detected in a group of 46 Bulgarian and 14 Romanian classical RTT patients. Eighteen patients were clarified on molecular level (30%). The point mutations in our sample account for 72.2%. Five patients carry missense mutations and 8 patients carry nonsense mutations. Two of the nonsense mutations (p.Arg168\* and p.Arg255\*) were found only in Romanian patients and were never detected among Bulgarians, while two other nonsense mutations (p.Arg270\* and p.Arg294\*) were private for Bulgarian patients. One intraexonic deletion was detected in the present study (5.6%). A

novel insertion c.321\_322insGAAG, p.(Lys107\_Leu108insGluAlafs2\*) was found (5.6%) in one Bulgarian patient. Large deletions and complex mutations account for 16.7%. A novel complex mutational event c.[584\_624del41insTT; 638delTinsCA] was detected in a Romanian patient. Complex gene rearrangements involving a combination of deletions and insertions have always been most difficult to detect, to specify precisely and hence to explain in terms of their underlying mutational mechanisms. The group of Romanian patients was better clinically characterized and 50% of the patients were clarified on molecular level. The Bulgarian group of patients contained mixed phenotypes and only 24% were genetically clarified.

## **P62. Sera protein profiling of schizophrenia treated patients**

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Schizophrenia is a severe, lifelong brain disorder, which incidence is estimated on about 1% worldwide. The disease is a serious social and economic burden to healthcare systems. Although it is thought to be a result of a complex interaction between genetic and environmental factors, the etiology remains to be elucidated. Currently, no etiological treatment is available. So far there isn't any confirmed diagnostic molecular marker for schizophrenia. The exact diagnostics is complicated since some of the symptoms also occur in several other psychiatric disorders. We have analyzed sera samples from schizophrenic patients - before and after treatment and at different stage of the disease and compare them to normal sera. We have extracted proteins and performed 2D-SDS PAGE and mass spectrometric analysis. Detailed proteomic analysis showed obvious differences before and after treatment of the patients. Since we have observed significant differences in patients' sera proteins after treatment according to the medication, we intend to analyze these findings by mass spectrometric techniques. Acknowledgements: This research is funded by Ministry of Education and Science - "Proteome screening in sera of patients with schizophrenia at different stages and courses of drug therapy" competition "Ideas", DO02-12/10.02.2009

## **P63. Genetic markers of oxidative stress in Serbian patients with type 2 diabetes mellitus and atherosclerosis: Paraoxonase 1 gene polymorphisms**

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Background. Paraoxonase 1 (PON1) is multifunctional enzyme associated with high-density lipoprotein particles (HDL). It is important in detoxification of some organophosphorus compounds and in

metabolism of oxidized lipids. Coding (Q192R and L55M) and promoter (C(-107)T) region polymorphisms of PON1 gene are responsible for catalytic efficiency, activity and the level of the enzyme, respectively. Due to increased oxidative stress, diabetes is risk factor for atherosclerosis and cardiovascular diseases. Because PON1 is characterized as an antioxidant, PON1 gene polymorphisms could be genetic markers for atherosclerosis in type 2 diabetes patients. Aim. The aim of this study was to investigate the role of PON1 polymorphisms Q192R, L55M and C(-107)T in patients with type 2 diabetes mellitus and atherosclerosis. Methods. Genomic DNA was isolated from peripheral blood cells of 100 patients and 100 healthy controls and genotyping of all three polymorphisms was performed using PCR-RFLP analysis. Results. RR genotype and R allele were significantly frequent in patients than in the control group (18% vs 7%,  $P=0.019$  and 51% vs 26%,  $P<0.01$ ). For L55M and C(-107)T polymorphisms, our results showed no statistically significant differences in distribution of the genotypes and alleles between patients and controls (all  $P >0.05$ ). Conclusions. Our results indicate that Q192R polymorphism is associated with oxidative stress and R allele could be used as genetic risk factor for atherosclerosis in diabetes patients.

Key words: Paraoxonase 1 gene polymorphisms, genetic risk factors, oxidative stress

#### **P64. Genetic mutations and increased risk of recurrence of venous thromboembolism in women with thrombophilia**

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Aim: New studies of coagulation disorders discovered an increasing number of mutations in the genes of the factors of coagulation, termed inherited thrombophilia. The study aimed to compare the risk for venous thromboembolism (VTE) recurrence in women with multiple thrombophilia to women with no known thrombophilia. Materials and methods: This retrospective study comprised 41 women (mean age 32 years) diagnosed with known thrombophilia and 40 age-matched women with no known thrombophilia. Complete blood count and coagulation tests were recorded in both groups. Cases and controls were also tested for the genetic mutations. Results: It was observed that 14 women were with MTHFR-PAI mutation, 3 women were homozygous for the C677T MTHFR mutation, 3 were homozygous for the A1298C MTHFR mutation and 2 were heterozygous for the same mutation. 6 women were with MTHFR heterozygous mutation combined with the factor V Leiden mutation, 5 women with protein C deficiency, 4 women with protein S deficiency and 2 women with combined deficit for protein C and S. 2 women were with a heterozygous prothrombin mutation (factor II). 5 cases (12.1%) from the group of women with known thrombophilia were with genetic mutations such as: 4 cases with satellites on chromosome 21 and one case with constituent heterochromatina at chromosome 9 (9qh +) and 16 (16qh +). We observed that the recurrence of VTE was significantly higher in patients with genetic thrombophilic defects compared to those with no thrombophilic defects ( $p<0.005$ ). The family history of

VTE was significantly higher in patients with genetic thrombophilia compared with patients with no known thrombophilia ( $p < 0.001$ ). Conclusions: The study proved an increase risk of VTE recurrence in women with multiple thrombophilic defects. Women with thrombophilic defects whether single or multiple, and recurrence of VTE are more likely to have a family history of VTE.

#### **P65. Analysis of -T786C eNOS gene polymorphism in hemodialysis patients**

Tatjana Damjanovic<sup>1</sup>, Biljana Jekic<sup>1</sup>, Nela Maksimovic<sup>1</sup>, Vjera Bunjevacki<sup>1</sup>, Sanja Simic<sup>2</sup>, Ljubica Djukanovic<sup>2</sup>, Ljiljana Lukovic<sup>1</sup>, Ivana Novakovic<sup>1</sup>

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Nitric oxide (NO) plays a key role in the relaxation of vascular smooth muscle, inhibits platelet and leukocyte adhesion to the endothelium and reduces vascular smooth muscle cell migration and proliferation. NO is constitutively produced by endothelial NO synthase (eNOS) in endothelial cells. Several polymorphisms in genes encoding for endothelial NO synthase (eNOS) have been associated with increased susceptibility to renal failure. The aim of the study was to investigate an allele and genotype distribution of eNOS -T786C gene polymorphism in hemodialysis patients and to find possible association of mutant allele with this disorder in Serbian population. The study included 128 hemodialysis patients and 104 healthy persons as control group. Genotypes of eNOS -T786C polymorphism were determined by PCR - RFLPs method. The C allele frequency of the -T786C eNOS gene polymorphism was 0.34 in the group of hemodialysis patients and 0.26 in the control group. In group of patients C allele frequency was higher than in control group without statistical significance ( $\chi^2=3, 27 DF=1, p=0.07$ ). The genotype frequencies of eNOS -T786C polymorphism were 44,9% TT, 41,6% CT, 13,5% CC in the group of patients, and 49,04% TT, 50,0% CT, 0,96% CC in the control group. The difference between genotype frequencies two analyzed groups (patients/control) is statistically significant ( $\chi^2=10.698, DF=2, p=0.001$ ). Patients with CC genotype have higher triglycerides and total cholesterol level without statistical significance. Also there were no association between CC genotype and thickness of carotids atherosclerotic plaque. Thus, CC genotype can be associated with renal failure.

#### **P66. Analysis of the association between RFC1 G80A gene polymorphism and efficacy and toxicity of methotrexate**

Biljana Jekic<sup>1</sup>, Vera Milic<sup>2</sup>, Ivana Novakovic<sup>1</sup>, Jelena Milosevic<sup>3</sup>, Nemanja Damjanov<sup>2</sup>, Goran Radunovic<sup>2</sup>, Ljiljana Lukovic<sup>1</sup>

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Background. Methotrexate (MTX) is the most common used disease modifying drug in the treatment of rheumatoid arthritis (RA). Reduced folate carrier gene (RFC-1) encodes for transporter protein necessary

for cellular uptake of the MTX and consequently its therapeutic action. Aim. Aim of this study was to determine whether G80A polymorphism of the RFC-1 gene modulates MTX efficacy and/or has impact on drug adverse effects. Methods. Study included 184 RA patients treated with MTX. Patients genotypes for selected polymorphism were detected by PCR-RFLP method. For the estimation of the MTX efficacy disease activity score in 28 joints (DAS28) based on EULAR criteria and relative DAS28 values (rDAS28) were used, while adverse drug events (ADEs) were recorded. Between efficacy and toxicity of MTX and detected genotypes an association studies have been performed. Results. When EULAR response criteria have been used to estimate efficacy of the MTX 89 patients (48.4%) were classified as responders (good/moderate response) and 95 (51.6%) as non-responders (poor response). Among 184 analyzed RA patients rDAS28 values ranged from -0.02 to 0.80 (mean value  $0.32 \pm 0.18$ ). No statistically significant correlation between RFC1 G80A gene polymorphisms and efficacy of MTX, based on EULAR and rDAS, have been found. Among 184 patients 53 experienced side effects. No statistically significant correlation between RFC1 G80A genotype and side events has been observed. Conclusions. According to our results RFC1 G80A gene polymorphisms genotypes do not have influence on efficacy or toxicity of the MTX in RA patients.

#### **P67. Variation in mitochondrial DNA of the ancient population in the Bulgarian lands**

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The extreme importance of studying the Bulgarian biological history comes from the understanding of the human as a biosociopsychological entity. The aim of our study is to describe the genetic structure of ancient Bulgarians and the anthropological characteristic in order to obtain full concept of the Bulgarian anthropo-genetic identity. The concept of the genetic evolution of the Bulgarians is supported by direct evidence, obtained from the phylogenetic analysis of ancient DNA retrieved from bone remains, dating from different periods. We have examined 100 mtDNA samples from ancient remains (teeth) dating from VII - VIII century A.C. by direct sequencing. Sequences have been obtained for 10 samples and the following polymorphisms have been detected in 5 of them toward Cambridge Referent Sequence : an SNP at position 16168 (C-T); 16195 (T>A); 16126 (T-C); 16352 (T-C); 16129(G-A); Other 5 samples were without changes toward CRS : at positions: 16017-16126 and 16161-16237; 16046-16158 and 16142-16237; 16017-16158 and 16142-16237; 16017-16158 and 16167-16237; 16043-16158 and 16139-16237. These findings clearly demonstrate that the investigated samples belong to unrelated people and could be helpful in characterization of the haplogroups in the ancient mtDNA. To the best of our knowledge this is the first study performed with ancient samples in Bulgarian population. So far our results are not enough to make any final conclusions about anthropo-genetic characterization in Bulgarians, but we

continue collecting the samples and we intend to improve the method, in order to avoid other contaminations in our future work.

### **P68. 33 years mass newborn screening programme for PKU in Bulgaria - past, current and perspectives**

Kremensky I., Ivanova M., Sinigerska I., Dimitrov D., Vazharova R., Bradinova I., Savov A., Andonova S., Raynova R., Bichev S., Georgieva V., Kalaydjieva L.

National Genetic Laboratory

Since 1978 until now mass newborn screening (MNS) for PKU is being provided by the National genetic laboratory. Up to now 2 185 981 newborns have been tested. 72 newborns with classic PKU and 77 with variant forms are diagnosed and treated. The PKU in Bulgaria is found to be very rare and affects 1 of 37 389 newborns. The newborn screening revealed differences of PKU incidence among Bulgarian subpopulations - in Bulgarians - 1 of 29736, in Bulgarian Turks -1 of 10000 and in Bulgarian gypsies 1 of 137000 newborns. Average 76% of the newborns have been screened trough the years. For the period 2003-2008 the coverage of the newborn population was 95%. In 2009-2010 a decrease in coverage is present (92% for 2010). Over 900000 newborns missed the screening test because no blood sampling was provided at the newborn departments. Among them 18 individuals were diagnosed with classical PKU later in life and developed irreversible disabilities. Since 1980 the MNS is under Japanese system for external laboratory quality control. Up to now 4600 control samples have been investigated. After 1997 there are not incorrectly reported control samples. The National genetic laboratory has technological resources to expand the newborn screening for further 20 metabolic genetic diseases. At the moment this possibility is offered to more than 5000 newborns from the intensive care departments.

### **P69. Analysis of -T786C eNOS gene polymorphism in hemodialysis patients**

Damnjanovic T.<sup>1</sup>, Lukovic Lj.<sup>1</sup>, Jekic B.<sup>1</sup>, Maksimovic N.<sup>1</sup>, Bunjevacki V.<sup>1</sup>, Simic S.<sup>2</sup>, Djukanovic L.<sup>2</sup>, Novakovic I.<sup>1</sup>

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Nitric oxide (NO) plays a key role in the relaxation of vascular smooth muscle, inhibits platelet and leukocyte adhesion to the endothelium and reduces vascular smooth muscle cell migration and proliferation. NO is constitutively produced by endothelial NO synthase (eNOS) in endothelial cells. Several polymorphisms in genes encoding for endothelial NO syntase (eNOS) have been associated with increased susceptibility to renal failure. The aim of the study was to investigate an allele and genotype distribution of eNOS -T786C gene polymorphism in hemodialysis patients and to find possible association of mutant allele with this disorder in Serbian population. The study included 128 hemodialysis patients and 104 healthy persons as control group. Genotypes of eNOS -T786C polymorphism were determined by PCR - RFLPs method. The C allele frequency of the -T786C eNOS



gene polymorphism was 0.34 in the group of hemodialysis patients and 0.26 in the control group. In group of patients C allele frequency was higher than in control group without statistical significance ( $\chi^2=3$ , 27 DF=1,  $p=0.07$ ). The genotype frequencies of eNOS -T786C polymorphism were 44,9% TT, 41,6% CT, 13,5% CC in the group of patients, and 49,04% TT, 50,0% CT, 0,96% CC in the control group. The difference between genotype frequencies two analyzed groups (patients/control) is statistically significant ( $\chi^2=10.698$ , DF=2,  $p=0.001$ ). Patients with CC genotype have higher triglycerides and total cholesterol level without statistical significance. Also there were no association between CC genotype and thickness of carotids atherosclerotic plaque. Thus, CC genotype can be associated with renal failure.

KEY WORDS: Nitric oxide, eNOS gene, SNP polymorphism, renal failure

#### **P70. Analysis of the association between RFC1 G80A gene polymorphism and efficacy and toxicity of Methotrexate**

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Background. Methotrexate (MTX) is the most common used disease modifying drug in the treatment of rheumatoid arthritis (RA). Reduced folate carrier gene (RFC-1) encodes for transporter protein necessary for cellular uptake of the MTX and consequently its therapeutic action. Aim. Aim of this study was to determine whether G80A polymorphism of the RFC-1 gene modulates MTX efficacy and/or has impact on drug adverse effects. Methods. Study included 184 RA patients treated with MTX. Patients genotypes for selected polymorphism were detected by PCR-RFLP method. For the estimation of the MTX efficacy disease activity score in 28 joints (DAS28) based on EULAR criteria and relative DAS28 values (rDAS28) were used, while adverse drug events (ADEs) were recorded. Between efficacy and toxicity of MTX and detected genotypes an association studies have been performed. Results. When EULAR response criteria have been used to estimate efficacy of the MTX 89 patients (48.4%) were classified as responders (good/moderate response) and 95 (51.6%) as non-responders (poor response). Among 184 analyzed RA patients rDAS28 values ranged from -0.02 to 0.80 (mean value  $0.32\pm 0.18$ ). No statistically significant correlation between RFC1 G80A gene polymorphisms and efficacy of MTX, based on EULAR and rDAS, have been found. Among 184 patients 53 experienced side effects. No statistically significant correlation between RFC1 G80A genotype and side events has been observed. Conclusions. According to our results RFC1 G80A gene polymorphisms genotypes do not have influence on efficacy or toxicity of the MTX in RA patients.

Key words: methotrexate, rheumatoid arthritis, RFC-1

#### **P71. Molecular testing for GJB2 mutations among Macedonian nonsyndromic hearing loss children**

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Hearing loss the most prevalent sensory defect is influenced by both genetic and environmental factors. Despite the enormous genetic heterogeneity, mutations in one gene, GJB2, located at the DFNB1 locus (13q12) are responsible for half of all cases with nonsyndromic hearing loss (NSHL). More than 100 different mutations in this gene have been described with specific prevalence in different ethnic groups. Due to the high incidence, molecular testing for GJB2 mutations became the standard of care, diagnosis and counseling of NSHL. The aim of our study was to determine the prevalence and spectrum of GJB2 mutations among 120 Macedonian children with profound deafness as well as the presence of del(GJB6-D13S1830) and mitochondrial DNA mutations. Molecular studies were performed using direct sequencing of GJB2 gene and specific PCR analysis for del(GJB6-D13S1830) mutation. Five common mtDNA mutations (A1555G, 961delT+C(n), T1095C, C1494T and A827G) were analyzed using SNaPShot method. GJB2 mutations were found in 55 patients, in homozygote (37), compound heterozygote (8) or heterozygote state (10). The predominant GJB2 mutations accounting for more than 91% of mutated chromosomes were 35delG and W24X found among Caucasian (Macedonian and Albanian patients) and Gypsy patients, respectively. Other GJB2 mutations: R127H, V153I, Cd120delGAG and P175T were found with allelic frequency of 1.7%, 0.8%, 0.8%, 0.4% and 0.4% respectively. Del(GJB6-D13S1830) and mtDNA mutations were not found. As the major cause of NSHL in Macedonian children with profound deafness, GJB2 mutations should be tested in each routine diagnostic approach.

## **P72. TT viruses prevalence and the association with pathology in the Romanian population**

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The TT virus (TTV) is a recently discovered DNA non-enveloped virus with single stranded circular genome included in the Anelloviridae family along with TT mini virus (TTMV) and TT midi virus (TTMDV). The aim of the study is to determine the prevalence of the TT viruses and to establish a connection with several pathologies within Romanian population. Methods: TTV DNA was studied in patients with diabetic nephropathy that underwent dialysis, patients with breast cancer and healthy individuals using nested-polymerase chain reaction (PCR). The primers used in the first round of the nested PCR amplify a highly conserved region of the viruses downstream of the TATA box and the primers used in the second round are specific to TTV, TTMDV and TTMV. The amplification products were analyzed using agarose gel electrophoresis. Results: The average frequency of the TT viruses was approximately 60% within diabetic nephropathy group, breast cancer group and healthy group. The highest frequency of the

viruses was found within the group of patients that underwent dialysis. The most commonly found virus was TTV and the least one was TTMDV. Conclusions: The prevalence of TT viruses is higher within the group of patients that underwent dialysis than in those with breast cancer or in healthy individuals. The estimated frequency is consistent with the values reported in other populations. Acknowledgements. This paper is supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/ 64109

### **P73. Distribution of eleven autosomal markers in South Romanian population**

Cimponeriu D.<sup>1</sup>, Panduru N.M.<sup>2</sup>, Apostol P.<sup>1</sup>, Toma M.<sup>1</sup>, Stavarachi M.<sup>1</sup>, Radu I.<sup>1</sup>, Serafinceanu C.<sup>3</sup>, Craciun A.M.<sup>3</sup>, Ion D.A.<sup>2</sup>

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The genetic structure of Romanian population is not well studied. The South part of the country presents minor cultural, linguistic and geographical differences. Aim. The aim of the present study is to assess the distribution of eleven common autosomal markers in healthy population living in South Romania. Materials and methods. Healthy Caucasian subjects (n=576) living in eight districts from South Romania were selected for this study. PCR or PCR-RFLP protocols were used for genotyping rs1801133, rs3767140, rs2229569, rs1805087, rs5186, rs3842752, rs680, rs2228570, rs4646994, rs1800469 and eNOS ID polymorphisms. PowerMarkerv3.25, Samova 1.0 and Arlequin3.11 software were used to calculate summary statistics and to compare genotypes distribution between districts. Results. No deviations from Hardy-Weinberg equilibrium were observed. The distribution of genotypes presents minor differences in the eight districts. Significant differences in distribution of rs1800469 and rs5186 related to gender were detected in samples from Dambovită and Teleorman respectively. Minor allele frequency of rs1801133 increases progressively to the younger age in subjects grouped according to the age. The frequency of allele and genotypes in Romanian population is in the range of values described in other Caucasian populations. Conclusions. The genetic distribution may be a factor that may influence the results of non-family-based association studies especially if the samples are collected from Bucharest districts. Acknowledgements. This paper is supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/ 64109 Key words: autosomal markers, healthy subjects, genotypes, South Romania

#### **P74. Copy number variants in infertile men detected by Array Comparative Genomic Hybridization**

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Spermatogenesis is a dynamic and multistep process of male germ cell proliferation and differentiation by which spermatozoa are produced from primordial germ cells. The causes of spermatogenic defects in infertile men include environmental, nutritional, behavioral and genetic factors. Despite enormous progress in the understanding of human reproductive physiology, the underlying cause of male infertility remains obscure in about 50% of cases. In order to determine the Copy Number Variants (CNVs) that may be involved in the origin of male infertility we performed high resolution array Comparative Genomic Hybridization (array-CGH) on eight patients with idiopathic infertility. Four patients were azoospermic with a histopathological diagnosis of maturity arrest, and four patients had sperm counts ranging from azoospermia to normozoospermia and carried gr/gr partial AZFc deletion. We used the Database of Genomic Variants (DGV, <http://projects.tcag.ca/cgi-bin/variation/>) to compare our findings to previously reported CNVs. A total number of 70 CNVs were detected with sizes ranging from 6 kb to 700 kb. Fifty six of them were considered as common CNV (DGV), 10 were in regions without known genes and 4 were CNVs in regions with candidate genes causing or being risk factors for spermatogenic failure (UGT2B17 on 4q13.2; STEAP2 on 7q21.13; TPTE on 21p11.2-11.1 and H2BFWT on Xq22.2). In conclusion, our initial results using array CGH analysis to study male infertility revealed several CNVs that may represent a risk factor for impaired spermatogenesis and male infertility.

Keywords: array-CGH, CNV, male infertility

#### **P75. Y chromosome microdeletions in infertile men with azoospermia in Serbia**

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Aim: To identify submicroscopic interstitial deletions in azoospermia factor (AZF) loci in infertile men with azoospermia with unknown etiology of infertility in Serbia. Methods: One hundred and twenty five infertile males with azoospermia were included in this study. The diagnosis of azoospermia was made on the basis of semen analysis according to WHO guidelines. Each patient was carefully examined to rule out other causes of infertility. DNA was extracted using peripheral blood. The STS primers tested in each case were sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc) and two control primers (SRY and ZFY). PCR amplifications found to be negative were repeated at least 2 times to confirm the deletion of a

given marker. The PCR products were analyzed on 8 % acrylamid gel. Results: 5 of the 125 cases (4%) showed AZFc microdeletion. Conclusion: It is recommended that all males with absence sperm counts seeking assisted reproductive technologies be screened for deletions of Y chromosome

#### **P76. CAG repeat length in the androgen receptor gene in infertile men with azoospermia in Serbia**

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The purpose of this study was to evaluate CAG repeat length in the androgen receptor gene in Serbian men with azoospermia compared to fertile men. Materials and methods: 125 infertile patients with azoospermia were included in the study. The diagnosis of azoospermia was made on the basis of semen analysis according to WHO guidelines. Patients were excluded if clinical evidence of obstructive azoospermia, known cytogenetic defects, Y chromosome microdeletion or abnormal hormonal parameters were present. DNA was extracted using peripheral blood. CAG repeat length of androgen receptor gene were analyzed comparing with samples with known CAG repeat length of androgen receptor gene. The number of CAG repeats was determined with PCR technique and fragment analysis. Results: Average number of CAG repeats in infertile men was higher than in fertile men. The difference was statistically highly significant. Conclusion: The number of CAG repeats in our population in infertile men match those obtained by researchers of Western Europe and parts of North America.

#### **P77. Reproduction failure in couples with mosaic chromosomal rearrangements**

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Balanced and unbalanced structural aberrations in mosaic states are rarely reported, their mechanism of appearance and clinical importance are not well understood. Mosaic chromosomal rearrangements were found when investigated the cause of reproductive failure. Here we report on three patients of couples referred for cytogenetic investigation after reproduction failure. Standard cytogenetic analysis followed by G-banding was performed on peripheral blood lymphocytes. The first patient was a healthy 34-year-old female, investigated because the couple had a plurimalformed child and a first trimester abortion. Cytogenetic investigation revealed an apparently balanced reciprocal translocation between chromosomes 2 and 11 in 20% of the cells, the karyotype was 46,XX (80%)/46,XX,t(2;11)(p23;q25)(20%). The second case was a 31-year-old healthy woman referred to the laboratory for chromosomal investigation before assisted reproductive technology because of primary infertility. Analysis of G banded metaphases from peripheral blood lymphocytes led to diagnosis of a der(18) in 8% of the cells,

the karyotype was 46,XX(92%)/46,XX,der(18)(8%). The third case was a 32 years-old male. The couple was referred for genetic diagnosis before FIV. The cytogenetic investigation revealed a robertsonian translocation (13;14) in 15% of the metaphases, the karyotype was 46,XY(85%)/45,XY,trob(13;14)(15%). Identification of chromosomal rearrangements mosaicism allows the optimization of medical follow up during pregnancy. For cases with mosaic structural rearrangement the theoretical risk is difficult to be calculated, it depends on the percentage of abnormal cells in different tissues. For correct genetic counseling is important that this cytogenetic findings to be confirmed in different tissues.

#### **P78. Double aneuploidy of Trisomy 18 and Klinefelter Syndrome - prenatal diagnosis**

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**Introduction** The simultaneous occurrence of double aneuploidy in the same individual is a relatively rare phenomenon. Such associations most frequently involved aneuploidy of a sex chromosome and trisomy of an autosome. Most of the previously reported cases of double trisomy were found in spontaneous abortions. **Methodology.** Case history: a 26 years old woman with maternal serum screening in the first trimester (combined test) which indicate an increased risk of Down syndrome. Ultrasound examination revealed intrauterine growth retardation and cardiomegaly and consequentially a genetic amniocentesis was performed. **Result.** Cytogenetic study showed karyotype 48,XXY,+18 and the patient decided to terminate the pregnancy. **Discussion and Conclusion.** The first case of double aneuploidy (48,XXY,+21) was reported in 1959 by Ford et al. Other double aneuploidy that are found frequently are 48,XXX,+21, 48,XXY,+18 and 48,XXX,+18. Double aneuploidy that leads to trisomy of the two different chromosomes occurs due to accidentally meiotic non -disjunction events, both could have a same or different parental origin.

#### **P79. Chromosomal abnormalities and polymorphic variants in couples with recurrent abortion**

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Numerous studies have shown that about 5.5% of couples experiencing recurrent abortions include one partner who carries a balanced chromosomal rearrangement, in comparison to less than 0.55% of the general population. The aim of the study is to determine the balanced chromosomal rearrangement etiology in couples with recurrent miscarriages. We perform a retrospective study on 49 couples with recurrent abortions whose karyotype was established on GTG-banded metaphases. A chromosomal aberration was detected in one partner of 4 couples represented by two reciprocal autosomal translocations and two Robertsonian translocations. A female to male ratio of 3:1 was observed. In addition 4 couples with the structural variants inversion 9qh and enlarged heterochromatic region of

chromosome 16 were detected. In conclusion chromosomal analysis is necessary for appropriate clinical management in these couples. Regarding the polymorphic variants detected in our cases we consider as coexistence.

#### **P80. Rapid prenatal diagnosis by QF-PCR technique in Romania - the report on 2615 cases**

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Background: The quantitative fluorescence PCR (QF-PCR) assay has been used for more than a decade in the prenatal diagnosis field, allowing the rapid detection of selected chromosomal aneuploidies. In this study we present the rapid prenatal diagnosis method currently employed in Romania and the results obtained after four years of testing. Aim: Our aim was to prove that this rapid prenatal diagnosis method is a reliable and accurate tool in relieving maternal anxiety by providing a diagnosis report in maximum 24 hours after sampling. Methods: We started by using two commercial kits designed to perform rapid prenatal diagnosis of trisomy 21, 18, 13 and sex chromosome aneuploidies. Last year we put into practice a test based on international published data on this subject. Results: We analyzed 2615 prenatal samples including amniotic fluid, CVS, products of conception and blood. We detected 118 abnormal results from which the majority were autosomal anomalies and 10 were sex chromosomes aneuploidies. 70.37% of the autosomal abnormalities were represented by trisomy 21 cases (n=76). We also detected trisomy 18 (n=21) and trisomy 13 (n=7). The sex chromosomes aneuploidies included trisomy XXY, trisomy XXX and monosomy X . We reported 17 fetuses with triploidy and also submicroscopic duplication, somatic microsatellite mutations and mosaicism cases. No false positive or false negative results were detected. Conclusion: Based on the results presented in this paper we are confident that the QF-PCR test can reduce the number of karyotype in pregnancies carefully evaluated by ultrasound and maternal serum screening tests.

#### **P81. Type and frequency of chromosome aberrations found in 1360 couples with Recurrent miscarriages**

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Chromosomal abnormalities are a major cause of spontaneous abortions, recurrent pregnancy loss and infertility, as previous studies have shown. Some of these genetic anomalies appear de novo or they are derived from a genetic anomaly present in one of the parents. The cytogenetic analysis offers a quick and easy method for the identification of chromosomal abnormalities and it can offer important information for genetic counselling. The prevalence of chromosomal aberrations is estimated between 1,3-13,1% in patients with fertility problems without morphologic phenotypic anomalies, between 5-7% in males with a sperm count of 10 x10<sup>6</sup> sperms/ml and up to 10-15% in azoospermic men. In our

laboratory, over the past decade, we analyzed the karyotype of over 1360 couples. A total of 370 of aberrant karyotypes were diagnosed, corresponding to an abnormality rate of 27,2 % per couple or 13,6 % per individual studied. The following chromosomal aberrations were observed: 37 sex chromosome aberrations (including mosaicism), 151 autosomal aberrations including 18 reciprocal translocations, 17 Robertsonian translocations, 68 inversions, 14 deletions and 34 duplications. Low-level mosaicism for numerical sex chromosome aberrations were diagnosed in 35 individuals. Another 140 patients showed single cell aberrations, but the significance of these cells is unclear at the moment. Our data confirms previous studies that a high number of infertile couples have a chromosome aberration which occur in both sexes. In conclusion it is recommended that a chromosomal analysis should be performed on both partners with reproductive problems. Key words: karyotype, chromosome aberrations, infertility, genetic counseling

### **P82. Case report: a rare missense mutation found in the CFTR gene**

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Background: Cystic fibrosis (CF) is the most common life limiting autosomal recessive hereditary disorder in the Caucasian population affecting 1:3000 live births. There are over 1600 mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene affecting the normal function of the CFTR protein. Different mutations results in different disease phenotypes, some mutations having mild or no effect on CFTR functions and some mutations resulting in severe forms of disease. Aim: Our goal was to investigate an 8 month old patient clinically diagnosed with cystic fibrosis (positive sweat test, mild digestive manifestations) by molecular biology tests. Methods: Initially, we test the patient for the 32 of the most frequently mutations within European population. The poly T status was also analyzed. Genomic DNA was amplified for CFTR gene by multiplex fluorescent PCR using a commercial kit. The amplicons were subjected to capillary electrophoresis on 310 Genetic Analyzer. In the second step we proceeded to gene sequencing. Results: We detected delF508 mutation in a heterozygous form and a 7T/9T polymorphism with 32 CFTR gene mutation test. After sequencing, a heterozygous c.3208C>T missense mutation was identified in exon 17b of the CFTR gene. This rare mutation was also found at the mother of the patient. Conclusions: In the case of patients with symptoms leading to suspicion of cystic fibrosis with no mutations detected by commercial tests, there is a real possibility of the existence of rare mutation. To confirm the CF diagnosis is recommended CFTR gene sequencing.

### **P83. Partial trisomy of chromosome 9 - case report**

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We present the case of a 8 years old patient, who is diagnosed with dup 9 (p+) after having done a genetic consultation and a karyotype. The trisomy 9p+ is a well defined and easy to recognise syndrome,



a rare illness whose cause is represented by the presence of extra genetic material on the short arm of the chromosome 9. It is described an easy preponderance of the feminine patients. Clinically speaking, the syndrome is characterized by: a psychomotor retardation in varying degrees and a typical facial dismorphie (high forehead, enoftalmy, bulbous nose with the widened, large and malformed ears, down inserted). 5% of the patients also associate it with cardiac abnormalities and less than 5% present lip or labiopalatine clefts. The consultant couple requires investigating the probing for the diagnosis, providing a genetic counseling and estimating the risk of recurrence. In the literature of speciality is mentioned the fact that, more frequently, the parents are carriers of mutual balanced translocations, where is also involved the 9 chromosome and that even fewer cases appear by duplications de novo which increase the risk of recurrence. Thereby, within the genetic counseling, the parents are recommended to make the karyotype because the risk of recurrence can be estimated correctly according to or not to the presence of some possible chromosomal abnormalities at one or both consultants. In the case they don't show chromosomal abnormalities, the risk of recurrence is minimal. It is also recommended a prenatal diagnosis (vilocentesis or amniocentesis) with the karyotype.

#### **P 84. Pharmacology, genetics and imaging working together to decrease the risk of thromboembolic complications after total hip arthroplasty**

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The risk of deep vein thrombosis and pulmonary embolism is of great concern after performing a total hip arthroplasty, because of the immediate threat and the possible long-term major dissability. The number of lower limb arthroplasties has increased significantly during the past few years leading to the necessity of improvement in establishing the amount of thromboembolic risk. Besides the wellknown risk factors such as age, cancer, immobility, smoking, estrogen use or obesity, the genetic factors are very important. Almost a half of the patients diagnosed with thromboembolism after total hip arthroplasty had no clinical predisposing factors. Thromboprophylaxis is performed to all this patients, but, in addition to the classical methods, preoperative genetic screening would be very useful in order to detect who needs mild anticoagulant therapy or a more aggressive one. The cost-effectiveness of imaging, such as the invasive venography or noninvasive ultrasonography, and genetic tests has to be established.

Key words: deep vein thrombosis, hip arthroplasty, genetic screening

## **P 85. A family with congenital neutropenia**

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**Introduction.** Chronic neutropenias are one of the problem found in hematological practice and among them, congenital neutropenias are rare and often need sophisticated laboratory test for establishing the diagnosis.

**Case presentation.** A 2 years old girl was admitted in the IIIrd Pediatric Clinic to establish the etiology of neutropenia occasionally discovered when she had pertussis. The patient didn't have a history of recurrent infections. The girl's mother was also diagnosed in childhood with severe chronic neutropenia of unknown etiology and ectopic right kidney with recurrent urinary tract infections. Laboratory explorations in our patient had shown normal values for hemoglobin and platelet but leucopenia of 2700/mm<sup>3</sup> with severe neutropenia of 240 granulocytes/mm<sup>3</sup>. In addition, at abdominal echo she presented the same urinary tract anomaly as founded in mother. Bone marrow aspiration was performed both in patient and mother and shows the same features: granulocytes hyperplasia with normal maturation. In this condition we had high suspicion of WHIM syndrome and molecular investigation show CXCR4 mutation in 2B exon c.1012 C>T p.338 R>X.

**Conclusions.** In all cases with chronic familial neutropenia with myelokathexis we must think to WHIM syndrome, a rare primary immunodeficiency.

## **P 86. The role of pharmacogenomics in the management of psychiatric disease**

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The ultimate goal of antipsychotic medication is for the physician to be able to chose the right drug treatment for a patient with psychiatric disease, knowing from the start how the patient will respond to medication and what are the chances to develop side effects. The merger of the fields of pharmacology and genetics aims to describe how the impact of genetic variation between individuals can predict the drug response and thus helping the physician to start drug treatment with the maximum response possible and minimum risk of harm. Although non-genetic factors like the severity of the disease being treated, the age of the patients, and other external factors can influence the treatment outcome, genetic polymorphism in liver metabolism enzymes, transporters, plasma binding proteins, drug targets are associated more strongly with efficacy and tolerance of drugs.