



ES-PCR

since 2007



European Society of Pediatric Clinical Research
Europäische Gesellschaft für klinische
pädiatrische Forschung

Medical Faculty Comenius University, Bratislava

June 21st - 22nd, 2018

Organized by

Department of Pediatrics Medical Faculty Comenius University
and National Institute of Children's Diseases

ES-PCR

Supported by

Ministry of Education, Science, Research and Sport of the Slovak Republic

Medical Faculty Comenius University in Bratislava

President of Conference

prof. Dr. Ľudmila Podracká, PhD.

prof. Dr. Radvan Urbánek

Organizing committee

Dr. Peter Musil, PhD.

doc. Dr. Juraj Staník, PhD.

doc. Dr. Peter Čížňár, PhD.

Dr. Tomáš Dallos, PhD.

Dr. Katarína Prochotská, PhD.

Dr. Alžbeta Lencsesová

Venue

Medical Faculty Comenius University in Bratislava

Moskovská 2

813 72 Bratislava

Language

English

Technical remarks

Oral presentations in English (10 minutes followed by 10 minutes discussion)

Posters will be displayed at the conference venue on June 22 from 7.00 AM

The posters should be not larger than 118 cm height and 84 cm width

Registration

June 21th 2018, from 2:00 p.m.–6:00 p.m.

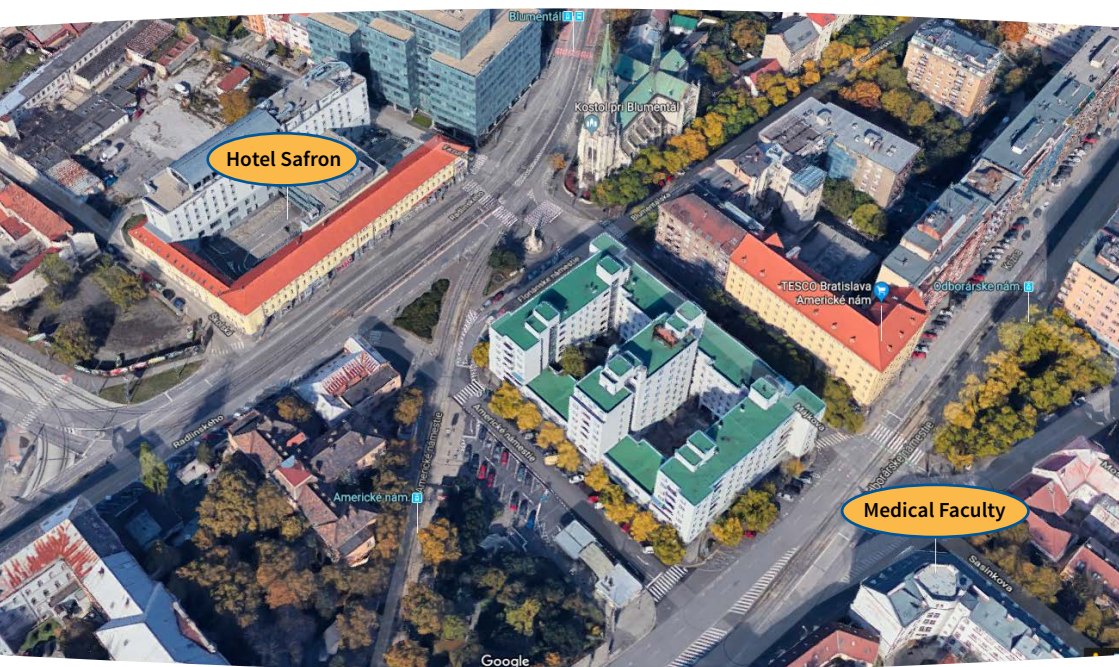
Medical Faculty, Moskovská 2, Bratislava

No registration fee

Get together party (free of charge)

7:30 p.m.

Hotel Saffron, Radlinského 27, Bratislava– just 5 min. walk from the Medical Faculty (300m)



Dear Friends,

we cordially welcome you in Bratislava. We are pleased to have you here and we are looking forward to exciting scientific presentations and stimulated discussion.

Sincerely,

L'udmila Podracká
Radvan Urbánek

THURSDAY, JUNE 21, 2018

19:00 **Opening, Key note lecture and get together at the Hotel Saffron**

New trends in the management of testicular germ tumors

Michal Mego, Bratislava- Slovakia

Welcome reception with Slovak folk music performance

FRIDAY, JUNE 22, 2018

Medical Faculty, Moskovská 2

8:00 – 8:15 **Welcome**

8:15 – 9:35 **GENETICS**

Aif deficiency in five patients: two novel mutations, heart involvement and aif and CHCHD4 level alterations in various autoptic tissues

Jan Kulhanek, Prague - Czech Republic

High prevalence of growth plate disorders among children with familiar short stature

Lukáš Plachý, Prague - Czech Republic

Clinical, cytogenetic and molecular assessment of Angelman-syndrome patients and description of a novel mutation in the UBE3A gene

Tímea Margit Szabó, Debrecen - Hungary

The change in the therapeutic approach based on genetic analysis in children with steroid-resistant nephrotic syndrome

Martin Bezdička, Praha - Czech Republic

9:35 – 9:45 **Coffee Break**

9:45 – 11:05 **NEPHROLOGY**

5 years follow-up of pediatric renal transplant recipients after joining Eurotransplant: a Hungarian retrospective analysis, early findings

László Berta, Budapest – Hungary

Dynamics of salivary urea and creatinine during the development of renal injury in animal models

Alexandra Gaál Kovalčíková, Bratislava - Slovakia

Role of nanovesicles in the development of peritoneal fibrosis during peritoneal dialysis

Beáta Szebeni, Budapest – Hungary

The role of IL-20 cytokine subfamily in the pathogenesis of chronic kidney disease

Domonkos Pap, Budapest - Hungary

11:05 – 11:15 **Coffee Break**

11:15 – 12:35 **CARDIOLOGY**

Correlation of pulse wave velocity parameters with kidney function markers in children

Mirjam Močnik, Maribor - Slovenia

Use of mitral inflow propagation velocity as a marker of diastolic function in preterm newborns

Gergely Balázs, Debrecen - Hungary

Comparison of preductal and postductal blood pressure values obtained by peripheral arterial lines in extremely preterm infants on the first day of life

Lukas Aichhorn, Wien - Austria

MRI validation of surface distance measurement for pulse wave velocity in children

Adrienn Bárczi, Budapest – Hungary

12:35 – 13:15 **Lunch**

13:15 – 14:00 **POSTER SESSION**

14:00– 15:20 **HEMATOLOGY, GASTROENTEROLOGY**

Improvement of the diagnostic workup based by Porto criteria: results of Hungarian prospective nationwide inception cohort pediatric IBD registry (HUPIR)

Orsolya Somodi, Debrecen – Hungary

Increment of exclusive enteral nutrition for children with Crohn's disease based on Hungarian pediatric IBD registry (HUPIR)

Veronika Kovács, Debrecen – Hungary

Czech national registry of Diamond-Blackfan anemia: search for novel molecular markers

Jana Volejníková, Olomouc – Czech Republic

Factor VII deficiency in children – interrelations between genotype, factor VII activity and bleeding signs

Marie Al-Muhanna, Debrecen – Hungary

15:20 – 15:30 **Coffee Break**

15:30 – 16:50 **ENDOCRINOLOGY**

Automated vs. manual bone age assessment: comparative study in children and adolescents with endocrine disorders

Klára Maratová, Prague – Czech Republic

Influence of high fat diet and treadmill training on bone metabolism in experimental model of female rats with and without ovariectomy

Katarína Krivošíková, Bratislava – Slovakia

Diabetes mellitus in infants and toddlers

Kristína Podoláková, Bratislava – Slovakia

Baclofen pump implantation and selective dorsal rhizotomy as surgical treatment modalities in pediatric spastic cerebral palsy

Michal Petřík, Bratislava – Slovakia

POSTER SESSION

Comprehensive development of the skin, oral and gut microbiome in extreme low birth weight infants during the first two weeks of life

Lukas Wisgrill, David Seki, Christos Zioutis, Angelika Berger, David Berry (Austria)

The feasibility of ECG screening in childhood: a population study

Vincze V., Papp Cs., Veres G., Mogyorósy G. (Hungary)

Changes in short-term blood pressure control after monthly spa stay in children with bronchial asthma

Tinka P., Líšková T., Svačinová J., Budinskaya K., Svízela V., Hrušková J., Rýdlová J., Hrstkova H., Nováková Z. (Czech Republic)

Malnutrition as a risk factor to impaired vaccine response in paediatric inflammatory bowel disease

Boros K., Müller K., Cseh Á., Dezsőfi A., Arató A., Veres G. (Hungary)

Inguinal hernia in adolescents: is there an ideal way of treatment?

Kecskés E., Hodosi K., Sasi-Szabó L. (Hungary)

Appraisal of the association between inflammatory bowel diseases and acute pancreatitis – a meta-analysis

Tél B., Stubnya B., Kiss Z., Gede N., Hegyi P., Veres G. (Hungary)

Long term follow-up on body composition, physical activity, quality of life in pediatric patients with inflammatory bowel disease (IBD)

Dohos D., Zsirai Z., Müller K., Boros K., Veres G. (Hungary)

Characteristics of the microbiome under biological therapy in pediatric patients with inflammatory bowel disease

Kiss Z., Makra N., Juhász J., Tél B., Boros K.K., Szabó A.J., Szabó D., Veres G. (Hungary)

Novel insights into the pathophysiology of growth retardation and other endocrine conditions. Outline of an upcoming research project analysing consanguineous families from the North- East of Iraq

Amaratunga S., Tayeb TH., Lebl J., Průhová Š. (Czech Republic, Iraq)

Our first case of Hutchinson-Gilford Progeria Syndrome (HGPS) due to a pathogenic LMNA variant c.433G>A (p.Glu145Lys)

Toni L., Dušátková P., Novotná D., Zemková D., Průhová Š., Lebl J. (Czech Republic)

Determination of some biological markers of obesity in children with cardiovascular risk

Medved M., Ojsteršek L., Marčun Varda N. (Slovenia)

Examination of cellular procoagulant function by thrombin generation test

Hudák R., Beke Debreceni I., Gál Szabó G., Hevessy Z., Antal-Szalmás P., Kappelmayer J. (Hungary)

Clinical and biological impact of factor XIII A expression in leukemic lymphoblasts

Gyurina K., Kárai B., Szegedi I., Hevessy Z., Scholtz B., Kappelmayer J., Kiss C. (Hungary)

Role of microRNAs and histone deacetylase enzymes in the pathogenesis of adult hematological malignancies

Gaál Z., Rejtő L., Erdődi F., Bálint B.L., Oláh E., Veres G., Csernoch L. (Hungary)

Alterations in bone mineral metabolism in survivors of childhood cancer

Lencsésóvá A., Tichá L., Sejnová D., Killinger Z., Payer J., Podracká L. (Slovakia)

Preventable and unpreventable risk factors of retinopathy of prematurity

Lenhartová N., Maťašová K., Lasabová Z., Mendelová A., Zibolen M. (Slovakia)

Vagus nerve stimulation in children – when time matters

Ramos Rivera GA., Foltán T., Švecová L., Kolníková M., Novotný M., Šteňo J., Sýkora P. (Slovakia)

7-dehydrocholesterol modifies the operation of Kv1.3 channels in T cells isolated from Smith-Lemli-Opitz syndrome patients

Balajthy A., Petho Z.D., Szabo G.P., Somod S., Varga Z., Veres G., Panyi G., Hajdu P. (Hungary)

Connections of PACAP signalling pathways in Alzheimer disease kidney of mice

Perényi H., Hinnah B., Horváth G., Zákány R., Reglődi D., Veres G., Juhász T. (Hungary)

Can probiotic supplementation change cytokine response of children with urinary tract infection during antibiotic therapy?

Meštrović Popović K., Langerholc T., Marčun Varda N. (Slovenia)

Renin-angiotensin-aldosterone system inhibitors ameliorate diabetic renal and cardiovascular complications

Molnar A., Koszegi S., Lenart L., Hodrea J., Balogh D.B., Szkibinszki E., Wagner L., Vannay A., Szabo A.J., Fekete A. (Hungary)

Sex differences and sex hormones in kidney functions of aging rats

Domonkos E., Kačmárová M., Borbélyová V., Gyurászová M., Gaál Kovalčíková A., Ostatníková D., Hodosy J., Celec P. (Slovakia)

APDS - rare primary immunodeficiency (case)

Trochanová I., Šoltýsová A., Kuková Z., Hricová M., Čížnár P. (Slovakia)

SIM1 and MC4R gene mutations in children with an early-onset severe obesity

Staníková D., Buzga M., Škopková M., Surová M., Ukropcová B., Tichá L., Lobotková D., Petrášová M., Piskorová L., Bozensky J., Ukropec J., Zavacká I., Klimeš I., Staník J., Gašperíková D. (Slovakia)

Identification of novel gene variants involved in HNF1A-MODY diabetes

Terézia Valkovičová, Martina Škopková, Lucia Valentínová, Miloslava Hučková, Iwar Klimeš, Juraj Staník, Daniela Gašperíková (Slovakia)

17.00 – **CONCLUDING REMARKS – BEST PRESENTATION & BEST POSTER AWARDS**

Guided tour to Bratislava Castle will be available on demand

NEW TRENDS IN THE MANAGEMENT OF TESTICULAR GERM CELL TUMORS

Michal Mego

*2nd Department of Oncology, Faculty of Medicine, Comenius University
National Cancer Institute Bratislava*

Testicular germ cell tumors (TGCTs) represent the most common malignancy in young men. Cisplatin treatment effect on TGCTs is more successful compared to any other solid tumors, what is likely associated with their cell of origin. Due to exquisite chemosensitivity, TGCTs are considered the model for curable cancer. Although they belong to the most chemosensitive solid tumors, approximately 5 % of all TGCTs patients and 10 – 20 % of patients with disseminated disease do not achieve a durable complete remission with cisplatin-based chemotherapy and develop de novo or therapy-induced resistance. Only 30 – 60 % of patients progressing/relapsing after first-line chemotherapy can be cured by standard dose or high-dose chemotherapy with autologous hematopoietic stem-cell rescue. Patients who fail to achieve remission after either high-dose chemotherapy or second-line salvage therapy carry an extremely poor prognosis and the vast majority of them will eventually die of disease. During the last two decades, only few chemotherapeutic agents such as paclitaxel, gemcitabine and oxaliplatin were introduced into clinical practice. Therefore, better understanding of resistance to chemotherapy and searching for a new and less toxic treatment strategies as well as finding of novel therapeutic targets are highly warranted. Moreover, identification of new biomarkers for personalization of treatment is another trend in TGCTs research as well. Furthermore, since majority of patients are cured, increasing number of GCT survivors suffer from late toxicity of treatment. Such consequences result in additional morbidity, decreased quality of personal, social and professional life altering the day-to-day functioning of survivors. Thus, the research initiative to explore the biology of GCTs, overcome the resistance to conventional treatments and address the long-term health issues is imperative.

AIF DEFICIENCY IN FIVE PATIENTS: TWO NOVEL MUTATIONS, HEART INVOLVEMENT AND AIF AND CHCHD4 LEVEL ALTERATIONS IN VARIOUS AUTOPTIC TISSUES

Jan Kulhánek, Martin Magner, Hana Štufkova, Alžběta Vondráčková, Tereza Daňhelovska, Viktor Stránecký, Lenka Dvořáková, Martin Řeboun, Hana Hansíková, Jiří Zeman, Tomáš Honzík, Markéta Tesařová

Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic

Background: AIF (Apoptosis-Inducing Factor) deficiency, caused by *AIFM1* gene mutations, is a rare X-linked disorder with a wide phenotypic spectrum; early-onset and rapidly progressing encephalomyopathy being the severest form. A novel pathophysiological pathway of AIF deficiency was proposed, involving a catastrophic disruption of the mitochondrial bioenergetics due to

the malfunction of AIF and CHCHD4 (Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 4), 2 indispensable mitochondrial proteins.

Methods: 5 patients from 2 unrelated families were thoroughly studied on a clinical, biochemical and molecular-genetic level.

Results: 3 male and 2 female patients manifested a profound neonatal-onset encephalomyopathy with lactic acidosis. Psychomotor retardation was present in all patients, hypertrophic cardiomyopathy developed in 4 and Leigh syndrome in 3 patients, 3 suffered from myoclonic epilepsy, optic atrophy was found in 1. Respiratory chain complexes deficiency was confirmed. Mitochondrial exome sequencing disclosed 2 novel missense *AIFM1* mutations, c.506C>T and c.1391T>G. Surprisingly, preferential inactivation of X-chromosome carrying the mutation was observed regardless to disease manifestation. AIF and CHCHD4 protein levels were severely diminished in 5 autaptic tissues.

Conclusion: We present the clinical, laboratory and molecular-genetic characterization of 5 patients, with the first female patients described and 2 novel *AIFM1* mutations. Heart involvement in our patients expands the clinical phenotype. Autaptic tissues were used for the first time to provide a biochemical evidence of the presumed pathophysiological pathway, studied only in yeast and animal models and human fibroblasts, so far.

Supported by AZV 16-32341A, SW 260367, GAČR 14-36804G, and RVO-VFN64165/2012.

HIGH PREVALENCE OF GROWTH PLATE DISORDERS AMONG CHILDREN WITH FAMILIAR SHORT STATURE

Lukáš Plachý, Veronika Straková, Lenka Elblova, Barbora Obermannova, Stanislava Koloušková, Marta Šnajderová, Dana Zemková, Petra Dušátková, Zdenek Sumnik, Jan Lebl, Stepanka Pruhova

Department of Pediatrics, 2nd Faculty of Medicine, Charles University in Prague and University Hospital Motol

Background: Familiar short stature (FSS) is a common growth disorder with heterogeneous etiology frequently considered as a normal variant of growth. Children are often excluded from further diagnosis and treatment. However, significant number of children from families with FSS comply even with the European criteria for growth hormone (GH) therapy – patients with SHOX-deficiency, growth hormone deficiency (GHD) or these born short for gestational age (SGA). The treatment response is variable, but the majority profit from the therapy. The aim of the study is to identify the genetic etiology of short stature in the families with severe FSS treated with rGH in our center excluding the families with SHOX deficiency.

Methods: Out of 555 children treated with GH due to GHD and/or SGA 32 (5.8 %) have severe FSS defined as live-minimum height ≤ -2.5 SD in patient and shorter parent. Twenty-one of them were born SGA, 24 had GHD (median of GH level after stimulation was 6.7 ug/l). In 4 children, the etiology is known (variant in *ACAN* 2 families, *NF1* 1 family, *PTPN11* 1 family). In remaining 28 patients with severe FSS (20 boys, median age 10.1 years, median age of the start of rGH therapy 7.2 years) no genetic cause of short stature was discovered despite detailed clinical examination including

genetic exclusion of SHOX gene defect prior the study. Their DNA was analyzed using whole exome sequencing methods, the results were evaluated using ACMG standards and guidelines.

Results: In 53.1 % (17/32) a causative variant was found. The etiology of growth failure was very variable. In 52.9 % (9/17), the variant was identified in the gene with a function in the growth plate (*COL2A1* 2 families, *COL11A1* 2 families, *ACAN* 2 families, *FLNB*, *FGFR3*, *IGF1R*) – mostly in probands born SGA. In 17.6 % (3/17) they affected IGF-proteins (IGFALS 2 families, *HGMA2*). Remaining genes could not be classified to neither of groups (*THRH*, *MBTPS2*, *GSHR*, *NF1*, *PTPN11*). Some variants fully explained phenotype in proband and his/her family, others definitely contributed to the proband's short stature but did not explain all the features of the complex phenotype and/or the growth disorder in the family.

Conclusion: In children from families with severe FSS who are classified as SGA and/or GHD, the genetic etiology of short stature is heterogeneous. However, the genes affecting the structure and function of the growth plate play an important role.

Supported by Ministry of Health, Czech Republic, grant numbers 16-31211A and NV18- 07-00283 and by research project of Grant Agency of Charles University of Prague, GAUK 976718. All rights reserved.

CLINICAL, CYTOGENETIC AND MOLECULAR ASSESSMENT OF ANGELMAN-SYNDROME PATIENTS AND DESCRIPTION OF A NOVEL MUTATION IN THE UBE3A GENE

Tímea Margit Szabó¹, Anikó Ujfaluši², Beáta Bessenyei², Éva Oláh¹, Gabriella P. Szabó¹, Bálint László Bálint³, Szilárd Póliska³, Kinga Hadzsiev⁴, Márta Czakó⁴, Gábor Veres¹, Katalin Szakszon¹

¹ *Pediatric Institute, University of Debrecen*

² *University of Debrecen, Faculty of Medicine, Inst. of Laboratory Medicine*

³ *University of Debrecen, Department of Biochemistry and Molecular Biology*

⁴ *University of Pécs, Inst. of Medical Genetics*

Background: Angelman syndrome (AS) is a rare neurodevelopmental disorder featuring intellectual disability, severely impaired expressive language, seizures, poor motor coordination and a characteristic behaviour with reduced attention span, hypermotility, excessive or inappropriate laughter. The underlying genetic mechanism is the deficient expression of the maternal allele of the *UBE3A* gene in the brain, located on chromosome 15. In healthy individuals, the paternal allele of *UBE3A* is subject to imprinting, and only the maternal copy is active. In AS, 1) microdeletion of the maternal 15q11.2-q13 chromosomal region, 2) paternal uniparental disomy of chromosome 15, 3) imprinting defects silencing the maternal allele, and 4) mutations in the maternally inherited *UBE3A* may account for the phenotype. The microdeletion can be classified into two main types according to the breakpoints (BP): in Class I the BP1-BP3 region, in Class II the BP2-BP3 region is deleted. A separate entity named BP1-BP2 microdeletion syndrome exists beyond the AS phenotype.

Methods: We tested 11 patients with clinical signs of Angelman syndrome. We used Fluorescent in situ hybridization (FISH), Methylation-Specific Multiplex Ligation-dependent Probe Amplification assay (MS-MLPA), array CGH, DNA microsatellita analysis and mutation testing of the *UBE3A*

gene. Sequence analysis of the whole coding region of the UBE3A gene was performed by Sanger sequencing.

Results: Seven patients had a detectable deletion in the 15q11-13 cytoband. Three patients had Class I deletions, four patients Class II; two had UPD; and two were found to have a mutation in the UBE3A gene: one being a novel pathogenic frameshift mutation (c.2309_2312delTCGT, p.Val771Ilefs*26) causing a premature stop codon, leading to a truncated protein. The other, a c.2503_2506dupCTTA, p.Lys836Thrfs*1 is a known pathogenic variant, already described in the literature. Both mutations arose de novo. Apart from AS patients, two children having BP1-BP2 deletions were diagnosed.

Conclusion: When comparing Class I and II patients the authors observed a more favourable, milder phenotype in Class II, albeit this observation should be handled with caution, due to the limited number of comparable cases.

Given its sensitivity to detect both deletions and disturbed methylation pattern, methylation-sensitive Multiplex Ligation Probe Amplification (MLPA) is now considered as the first-tier diagnostic test in the identification of AS. In FISH negative or MLPA negative cases, sequencing the UBE3A gene may confirm or disapprove the diagnosis if suggestive symptoms persist. The molecular genetic heterogeneity of AS does not always allow for a clear conclusion regarding genotype-phenotype correlation and calls for a careful approach.

THE CHANGE IN THE THERAPEUTIC APPROACH BASED ON GENETIC ANALYSIS IN CHILDREN WITH STEROID-RESISTANT NEPHROTIC SYNDROME

Martin Bezdička, Šárka Štolbová, Tomáš Seeman, Ondřej Cinek, Michal Malina, Naděžda Šimánková, Štěpánka Průhová, Jakub Zieg

Charles University in Prague, 2nd Faculty of Medicine, and University Hospital Motol

Background: Nephrotic syndrome (NS) is characterized by nephrotic range proteinuria, hypoproteinemia and oedema. The occurrence of NS is 1.5/100 000 children in Europe. The most common aetiology of steroid-resistant nephrotic syndrome (SRNS) are idiopathic and genetic forms (there are 56 identified genes, the most common are *NPHS1*, *NPHS2* and *WT1*). The proportion of genetic SRNS in Czech and Slovak population has not been studied yet. The aim of the study was to perform a genetic analysis of Czech and Slovak children with SRNS and to change their current therapy based on the results of the genetic examination.

Methods: The study contained 74 children (38 boys and 36 girls, the samples were collected in 2000-2017 (inclusive)) with congenital (11/74, 0-3 months of age onset), infantile (10/74, 3-12 months of age onset) and childhood (52/74, after 1 year of age onset) NS. The DNA samples were first analysed by Sanger sequencing (*NPHS1*, *NPHS2*, *WT1* genes) and then the negative samples were analysed by next generation sequencing (NGS) using a targeted panel of 48 genes previously associated with SRNS.

Results: Genetic diagnosis was established in 28/74 (38 %) patients. Sanger sequencing diagnosed 19/74 (26 %) children and NGS diagnosed further 9/74 (12 %) children. The most frequent causative genes were *NPHS2* (N=11/74), *WT1* (N=7/74) and a novel SRNS associated NUP93 gene (N=4/74).

Further causative genes were *COQ2* (N=2/74), *NPHS1*, *INF2*, *DGKE* and *LMX1B* (one patient for each gene). The girl with *COQ2* causative variant (encodes a catalytic enzyme in coenzyme Q10 biosynthesis) received oral coenzyme Q10 therapy leading to significant decrease of proteinuria (from 1.2 g/day to 0.17 g/day after 3 months). The immunosuppressive therapy could be terminated in some other patients with causative genetic finding.

Conclusion: The unexpectedly high proportion of causative variants in novel *NUP93* gene demonstrates the suitability to start analysing *NPHS2*, *WT1* and *NUP93* genes in Czech and Slovak patients with SRNS preferably. Genetic findings in SRNS clarify the resistance to corticosteroid therapy or an absence of recurrence after kidney transplantation and thereby found causative variants underline the clinical significance of genetic analysis of well-known and minor genes in patients with SRNS. The genetic results may lead to optimization of therapeutic approach in children with SRNS.

The study was supported by the Ministry of Health of the Czech Republic, grant number 15-31586A.

5 YEARS FOLLOW-UP OF PEDIATRIC RENAL TRANSPLANT RECIPIENTS AFTER JOINING EUROTRANSPLANT : A HUNGARIAN RETROSPECTIVE ANALYSIS, EARLY FINDINGS

László Berta¹, György Reusz¹, Péter Sallay¹, Kata Kelen¹, Petronella Pethő-Orosz¹, Attila Szabó^{1,2}

¹ I. Department of Pediatrics, Budapest

² MTA-SE Pediatric and Nephrology Research Group, Budapest

Background: Five years have passed since the first transplantation (Tx) within the confines of Eurotransplant. The results of pediatric renal Tx have improved markedly in the last decade. However, a number of relevant clinical problems remain unrevealed. Our main goal was to collect and analyze data of children, receiving renal transplant in Hungary and provide detailed information about relevant clinical aspects.

Methods: We would like to present our latest findings between 2013 and 2018. We analyzed our retrospectively maintained data, in the multinational, web-based CERTAIN-Registry. The patients were treated at the I. Department of Pediatrics, Semmelweis University. Data were collected before the Tx, and 7 days -, 3 months and in every half of year following Tx.

Results: 45 Tx were performed (11 living related, 29 deceased) since 2013. 40 patients were included in the study (19 female, 21 male, mean age, 11 years). The most common primary diagnosis was congenital disorder of the kidney and urinary tract in 12 patients, followed by nephrotic syndrome in 9 cases and cystic kidney disease in 5 cases. 16 patients were on hemodialysis, 16 on peritoneal dialysis before Tx, and in 8 cases pre-emptive Tx was performed. In 6 cases delayed graft function was observed. In terms of outcome, 1 death, 3 graft loss occurred. We have got 5 biopsies taken (2 borderline, 3 acute T-cell rejection) from 9 rejection episodes. All rejection episodes were resolved with steroid bolus therapy. Medication, hospitalisation, laboratory parameters and other detailed informations will be elaborated in the near future.

Conclusion: The basic steps were made for a good source of our further investigations and for international collaborations.

DYNAMICS OF SALIVARY UREA AND CREATININE DURING THE DEVELOPMENT OF RENAL INJURY IN ANIMAL MODELS

Alexandra Gaál Kovalčíková¹, Emese Domonkos¹, Róbert Lipták¹, Kristína Ploth³, Marianna Hladová³, Ľubomíra Tóthová¹, Ľudmila Podracká⁴, Peter Celec^{1,2,3}

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

² Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

³ Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

⁴ 1st Pediatric department, Bratislava

Background: Recently, saliva is gaining popularity as an alternative biofluid to blood for the assessment of various diseases. Monitoring kidney disease in home conditions would be of major interest. Therefore, the aim of our study was to describe the dynamics of salivary urea and creatinine during progression of acute kidney injury (AKI) and chronic kidney disease (CKD) in rats.

Methods: Ninety adult Wistar male rats underwent bilateral nephrectomy (BNX), ischemic-reperfusion injury (IRI) or glycerol nephropathy injury to induce acute kidney injury. Blood and saliva were collected at baseline and 3, 6, 12 and 24 hours after induction of kidney injury. Chronic kidney disease was induced by 5/6 nephrectomy. Blood and saliva were collected at baseline and 2, 4, and 6 months after surgery. Creatinine and urea concentrations in plasma and saliva samples were measured using commercially available spectrophotometric assays.

Results: We observed the most rapid increase of plasma urea in models of BNX and IRI in which urea was elevated by almost 50 % 3 hours after AKI induction. A subsequent increase of urea salivary concentrations to approximately 10mmol/l was observed after 12 hours when plasma levels were increased more than 1-fold and reached the level of 30mmol/l. Plasma creatinine level in these models was elevated by 200 % only after 12 hours. An increase of its salivary levels to approximately 50µmol/l was observed after 24 hours when plasma levels were elevated 3-fold and reached the concentration of 300µmol/l. In glycerol nephropathy, plasma urea was elevated by 130 % after 24 hours. Its salivary levels were increased to 10mmol/l after 24 hours when plasma levels reached more than 20mmol/l. Plasma creatinine was elevated by more than 100 % only after 48 hours and reached the concentration of 150µmol/l. No changes in salivary creatinine were observed. In 5/6 nephrectomy, plasma urea was increased by 50 % 2 months after CKD induction. Salivary urea was elevated the most after 6 months when plasma levels reached more than 20mmol/l. The concentration of plasma creatinine was raised by 50% after 6 months, while no changes in its salivary concentrations were found.

Conclusion: The salivary markers copy the plasma concentrations of urea and creatinine. However, the rise in plasma concentrations must be sufficient to reflect changes in saliva. Thus, an increase of salivary urea and creatinine is observed later in comparison to plasma. Moreover an elevation of salivary markers depends on the experimental model of renal failure and its severity. The most rapid increase of salivary urea and creatinine was observed in the most severe model of kidney injury, BNX. The sensitivity of salivary urea seems to be superior to creatinine. Saliva proved to be obtainable in sufficient amounts from rats and salivary creatinine and urea were measurable. To conclude, our study revealed the detailed dynamics of salivary creatinine and urea during renal failure in various animal models. It seems that salivary markers of renal functions could be used

in clinical practice only for the monitoring of advanced stages of renal diseases, but not for the diagnosis of their early stages.

ROLE OF NANOVESICLES IN THE DEVELOPMENT OF PERITONEAL FIBROSIS DURING PERITONEAL DIALYSIS

Beáta Szebeni^{1,2}, István Márton Takács², Zoltán Varga³, Domonkos Pap^{1,2}, Apor Veres-Székely², Attila J Szabó^{1,2}, György Reusz², Ádám Vannay^{1,2}

¹ MTA-SE, Pediatrics and Nephrology Research Group, Budapest, Hungary

² 1st Department of Pediatrics, Semmelweis University

³ MTA-TTK Biological Nanochemistry Research Group

Background: Nanovesicles (NVs) have significant therapeutic potential. They can protect against myocardial infarction, acute kidney injury or liver fibrosis as well. In 2017 Pearson et al successfully isolated extracellular vesicles from dialysis effluents (PDE) of PD patients, however their impact on the development of peritoneal fibrosis during PD remained unclear. Our aim was to determine, whether NVs isolated from PDE can affect/alter progression of peritoneal fibrosis.

Methods: Three hundred ml of PDE have been collected from PD patients. NVs were isolated by different ways (ultrafiltration with MWCO filters or size exclusion chromatography) to obtain the most homogenous/concentrated NV fractions. NVs were analysed by dynamic light scattering (DLS) and Fourier transform infrared spectroscopy (FTIR). NRK-49F fibroblast cell line was treated with PDE derived NVs and their effect on the endogenous as well as platelet derived growth factor (PDGF) induced proliferation of this fibroblast cell line were measured by MTT test after 24h.

Results: The average size and size distribution of NVs was about 80 [40-150] nm (DLS). FTIR analyses revealed that protein and lipid content and protein to lipid ratio of PDE derived NVs was typical for this type of extracellular vesicles. Incubation of NRK-49F with PDE derived NVs significantly reduced both the endogenous and PDGF induced proliferation rate of these fibroblast cells.

Conclusion: NVs isolated from dialysis effluents of PD patients may inhibit peritoneal fibrosis contributing to the preservation of the peritoneal membrane structure. However, future in vivo and in vitro experiments are required for testing their therapeutic potential in PD and their impact as potential biomarker.

This project was supported by ÚNKP-17- 4-IV- SE-60 New National Excellence Program of the Ministry of Human Capacities and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences

THE ROLE OF IL-20 CYTOKINE SUBFAMILY IN THE PATHOGENESIS OF CHRONIC KIDNEY DISEASE

Domonkos Pap¹, Apor Veres-Székely², Rita Lippai², István Márton Takács², Erna Sziksz¹, Beáta Szebeni¹, Attila J. Szabó^{1,2}, Ádám Vannay¹

¹ MTA-SE, Pediatrics and Nephrology Research Group, Budapest, Hungary

² 1st Department of Pediatrics, Semmelweis University, Budapest, Hungary

Background: Regardless of the etiology kidney fibrosis is a common outcome of progressive chronic kidney diseases. Our recent study showed that levels of interleukin (IL)-20 subfamily members, including IL-19 and IL-24 significantly increased in kidneys underwent unilateral ureteral obstruction (UUO). However, their precise role in the pathomechanism of renal fibrosis is not clearly understood.

Methods: To study the role of IL-20 cytokine subfamily we applied a mouse model of UUO induced kidney fibrosis on wild type and IL-20 receptor beta gene knockout (IL-20R β KO) mice. Masson's trichrome and Picro-Sirius Red staining were used to investigate the renal accumulation of extracellular matrix proteins. Real-time RT-PCR and western blot method were performed to measure the renal expression of fibrosis associated molecules. We also investigated the in vitro effect of IL-24 treatment on transforming growth factor beta (TGF- β) and platelet derived growth factor B (PDGF-B) expression of human proximal tubular epithelial (HK-2) cells by real-time RT-PCR and flow cytometry.

Results: We found elevated level of IL-19, IL-24 and IL-20R β in the fibrotic kidneys. IL-20R β KO mice showed reduced extracellular matrix deposition and decreased α -smooth muscle actin expression compared to wild-type mice following UUO. Treatment of renal epithelial cells with IL-24 increased their TGF- β and PDGF-B production.

Conclusion: Our study provides direct evidence of the pathogenic role of IL-20 cytokine subfamily in the development of renal fibrosis, possibly through the IL-24 mediated production of pro-fibrotic factors. Therefore, inhibition of IL-24 may have therapeutic effect in treatment of chronic kidney diseases.

Grants: OTKA K116928 and VKE-2017-00006. This project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

CORRELATION OF PULSE WAVE VELOCITY PARAMETERS WITH KIDNEY FUNCTION MARKERS IN CHILDREN

Mirjam Močnik¹, Nataša Marčun Varda^{2,3}

¹ Dr. Adolf Drolc Healthcare Centre, Vošnjakova ulica 2–4, 2000 Maribor, Slovenia

² Department of Paediatrics, University Medical Centre Maribor, Ljubljanska 5, 2000 Maribor, Slovenia

³ Medical Faculty, University of Maribor, Taborska 8, 2000 Maribor, Slovenia

Background: One of the important aims of preventive nephrology is search for early markers of cardiovascular risk as cardiovascular diseases present important cause of morbidity in children

with chronic kidney disease. Studies in adults have demonstrated the correlation of mildly decreased kidney function and even its normal values with pulse wave velocity (PWV), a measure of arterial stiffness and predictor of cardiovascular events. The aim of our study was to evaluate the connection between parameters of PWV and kidney markers – serum cystatin C, serum creatinine and microalbuminuria in children and adolescents.

Methods: 191 children and adolescents were included in the study, collecting the following data in the retrospective manner: subject's age, height, weight, body mass index (BMI), systolic blood pressure (SP), diastolic blood pressure (DP), PWV, serum cystatin C, creatinine and microalbuminuria. Parameters of PWV were measured using applanation tonometry and correlations calculated.

Results: PWV significantly correlated with age ($p < 0.001$), height ($p < 0.001$), weight ($p = 0.001$), SP ($p < 0.001$), DP ($p < 0.001$), but not with BMI ($p = 0.134$), serum cystatin C ($p = 0.547$), serum creatinine ($p = 0.179$). The correlation with microalbuminuria almost reached statistical significance ($p = 0.055$). In multiple regression analysis with PWV as dependent variable, only age and DP were found statistically significant.

Conclusion: We were not able to demonstrate a connection between PWV and markers of kidney function in children, although correlation between PWV and microalbuminuria could be promising. For confirmation of our results, a prospective study is needed.

USE OF MITRAL INFLOW PROPAGATION VELOCITY AS A MARKER OF DIASTOLIC FUNCTION IN PRETERM NEWBORNS

Gergely Balázs, Z. Papp, Gy. Balla, T. Kovács

Department of Pediatrics, Faculty of Medicine, University of Debrecen, Hungary

Background: Immature myocardium is less compliant than that in later of life and there are apparent early maturational changes. In contrast to traditional Doppler parameters early diastolic mitral inflow propagation velocity slope (Vp) is considered to be a largely load-independent marker of diastolic function. There is no experience with Vp in preterm newborns. The aim of our study were to determine feasibility, reproducibility of Vp measurement and to examine age dependent alterations of Vp during the early postnatal adaptation process.

Methods: The study group comprised a total of 37 extremely low birth weight infants (GA: 25.4 $\pm$ 1.4 wks; BW: 737 $\pm$ 217 g) born at Clinical Centre University of Debrecen, Hungary. As a control group 13 healthy term newborns were recruited (GA: 38.4 $\pm$ 1.3 wks; BW: 3190 $\pm$ 380g). The study protocol employed a repeated measures design, with echocardiography taking place on the 1st, 4th days and at the age of 2-3 weeks. Identical measurements were performed at each assessment, including Vp, peak E wave, peak A wave, LVOT Vti, and E/A, E/Vp ratios were calculated. Images from all 92 studies were re-analyzed by the same observer after 2 month for intra-observer variability.

Results: Measurements of peak E velocities and E/A were similar to previously published values. Premature infants had faster heart rate (HR) at each examination than term infants. We found no correlation between Vp and HR ($r = 0.2384$; $p = 0.0715$). Mean Vp values for either term or preterm newborns (preterm: 27.2 $\pm$ 9.1 cm/s, term: 34.8 $\pm$ 9.4 cm/s) were substantially lower than reported adult values, even if Bazett's HR correction applied. Vp shows a significant correlation to gestational

age ($r = 0.3771$; $p = 0.0003$) and preterm newborns had lower Vp values than their full term counterparts. In preterm newborns Vp increased significantly by day4 and this trend continued on week2. This highly significant linear correlation of Vp to postnatal age ($r = 0.6219$; $p < 0.0001$) was not observed in term newborns ($r = 0.2541$; $p = 0.1835$). The ratio of E/Vp did not significantly alter over the study period in either term or preterm groups ($r = -0.2445$; $p = 0.0225$, and $r = -0.1403$; $p = 0.2004$). A significant linear correlation of Vp to LVOT VTI was noted in a subset of patients with closed ductus arteriosus ($r = 0.6754$; $p = 0.0029$). Intraobserver standard deviation was 3.098 cm/sec.

Conclusion: Lower Vp in newborns compared to adults indicates differences in myocardial relaxation. Our data suggest that LV diastolic myocardial properties of preterm newborns improves in the early postnatal adaptation period in a manner that maintains constant filling pressures. Vp is a feasible and reproducible technique in analyzing newborn diastolic cardiac function, and can give new insights in transitional physiology, however interobserver variability needs to be determined.

COMPARISON OF PREDUCTAL AND POSTDUCTAL BLOOD PRESSURE VALUES OBTAINED BY PERIPHERAL ARTERIAL LINES IN EXTREMELY PRETERM INFANTS ON THE FIRST DAY OF LIFE

Lukas Aichhorn, Ulrike Salzer-Muhar, Sigrid Baumgartner, Katrin Klebermaß-Schrehof, Peter Knaus, Angelika Berger, Tobias Werther

Universitätsklinik für Kinder- und Jugendheilkunde, Medizinische Universität Wien

Background: When evaluating the need for antihypotensive treatment, neonatologists are confronted with complex, rapid cardiovascular transition processes which take place in extremely preterm infants (EPI) immediately after birth. The description of normative blood pressure (BP) values in EPI has been the focus of several studies in the past decades, and findings indicated that BP evolves differently during the first 12 hours compared to the following 12 hours of life. However, it remains unclear whether pre- and postductal BP values obtained by peripheral arterial lines differ from one another during the first day of life.

Methods: In this retrospective observational study, data were collected from preterm infants born at 23+0 to 27+6 weeks of gestation admitted to our neonatal intensive care unit from 10/2011 to 12/2015. A total of 40.959 BP readings from 143 infants were analyzed. Clinical factors included in our model were gestational age, birthweight, patent ductus arteriosus, sedation, intubation and inotropic support. Panel regressions were estimated for the first 12 hours and second 12 hours of life separately.

Results: During the first 12 hours, estimations for mean, systolic and diastolic preductal values were lower (-0.43mmHg, -0.32mmHg, -0.62mmHg respectively, $p < 0.01$) compared to postductal values. During the second 12 hours, preductal mean, systolic and diastolic BP were estimated higher (0.85mmHg, 1.25mmHg, 0.4mmHg, respectively, $p < 0.001$) than postductal values. In infants with spontaneous duct closure, mean preductal values were 5.83mmHg and 3.3mmHg lower than mean postductal BP values ($p < 0.001$) during the first and second 12 hours, respectively. In infants with subsequent ductal patency (PDA), mean preductal BP values were higher (0.4mmHg and 1.11mmHg, $p < 0.01$) during the first and second 12 hours, respectively.

Conclusion: Our data show that there is a significant difference in pre- and postductal values of EPI on the first day of life. The postnatal transition process may explain why preductal BP values were initially lower than postductal values but rose gradually and eventually surpassed postductal BP values: As pulmonary resistance decreases and ductal flow reverses, the left ventricle, having played a minor role prenatally, gradually takes on responsibility for providing systemic blood flow. The higher postductal BP values in infants with subsequent spontaneous ductal closure may be clinically relevant and could indicate higher than expected systemic vascular resistance. In infants with subsequent PDA, preductal BP may reflect duct-related hemodynamics, namely the extent of recirculation and lowered systemic vascular resistance.

MRI VALIDATION OF SURFACE DISTANCE MEASUREMENT FOR PULSE WAVE VELOCITY IN CHILDREN

Adrienn Bárczi¹, Arianna Dégi¹, Orsolya Cseprekál², Eva Kis³, Ádám György Szabó⁴, Gábor Rudas⁴, György Reusz¹

¹ Semmelweis University, 1st Department of Pediatrics, Budapest

² Semmelweis University, Department of Transplantation and Surgery, Budapest

³ Gottsegen György Hungarian Institute of Cardiology, Budapest

⁴ Semmelweis University, MR Research Center, Budapest

Background: Determination of aortic pulse wave velocity (PWV) is the gold standard measurement for arterial stiffness. PWV is defined as the velocity of the pressure waves in the aorta assessed by dividing travelled distance by travel time. The method of estimating the path length taken by the pulse wave is of crucial importance, however it is not standardized yet. The formula of 0.8 x surface distance (L0.8) based on MRI validation is used in adults. Normative values exist for PWV in children using the subtracted method (LSM) for the estimation of the distance, however there are no imaging validation studies in the growing pediatric population.

Our aim was to compare the different techniques of surface measurement and distances determined by MRI in pediatric subjects.

Methods: 21 patients were included (3.2-17.8 years, 16 males) in the study. PWV measurement was performed before MRI, and the pulse wave travel distance was assessed by surface tape measurements (L0.8 and LSM). Carotid, femoral and additional sampling sites were labelled by MRI detectable vitamin A capsule. Carotid-femoral path length was also measured by MRI.

Images were analysed by Philips Extended MR WorkSpace 2.6.3.5 software. To calculate the reference distance (LREF) for the real travelled aortic path length, centerpoints were placed manually in each slice and a centerline was reconstructed. Bland Altman plots (BA) were used to assess the difference between the distances measured on surface or intra-arterial.

Results: There was a high correlation ($r>0.86$, $p<0.001$) between surface and MRI distance measurements. According to L0.8 and LREF values the average difference was 35.9 cm (8.7 %) and BA plots showed significant difference. The difference between LSM and LREF was 2 cm (0.5 %) and in the BA analysis LSM and LREF were in good accordance. There was a proportional error in the BA plots in patients over 13 years.

Conclusion: In our study group the path length assessed by the subtracted method showed excellent correlation with intra-arterial MRI measurements. The small number of cases does not allow to conclude the difference in postpubertal patients, which needs further evaluation

„Supported BY the ÚNKP-17-3 New National Excellence Program of the Ministry of Human Capacities”

IMPROVEMENT OF THE DIAGNOSTIC WORKUP BASED BY PORTO CRITERIA: RESULTS OF THE HUNGARIAN PROSPECTIVE NATIONWIDE INCEPTION COHORT PEDIATRIC IBD REGISTRY (HUPIR)

Orsolya Somodi¹, Katalin Muller², HUPIR Registry, Gabor Veres¹

¹ Dept. Of Pediatrics, University of Debrecen, Debrecen, Hungary

² 1st Dept. Of Pediatrics, Semmelweis University, Budapest, Hungary

Background: The diagnostic yield of esophagogastroduodenoscopy (EGD) was never evaluated in any nationwide inflammatory bowel disease (IBD) registry. We evaluated whether diagnostic workup of paediatric IBD patients fulfils Porto Criteria in Hungary. In addition, we analyzed the diagnostic yield of EGD.

Methods: Newly diagnosed paediatric patients with IBD (ages 0 – 18 years) are registered in this prospective registry. All the 27 paediatric gastroenterology services participated ensuring a nationwide approach. The questionnaire includes epidemiological data, disease extension, disease activity (PCDAI, PUCAI) and initial therapy.

Results: Data of 1488 patients recorded between 2007 - 2016 were analyzed (922 Crohn disease (CD), 468 ulcerative colitis (UC), 98 IBD-U). Upper endoscopy (EGD) was performed in 52 % of the patients in 2007, and this rate increased to 91 % by 2016 ($p < 0.0001$). Proportion of ileoscopy changed from 50 % to 83 % ($p < 0.0001$). Imaging of the small bowel did not change in the observation period (range 30 - 60 %), but rate of MRI increased from 7 % to 38 % ($p < 0.0001$).

In addition, characteristics of EGD of 858 newly diagnosed IBD patients were analyzed. IBD characteristic lesions (aphtha, ulcer, cobblestoning) occurred in 272 (32 %). The diagnostic yield of EGD was 9 % in children with newly diagnosed CD (colonic disease without or unknown small bowel involvement, without intraabdominal complication or perianal disease, and without granuloma).

Conclusion: The quality of diagnostic workup in paediatric IBD patients improved in the last 10 years based on the database of HUPIR. The diagnostic yield of EGD was 9 % in CD patients confirming the importance of EGD in the classification of IBD.

INCREMENT OF EXCLUSIVE ENTERAL NUTRITION FOR CHILDREN WITH CROHN'S DISEASE BASED ON HUNGARIAN PEDIATRIC IBD REGISTRY (HUPIR)

Veronika Kovacs, Katalin Müller, HUPIR Group, Gabor Veres

Department of Pediatrics, University of Debrecen, Hungary

Background: The treatment of inflammatory bowel disease (IBD) in pediatric patients has changed considerably in the last decade. According to international guidelines, exclusive enteral nutrition (EEN) is the first choice for inducing Crohn's disease (CD) instead of the steroid therapy. The studies shown that EEN is just as effective on clinical remission as steroids, but the mucosal healing, which is considered to be the primary therapeutic goal, can be achieved in a higher ratio with EEN, compared to steroids. Below, we show the introduction of EEN based on the Hungarian Pediatric IBD Registry (HUPIR).

Methods: HUPIR is a nationwide database for prospectively registering newly diagnosed pediatric IBD cases. 27 pediatric gastroenterology institutes are involved in the management of HUPIR. Patients are enrolled by the diagnostic pediatric gastroenterologist and after the two levels of validation, the data is entered into the register. The patients demographic data, localization, activity, and therapy are recorded. The use of the EEN was evaluated in children with CD, registered between 1 January 2010 and 31 December 2016.

Results: In the inclusion period, a total of 662 CD were registered. In 2010, as induction therapy, systemic steroids were used by 56 % of the children with CD. EEN has been gradually increasing from 2 % in 2010 to 53 % in 2016 as induction therapy. At the same time, the use of systemic steroids decreased to 20 % as induction treatment. Under this 6-year period, 177 CD patients started EEN feeding, 16 % of these patients needed steroid therapy (systemic steroid or budesonide) within 3 months after the diagnosis. 62 % of 485 children started steroid therapy as the induction therapy. At the establishing of the diagnosis, the activity index, mean age, and localization between the children who did or did not receive EEN as induction therapy showed no significant difference. Three months after the diagnosis, the rate of clinical remission (activity index) did not reveal any significant differences (78 % vs. 82 %, $p = 0.406$).

Conclusion: New evidences and guidelines have significantly modified the treatment of CD. EEN became the first choice for induction therapy reducing the need for initial steroid treatment.

CZECH NATIONAL REGISTRY OF DIAMOND-BLACKFAN ANEMIA (DBA): SEARCH FOR NOVEL MOLECULAR MARKERS

Jana Volejnikova^{1,2}, Petr Vojta², Zuzana Maceckova², Pavla Koralkova³, Helena Urbankova⁴, Monika Horvathova³, Barbora Ludikova⁴, Martina Sukova⁵, Pavel Timr⁶, Marian Hajduch², Dagmar Pospisilova¹

¹ Department of Pediatrics, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Czech Republic

² Institute of Molecular and Translational Medicine, Palacky University Olomouc

³ Department of Biology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic

⁴ Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Czech Republic

⁵ Department of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

⁶ Department of Pediatrics, Hospital Ceske Budejovice, Ceske Budejovice, Czech Republic

Background: Diamond-Blackfan anemia (DBA) is a rare congenital erythroid aplasia manifesting usually during the first months of age. Approximately 50 – 70 % cases of DBA are underlied by mutations in genes coding for ribosomal proteins (RP), however, the etiology remains unexplained in the remaining patients. Also larger deletions involving some of the RP genes, and aberrations involving extraribosomal genes (e.g., *GATA1*, *TSR2*) are causative for DBA. Our aim was to identify underlying molecular pathology in patients without any previously identified mutation.

Methods: Peripheral blood of patients and their selected family members was examined by exome sequencing (Agilent SureSelect v6 Kit, HiSeq 2500 sequencer, Illumina) and array comparative genomic hybridization (aCGH, SurePrint G3 CGH+SNP microarray, 4x180K, Agilent). Identified mutations were verified by Sanger sequencing at the gDNA and cDNA level. Functional impact was verified in a cell culture (*RPS11*-knock-out UOS cell line).

Results: Czech DBA Registry currently contains 63 patients with DBA from Czech and Slovak Republic (children and adults). Detection rate of RP gene mutations is 73 %.

Novel mutations, previously unpublished and unrecorded in gene databases, or large deletions involving RP genes were found in 5 patients with DBA:

- 1) In a 10-year-old female with growth and developmental delay, severe skeletal anomalies (hypertelorism, high-arched palate, low-set ears, triphalangeal thumbs) and with a markedly elevated activity of erythrocytic adenosinedeaminase (eADA), a large deletion in 1p36 chromosomal region - arr[hg19] 1p36.12p36.11(22308520_24074348)x1 - involving the *RPL11* gene was found. Depletion of *RPL11* in a cellular model resulted in attenuation of proteosynthesis and cell cycle and in the disruption of nucleolar morphology.
- 2) In a 4-year-old female with transfusion-dependent DBA, large deletion of the 3q29 region - arr[hg38]3q29(194309533_197837049)x1 - including the *RPL35A* gene was detected. The patient's height is at the 10th percentile, but her weight is below the 3rd percentile. Mild intellectual disability and both atrial and ventricular septal defect are present.
- 3) Another deletion in 3q29 involving the *RPL35A* locus, together with 9p duplication including *JAK2* (arr[hg38] 3q29(194946946_197837049)x1, 9p24.3p23(214367_11610300)x3) was found in a 30-year-old female with steroid dependent DBA, short stature, mental retardation, epilepsy and somatic malformations (pterygium colli, thenar hypoplasia, fish mouth).
- 4) 4-month old girl, slightly hypotrophic, with transfusion-dependent DBA and atrial septal defect with mild mitral insufficiency has a deletion with loss of heterozygosity involving regions for *RPS29* and *RPL36AL* - arr[hg38] 14q21.3q22.1(49134547_53976945)x2 hnz
- 5) A 4,5-year-old male with transfusion dependency, iron overload and hepatopathy harbors mutation in *RPS19* - hg38[19:41869031_C>T], *RPS19* p.A58V. The patient's father with an

identical mutation required repeated blood transfusions in childhood (until 6 years of age) due to hypoplastic erythropoiesis. To date, all parameters of his blood count are normal without any intervention, only his eADA level is slightly elevated. All above mentioned patients are treated with leucine.

6) A 3-year-old male with normal phenotype harbors mutation hg38[19:41860841_A>T] in *RPS19* leading to premature stop codon K23Stop.

7) Mutation in *RPS19*: c.[3G>A], p.M1I in an 1 year old boy whose DBA is transfusion dependent and resistant to steroids.

Conclusion: Together with next generation sequencing, aCGH is important in detection of novel genetic defects in DBA due to its capacity to detect larger chromosomal aberrations. Reports on 3q29 deletions involving RPL35A are scarce (over 10 cases published to date). Connection between the presence of deletions and growth retardation (Kuramitsu et al., Blood 2012) is likely. Phenotypic differences between family members harboring the novel identical *RPS19* aberration are probably influenced by epigenetic regulation or revertant mosaicism and we have already observed this phenomenon in individuals with *RPS7* and *RPS19* mutation.

Supported by grants: AZV 16-32105A, NPU LO 1304, GA15-13732S

CZECH NATIONAL REGISTRY OF DIAMOND-BLACKFAN ANEMIA (DBA): SEARCH FOR NOVEL MOLECULAR MARKERS

Volejnikova J.^{1,2}, Prochazkova K.¹, Ludikova B.¹, Novak Z.¹, Sulovska L.¹, Ambruzova Z.³, Prochazkova D.⁴, Pospisilova D.¹

¹ Department of Pediatrics, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Czech Republic

² Institute of Molecular and Translational Medicine, Palacky University Olomouc

³ Department of Immunology, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Czech Republic

⁴ Department of Pediatrics, Masaryk Hospital Usti nad Labem, Czech Republic

Background: Immune thrombocytopenic purpura (ITP) is the most frequent bleeding disorder in childhood with an incidence of approximately 4-8/100,000 children per year. It is caused by immunologically mediated peripheral destruction of platelets, but the exact mechanism of immune dysfunction is often unclear. Acute ITP is far more prevalent in children, but transition to chronicity occurs in 10-20% of cases. Markers for prediction of chronic ITP development are lacking. We aimed at evaluation of anti-platelet antibodies in children with both acute and chronic ITP.

Methods and patients: One-hundred and two children (58 male, 44 female, age 6 months - 18 years) were treated at our center between 2008 and 2016. 71 patients had acute ITP and 31 patients chronic ITP. In all of them, antibodies against thrombocytic glykoproteins (GP), i.e., anti- GP Ib/IX, IIb/IIIa, Ia/IIa and V, were assessed by a MAIPA method in addition to routine clinical and laboratory examination. Results were statistically evaluated using Pearson test and p-values under 0.05 were reported as significant.

Results: Anti-GP antibodies were significantly more frequent in chronic ITP (26/31 patients) than in acute ITP (44/71 patients; $p=0.005$). Also distribution of respective types of anti-GP antibodies was different between acute and chronic ITP: anti-GP Ib/IX was in 23/71 acute ITP and 22/31 chronic ITP ($p=0.001$); anti-GP Ia/IIa in 21 acute and 19 chronic ITP ($p=0.005$), and anti-GP IIb/IIIa in 33 acute and 23 chronic ITP ($p=0.012$). In 48 patients (47%), combinations of anti-GP antibodies were present, 22 patients (21.6%) had a single antibody type and in 32 patients (31%) no antibodies were detected.

Conclusion: Anti-platelet antibodies occur more often in chronic ITP, with a most striking difference in the GPIb/IX subtype. This is interesting considering new data on the mechanism of action of GPIb antibodies, which is supposed to be different than in anti-GP IIb/IIIa and V antibodies: it acts through an Fc-independent platelet clearance with translocation and desialylation of neuraminidase 1. This leads to platelet clearance in the liver via hepatic Ashwell-Morell receptors. In adult patients with anti-GP1b positivity, worse response to intravenous immunoglobulins (IVIG) and steroids was observed, therefore, they may be in a higher risk of a chronic ITP development. Our results confirm this hypothesis, however, larger prospective studies of childhood ITP are needed.

Supported by grants: MH CZ – DRO (FNOI, 00098892), NPU LO 1304

FACTOR VII DEFICIENCY IN CHILDREN – INTERRELATIONS BETWEEN GENOTYPE, FACTOR VII ACTIVITY AND BLEEDING SIGNS

Marie Al-Muhanna¹, Marianna Speker², Csongor Kiss¹, Zsuzsanna Bereczky²

¹ Department of Pediatric Hematology-Oncology, Institute of Pediatrics, University of Debrecen

² Division of Clinical Laboratory Science, Department of Laboratory Medicine, University of Debrecen

Background: Factor VII (FVII) deficiency is either congenital or acquired. Congenital FVII deficiency is the second most common rare bleeding disorder (RBD) after FXI deficiency and it is the most common RBD in certain countries, such as Hungary and Slovakia. It is transmitted by autosomal recessive inheritance. The FVII gene mutations and the clinical manifestations of the disorder are highly variable. At present, the most effective treatment for FVII deficiency is recombinant FVIIa (rFVIIa) replacement. Our goal was to examine the children registered with FVII deficiency in the database of the Department of Pediatrics and Division of Clinical Laboratory Science, Department of Laboratory Medicine, University of Debrecen Clinical Center, between 2005 and 2018. Our secondary aim was to characterize the mutation spectrum of gene *F7* of all patients referred to Division of Clinical Laboratory Science, Department of Laboratory Medicine.

Methods: Data of patients were obtained retrospectively from medical documentation. Hemostasis screening tests were performed. FVII activity was determined with FVII:C one- stage clotting assay. *F7* genotyping was done by Sanger sequencing.

Results: We identified 15 children with congenital FVII deficiency (9 boys and 6 girls; age 0 - 18 yrs, mean 8,5 yrs) and one with acquired FVII deficiency (14 yrs-old male). All the pediatric patients had mild clinical presentation. The major bleeding symptoms were epistaxis and bleeding after dental extraction or trauma. All patients presented prolonged prothrombin time (PT). FVII activity of patients was variable (< 1 - 55 %). We examined the genotype of 13 pediatric patients, four of them were compound heterozygotes, four were combined homozygotes, four were combined hetero/

homozygotes and one was heterozygote. In the case of five probands, we were able to perform family tree analysis, which demonstrated the inheritance pattern among probands and their parents. We used rFVIIa in four patients to prevent intra- and postoperative bleeding. Response to rFVIIa treatment was excellent in each case and there was no any other complication. Among 33 patients (15 children and 18 adults) we identified 16 different mutations, including three were novel ones (c.200A>G, p.Glu67Gly; c.1283G>T, p.Cys428Phe; c.1303G>C, p.Gly435Arg). Ten of the 16 mutations were located in the catalytic serine protease domain, two in the Gla domain, two in the promoter region, one in the second epidermal growth factor domain and one in intron 7. There was no correlation between genotype, FVII activity and bleeding manifestations except for the fact that we were able to show the presence of pathogenic mutations in the F7 gene in every patient with FVII deficiency.

Conclusion: FVII deficiency is a RBD and most of the time the clinical picture is moderate. However, we have to set up the diagnosis as soon as possible to prevent the sever bleeding complications during injuries or surgeries. In case of bleeding symptoms with isolated, prolonged PT the FVII deficiency examination is necessary.

AUTOMATED VS. MANUAL BONE AGE ASSESSMENT: COMPARATIVE STUDY IN CHILDREN AND ADOLESCENTS WITH ENDOCRINE DISORDERS

Klára Maratová, Daniela Zemková, Jan Lebl, Onřej Soucek, Štěpánka Průhová, Stanislava Koloušková, Marta Šnajderová, Hana Krásničanová, Zdeněk Šumník

Department of Pediatrics Second Faculty of Medicine and Motol University Hospital, Prague, Czech Republic

Background: Skeletal maturation is the most reliable indicator of biological age in children and adolescents. The evaluation of hand and wrist X-Ray according to Tanner-Whitehouse (TW3) or Greulich-Pyle (GP) are the most commonly used methods for biological age assessment. Automated bone age assessment has recently become increasingly popular, however a large independent study comparing automated and manual evaluation of bone age is still missing. The aim of this study was to assess the differences between automated and manual evaluation of bone age using TW3 and GP method.

Methods: In this cross-sectional study we evaluated bone age scans using TW3 and GP methods in 1285 children and adolescents (659 boys, range 5.0 - 15.9 years, median 10.3, IQR 4.9 years) with various endocrine conditions in parallel manually and using BoneXpert software (Visiana, Holte, Denmark). Root mean square errors (RMSE) were calculated for the whole group and for sex-specific one-year age categories (girls between 5 and 15 years, boys between 5 and 16 years, over 50 children in each category).

Results: In total RMSE were 0.61 years and 0.58 years in boys and 0.79 years and 0.60 years in girls, respectively for TW3 and GP. Sex- and age-specific analysis showed the greatest differences between manual and automated TW3 evaluation in girls between 6 - 7, 12 - 13 and 13 - 14 years with RMSE 0.90, 0.90 and 1.05 years, respectively. Manual and automated evaluation differed by more than 1 year in 9.7 % and 7.0 % boys and 18.2 % and 8.6 % girls, respectively for TW3 and GP.

Conclusion: Automated bone age assessment provides sufficient agreement with manual evaluation in most scans of children with common endocrine disorders. Bone age assessment provided by BoneXpert tends to be underestimated, especially in girls during puberty using TW3 method. Further analysis is required to identify the source of these differences.

BACLOFEN PUMP IMPLANTATION AND SELECTIVE DORSAL RHIZOTOMY AS SURGICAL TREATMENT MODALITIES IN PEDIATRIC SPASTIC CEREBRAL PALSY

Michal Petrík¹, Dana Kuniaková¹, Zuzana Humpolcová², František Horn¹

¹ Department of paediatric surgery, National Institute of Children's Diseases, Limbova 1, Bratislava, 83340, Slovak republic, EU

² Department of Pediatric Neurology, National Institute of Children's Diseases, Limbova 1, Bratislava, 83340, Slovak republic, EU

Background: Functional neurosurgical approaches offer a number of possibilities to reduce spasticity in children with cerebral palsy, resulting in improvement of quality of life of children and their families. Spasticity in children's cerebral palsy occurs due to lesions of the central nervous system. Surgical procedures that affect central nervous system regulation after upper motor neuron lesion include the implantation of a programmable pump for intrathecal baclofen therapy and the disruption of pathological pathway activation by selective dorsal rhizotomy.

Methods: At Department of Pediatric Surgery at the National Institute of Children's Diseases in Bratislava, we diagnosed and treated 47 children with spasticity. Patient selection and surgical procedures were performed between 2012 - 2017. First baclofen test (bolus administration of 50 mcg of Baclofen) was performed in all 47 children. Of the total, a programmable pump was implanted in 19 children, and 10 children underwent selective dorsal rhizotomy. We evaluated spasticity according to the Modified Ashworth Scale prior the test, after the test and after the surgery.

Results: Significant drop ($p < 0.001$) in Modified Ashworth Scale was recognized in all candidates. A programmable pump was indicated in cases with quadruple spastic cerebral palsy. In most cases with lower limb diparesis, selective dorsal rhizotomy led to gait improvement.

Conclusion: In well-indicated intervention, muscle contraction, spasms, dystonia and desorganised gait patterns in patients with spasticity can be avoided. Surgical therapy and diagnosis of spasticity require a multidisciplinary approach. We recommend intrathecal application of baclofen using a programmable pump and selective dorsal rhizotomy as a safe and effective choice for the treatment of spasticity in carefully selected cases.

INFLUENCE OF HIGH FAT DIET AND TREADMILL TRAINING ON BONE METABOLISM IN EXPERIMENTAL MODEL OF FEMALE RATS WITH AND WITHOUT OVARECTOMY

Katarína Krivošíková¹, Zuzana Krivošíková², Martin Štelzer³, Martin Gajdoš², Joanna Folwarczna⁴, Aleksandra Janas⁴, Maria Pytlik⁴, Juraj Payer⁵

¹ Department of Paediatrics, Faculty of Medicine at Comenius University and National Institute of Children's Diseases, Bratislava

² Department of clinical and experimental pharmacotherapy, Slovak Medical University, Bratislava

³ Department of Prion Diseases, Faculty of Medicine at Slovak Medical University, Bratislava

⁴ Śląski University of Medicine, Katowice

⁵ 5. Department of Internal Medicine, Faculty of Medicine at Comenius University and University Hospital Bratislava

Background: Traditionally, it was believed that osteoporosis is actually prevented by obesity. Lately, the inverse relationship between fat and osteoporosis was found. It is now assumed that obesity is one of the most important risk factors for osteoporosis. On the contrary, regular exercise is known to induce beneficial effects on bone. More and more children are, nowadays, suffering from overweight and obesity all over the world. Therefore, already in childhood, conditions are created for onset of osteoporosis.

Methods: Forty-eight female rats were divided into two groups and fed with standard (n=16, SD) or high fat diet (n=32, HFD) for 8 weeks. 8 rats from each group underwent ovariectomy and sham surgery was performed on the remaining rats. Feeding continued for another 10 weeks and 8 rats with HFD from sham and ovariectomized groups regularly exercised (1h, 20m/min, 5d/week). Data obtained from densitometry measurements and bone biomechanical characteristics tested by three point bending were analyzed.

Results: The bone area was significantly increased in both adolescents and adults under the influence of high fat diet, with an increase in mineral content sufficient to maintain bone density in childhood but not sufficient in adulthood, resulting in a significant reduction in bone density. The decrease in bone density was accompanied by increased bone fragility with acceleration after ovariectomy. Physical activity had a slight beneficial effect on bone.

Conclusion: Obesity has a positive impact on bone density in childhood, but it is unknown what effect it has on bone quality. In adulthood, obesity has a negative effect on the quantity and quality of bone, and this condition is even worse in menopause. The exercise has a partially positive effect.

This experiment was prepared by the frame work of realization of the project "Center of excellence of environmental health", ITMS No.26240120033, based on the supporting Operational Research and Development Program financed from the European Regional Development Fund.

DIABETES MELLITUS IN INFANTS AND TODDLERS

Kristína Podoláková, Ľubomír Barák, Ľudmila Podracká

Department of Paediatrics, Faculty of Medicine at Comenius University and National Institute of Children's Diseases, Bratislava

Background: Diabetes mellitus in children under 3 years of age is not more a rare disease. Over the last 10 years, the age of manifestation has shifted from puberty and adolescence to lower age categories. Alarming demographic data demonstrate the most intensive increase in the incidence of diabetes in children under 5 years of age.

Methods: In the Children's Diabetes Center of the Slovak Republic, we monitor 90 children with manifestation of the diabetes during infancy. Retrospectively, we evaluated history of the patient, laboratory parameters at the time of manifestation, clinical findings, and period from onset to the end of postinitial remission.

Results: The age groups (0 - 1 year $n = 4$, 1 - 2 years $n = 36$, 2 - 3 years $n = 51$) were different in the presence and degree of diabetic ketoacidosis (age 0 - 1 year 100 %, age 1 - 2 years 69 % and age of 2 to 3 years 67 %), but not in length of history, mean glycemia or HbA1c, baseline C peptide, or autoantibody positivity. Significantly lower probability of the onset of the postinitial remission were patients with manifestation of the diabetes up to 2 years of age ($p = 0.004982$) and children with diabetic ketoacidosis ($\text{pH} < 7.3$ or $\text{HCO}_3^- < 15 \text{ mmol/l}$) $p = 0.0013$. In the case of remission, its duration did not correlate with any of the monitored parameters. Postinitial remission was achieved by 52.2 % of the patients. The mean time to the onset of the remission was 9.3 ± 6.2 days and the mean duration was 17 ± 12.4 months. The complete postinitial remission was achieved by 5.6 % of patients, with a significantly lower HbA1c at the beginning of the diabetes.

Conclusion: The frequency of DKA in children with newly diagnosed type 1 diabetes in Slovakia is still high and children less than 3 years of age have a high risk of DKA at onset. In order to reduce the rate of onset DKA some nationwide campaign are needed.

THE FEASIBILITY OF ECG SCREENING IN CHILDHOOD: A POPULATION STUDY

Viktória Vincze¹, Cs. Papp², G. Veres¹, G. Mogyorósy¹

¹ *Department of Pediatrics, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

² *Miskolc Institute for Health Development, Miskolc, Hungary*

Background: ECG screening decreased the frequency of sudden cardiac death among young athletes. However, there is little experience with general ECG screening for children and adolescents. Telemedicine ECG systems may improve the access and reduce the costs of population ECG screening.

Methods: Between October 2016 and December 2017, 12-lead ECG was made in 16 816 school children and adolescents attending schools in Miskolc, Hungary. The age of schoolchildren was between 7 and 21 (median 14 years). The ECG-s were recorded with a Heartview Transtelephonic ECG equipment. A pediatric cardiologist at the Pediatric Institute of the University of Debrecen

conducted their evaluation. The findings were received by the school nurses electronically and delivered to the children's parents.

Results: The ECG screening at 16 816 schoolchildren proved abnormality in 270 cases (1.6 %) that justified further evaluation. The most common differences were the right ventricular hypertrophy (122 cases), ventricular extrasystoles (34 cases), abnormal repolarization (28 cases) and preexcitation (18 cases). In one case a known hypertrophic cardiomyopathy has been identified. An uninterpretable curve was obtained 3 times (0.2 ‰).

Conclusion: The applied telemedicine ECG screening was easy to perform. In only a few cases, the curve was unacceptable. We found abnormal ECG of 1.6% of schoolchildren, which justified further investigation.

CHANGES IN SHORT-TERM BLOOD PRESSURE CONTROL AFTER MONTHLY SPA STAY IN CHILDREN WITH BRONCHIAL ASTHMA

Pavel Tinka¹, Tara Líšková¹, Jana Svačinová¹, Ksenia Budinskaya¹, Vojtěch Svízela¹, Jana Hrušková¹, Jana Rýdlová², Hana Hrstková³, Zuzana Nováková¹

¹ Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

² Children's Medical Institution Miramonti, Spa Luhačovice, Czech Republic

³ Department of Pediatric Oncology, Faculty Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Background: Monitoring of blood pressure is important in management of many clinical diseases. In our study we focused on differences in short-term blood pressure regulation through the baroreflex before and after one month long therapeutic stay in Spa Luhačovice in Czech Republic.

Methods: Our targeted group consisted of children and adolescents of age from 8 to 18 years with diagnosis of asthma bronchiale. We examined 110 persons (60 girls). Average age was 13.71±2.13 years. Blood pressure was measured by continuous fingertip photoplethysmographic method, Finometr Pro (Netherland). Protocol consisted of measuring during calm breathing, sitting and after standing up. This measurement was done at the beginning and after one month of spa intervention. We were interested in parameters like: inter-beats intervals (ms), systolic and diastolic pressure (mmHg), baroreflex sensitivity (ms/mmHg) and their changes after verticalization. Baroreflex sensitivity was calculated from cross spectral analysis of relationship between changes in systolic blood pressure and inter-beat intervals. Comparison of results acquired before and after spa was taken by Wilcoxon nonparametric paired statistical test.

Results: The median of the inter-beat intervals after spa intervention became significantly longer. At the beginning in the sitting it was 642.82 ms (bottom quartile: 603.15 ms; upper quartile: 744.33 ms) vs one-month in spa: 685.86 ms (644.71; 745.86 ms) (p=0.0002); after verticalization it was increased from 576.96 ms (530.26; 615.75 ms) to 594.74 ms (558.58; 654.36 ms) (p=0.0006). The median of baroreflex sensitivity in the sitting according to Wilcoxon test increased non-significantly (p=0.16) from 6.55 ms/mmHg (4.86; 9.68 ms/mmHg) vs 7.29 ms/mmHg (6.05; 9.31 ms/mmHg) at the end of spa intervention. The median of decrease of systolic blood pressure after verticalization was bigger

at the beginning 4.50 mmHg (-1.79; 9.57 mmHg) than at the end of spa accommodation 1.39 mmHg (-3.67; 9.37 mmHg; $p=0.01$). The median of increase of diastolic blood pressure after verticalization was at the beginning smaller 0.37 mmHg

(-3.84; 5.79 mmHg) than at the end 3.01 mmHg (-2.49; 7.57 mmHg; $p=0.002$).

Conclusion: We found positive influence of one-month long spa stay to short-term blood pressure regulation in children with bronchial asthma.

Supported by: Research study for Union of Czech Spa; by projects: MUNI/A/1355/2016 and MUNI/A/1157/2017.

MALNUTRITION AS A RISK FACTOR TO IMPAIRED VACCINE RESPONSE IN PAEDIATRIC INFLAMMATORY BOWEL DISEASE

Kriszta Katinka Boros¹, Katalin Müller¹, Áron Cseh¹, Antal Dezsőfi¹, András Arató¹, Gábor Veres²

¹ 1st Department of Paediatrics, Semmelweis University, Hungary

² Paediatrics Institute, University of Debrecen, Hungary

Introduction: Patients with inflammatory bowel disease (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC) may have a higher risk for infections due to underlying disease, malnutrition or immunosuppressive therapy. The fat free mass (FFMI) shows the metabolically active part of the body, and therefore it seems to be a better marker for estimating the nutritional status. Aims: The aim of our study was to investigate the responsiveness of hepatitis B vaccinations depending on the patients nutritional status. According to our knowledge there is no other study analysing FFMI to specify the nutritional status by estimating the level of malnutrition

Method: Weight, Body Mass Index (BMI) and FFMI values were investigated to estimate the nutritional status of our patients. FFM was measured using bioimpedance. Patients FFMI values were compared with age and sex paired controls (C). To check the response to the previously administered hepatitis B vaccine anti-HBs titers were determined. A level >100 IU/l was accept as seroprotective.

Results: Our study involved 25 IBD patients (CD: 16, male: 14, mean age: 14.72, range: 12.11-18.78). Only 8 patients had a seroprotective anti-HBs titers (responder group, R, n=8). 13 patients did not have a seroprotective anti-HBs titer (non-responder group NR, n=13) following immunisation. However, weight (R: -0.71; NR: -1.13), and BMI (R: -0.99, NR: -1.24) z-scores were in normal range. In addition, FFMI was lower in both R and NR group compared to C (R vs. C: $p<0.005$, NR vs. C: $p<0.0005$). Related to nutritional status, there was no difference between the R and NR group.

Conclusion: Interestingly, in our study the nutritional status was not a significant factor in the anti-Hbs seroprotection level. It seems that immunosuppression in paediatric IBD patients did not have a significant effect on malnutrition status.

INGUINAL HERNIA IN ADOLESCENTS: IS THERE AN IDEAL WAY OF TREATMENT?

Edit Kecskés¹, Katalin Hodosi², László Sasi-Szabó³

¹ University of Debrecen, Faculty of Medicine

² University of Debrecen, Department of Internal Medicine

³ University of Debrecen, Institute of Pediatrics, Department of Pediatric Surgery

Background: The technique of the inguinal hernia repair is different in pediatric and general surgery. While in pediatric surgery the only goal of the operation is high ligation of the hernia sac, traditional (adult-type) hernia repair also includes strengthening of the posterior wall of the inguinal canal as well either through an inguinal or laparoscopic approach. Surgical attendance of the adolescent age group is different in the countries; in many places both general and pediatric surgeons treat these children.

Objective: The purpose of this study was to identify which way of treatment can provide the best result in inguinal hernia repair in adolescents.

Patients and methods: We analyzed the data of all the adolescent patients (14 - 18 years) who underwent inguinal hernia surgery at the Pediatric Surgery Department and at the Institute of Surgery in our University between 2001 - 2017, retrospectively. Patients were classified into 3 groups according to the type of surgery: high-ligation only, adult-type inguinal interventions (Bassini, Lichtenstein) and TAPP (transabdominal preperitoneal). We analyzed the mean operative time, the perioperative complications, the rate of recurrence after each surgical procedure and the role of the operating institution. In addition, we have sent general and hernia-specific quality of life surveys to the patients via email as well.

Results: In the study period 81 adolescent patients were identified who underwent inguinal hernia repair (16 patients in the Institute of Surgery, and 65 patients in Department of Pediatric Surgery). There were 11 TAPP operations, in 33 patients adult-type repair was performed and 37 patients received high ligation of the hernia sac only. The length of the surgeries were significantly longer in the TAPP group (high ligation only: 34.28 ± 20.97 , TAPP: 66.00 ± 16.36 , adult-type inguinal interventions: 40.76 ± 9.93). There were no perioperative complications, and during an average follow-up of 6,77 years we did not find any recurrence. To the questionnaires we received 26 valid replies (32 %). The hernia-specific questionnaires showed the best results in the TAPP group although the result was not significant. The general quality of life was better in the high-ligation group especially regarding physical functions and post-op pain episodes. Those patients who were operated in the Department of Pediatric Surgery scored better on both tests irrespectively of the type of surgery they have received.

Conclusion: Our data are not sufficient to clearly identify the best technique for inguinal hernia repair in adolescents but we do recommend to perform them in pediatric surgical units.

APPRAISAL OF THE ASSOCIATION BETWEEN INFLAMMATORY BOWEL DISEASES AND ACUTE PANCREATITIS – A META-ANALYSIS

Bálint Tél¹, Bence Stubnya¹, Zoltán Kiss¹, Noémi Gede², Péter Hegyi², Gábor Veres³

¹ 1st Department of Paediatrics, Semmelweis University, Budapest, Hungary

² Institute for Translational Medicine, University of Pécs, Pécs, Hungary

³ Paediatric Institute, University of Debrecen, Debrecen, Hungary

Background: Inflammatory bowel diseases (IBD) can be associated with several extraintestinal manifestations, such as primary sclerosing cholangitis, or acute pancreatitis (AP). Severe forms of acute pancreatitis are potentially life-threatening events, and even the milder cases can lead to recurrent acute pancreatitis, chronic pancreatitis and exocrine pancreatic insufficiency. There was no previous study performed on the chances of AP in IBD, nor on the etiology of the association between the two disorders. Therefore, our aim was to explore and comprehensively appraise the currently available body of evidence by means of a meta-analysis.

Methods: We performed a database search on the PubMed/MEDLINE, EMBASE, Cochrane Library, SCOPUS and Web of Science online databases, with filters applied for 'English' and 'Human', from database inception to October 30th 2017. Case-control studies reporting odds ratios and other clinical studies reporting the occurrence rates or incidences of AP in any IBD group were included. Two reviewers independently assessed, screened and extracted the data of interest from the records. The primary outcomes were occurrence rates or odds ratios of AP in IBD. Results were synthesized and pooled for meta-analysis using random effects model.

Results: After screening 2679 entries from the database search, a total of 29 studies were included in this meta-analysis. Six of the 29 publications were case-control studies (including 120 393 patients), assessing the proportion of comorbid IBD within the group of patients with their first AP episode. The other 23 studies reported the events of AP among IBD patients. AP was associated with an increased chance for comorbid IBD (OR = 2.13 [CI: 1.96-2.32], $p < 0.00001$). Also, an AP episode was seen in approximately 1% of the IBD population. (event rate: 1.4 [CI: 1.1-2.0%; $p < 0.0001$]). Only two case control studies appraised the odds of AP in IBD, which was not enough for pooling. A subgroup analysis of the cohort studies is currently in progress.

Conclusion: Based on our study, AP is more frequent in IBD, but due to the low number of studies focusing on this specific population, pooled odds ratios could not be established. Nevertheless, prospective, multi-centric studies on the association of AP and IBD are clearly needed.

LONG TERM FOLLOW-UP ON BODY COMPOSITION, PHYSICAL ACTIVITY, QUALITY OF LIFE IN PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE (IBD)

Dóra Dohos¹, Zsófia Zsirai¹, Katalin Müller¹, Kriszta Boros¹, Gabor Veres²

¹ 1st Dept. of Pediatrics, Semmelweis University, Budapest

² Pediatric Institute, University of Debrecen, Debrecen

Background: The incidence of pediatric inflammatory bowel disease (IBD) is increasing. Multifactorial reasons, including inflammation effect on malnutrition, impaired growth and altered body composition (BC) lead to impaired quality of life (QoL) and physical activity (PA). Those are important for normal growing and bone strength, however, there is no prospective, well-designed study in the literature. Our aim was to characterise and follow-up on disease activity index, body composition (BMI, fat mass, fat free mass), PA and QoL in pediatric patients with IBD.

Methods: Patients were divided into four groups: children with newly diagnosed Crohn's disease (ndCD, n=11); newly diagnosed ulcerative colitis (ndUC, n=12); responder to biologicals (rBT, n=6) and non-responder to biologicals (nrBT, n=6). BC, PA and QoL were measured three times in a 6-month period. Children's data from the control group (C) was chosen by age and sex. Mann-Whitney U- and Wilcoxon tests were used for statistics.

Results: Values of fat free mass index (FFMI) were increased in the ndCD ($p<0,0005$), ndUC ($p<0,05$) and nrBT ($p<0,05$) groups compared to C. PA increased in the ndCD ($p<0,005$) group during the study period. PA in the ndUC ($p<0,0005$) and nrBT ($p<0,05$) groups was lower at the beginning and at the end of the study period compared to C's ($p<0,05$). QoL improved in ndCD, ndUC and rBT groups.

Conclusion: Increasing values of FFMI and BMI indicate improved nutritional status of our patients. In addition, in the 6 months of follow-up, parallel recovered QoL may show the success of the therapy.

CHARACTERISTICS OF THE MICROBIOME UNDER BIOLOGICAL THERAPY IN PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Zoltán Kiss^{1,3}, Nóra Makra², János Juhász⁴, Bálint Tél¹, Kriszta Katinka Boros¹, Attila J. Szabó¹, Dóra Szabó², Gábor Veres^{1,5}

¹ 1st Department of Pediatrics, Semmelweis University, Budapest, Hungary

² Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary

³ MTA-SE Pediatrics and Nephrology Research Group, Budapest, Hungary

⁴ Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Hungary

⁵ Institute of Pediatrics, University of Debrecen, Hungary

Background: Inflammatory bowel disease (IBD) is an idiopathic gastrointestinal disease caused by a dysregulated immune response to host intestinal microflora. The changes in the gut microbiome in pediatric patients receiving biological therapy is not characterized. We aimed to establish a stool sample bank for investigating the alterations of the gut microbiome under biological therapies

in pediatric patients with IBD. In the first phase of the study we characterized the diversity and taxonomic differences between different treatment regimens and healthy controls in order to investigate if there is a shift towards eubiosis in the therapy responder patients who achieved remission.

Methods: The stool samples were collected in an inpatient setting, aliquoted in native form and stored frozen on -80 C. Stool samples were enrolled in the study in four groups: control patients, therapy-naïve Crohn's disease (CD) patients, CD patients in remission under conventional therapies, and under biological therapy. Sequencing of the V4-V5 spanning 16S RNA gene was carried out on Illumina MiSeq platform. Bioinformatic analysis and visualisation of the results were conducted using the Qiime2 and Qiime2 view softwares.

Results: Analysis of results showed significant difference in the taxonomic composition, species richness of control patients and CD patients under different therapeutic interventions. The most differently abundant taxa were prevotellaceae, bacteroidaceae, and lachospiraceae. The most significant difference was seen in the abundance of the prevotella genus, which was almost completely missing from the samples of CD patients.

Conclusion: Our measurements on limited sample size suggests that, microbiome of conventional and biological therapy responder pediatric patients is disbiotic, and do not differ significantly from each other. This suggests, that biological therapy does not effectively facilitate the shift of the microbiome to eubiosis. Further analysis on extended sample number are needed to corroborate the initial findings of the study.

Supported by ÚNKP-17- 3-III New National Excellence Program of the Ministry of Human Capacities.

NOVEL INSIGHTS INTO THE PATHOPHYSIOLOGY OF GROWTH RETARDATION AND OTHER ENDOCRINE CONDITIONS. OUTLINE OF AN UPCOMING RESEARCH PROJECT ANALYSING CONSANGUINEOUS FAMILIES FROM THE NORTH- EAST OF IRAQ

Shenali Amaratunga¹, Tara Hussein Tayeb², Jan Lebl¹, Štěpánka Průhová¹

¹ Department of Pediatrics, 2nd Faculty of Medicine, Charles University in Prague

² Department of Pediatrics, Sulaymani University, College of Medicine

Background: Many paediatric endocrine conditions, including severe short stature, result from disturbed molecular mechanisms that are only partly elucidated thus far. For example, normal growth requires adequate nutrition, physiological balance of the immune system, normal hormonal signalling (GH – IGF-I system), glucocorticoids, thyroid hormones, sex hormones and normal local signalling within the epiphyseal growth plate as well. Recent studies show that multiple other factors regulate chondrogenesis in the growth plate. These factors include paracrine signalling, extracellular matrix formation and integrity of fundamental cellular processes. When pathological states such as short stature occur, there could be a disturbed mechanism in any of the above-mentioned factors that govern growth. Consanguinity within the families clearly suggests that a single-molecule mechanism is the pathophysiological mechanism of the individual conditions.

Therefore, consanguineous families provide the best chances to successfully encounter novel genes which have a close link to metabolic and/or endocrine conditions. As consanguinity in Europe is rare, we used this unique opportunity to collaborate with the Sulaymani University in Iraq to gather a cohort of children with endocrine conditions born within consanguineous families.

Methods: The cohort currently comprises of thirty-nine consanguineous families who have one or more children with endocrine disorders ranging between the ages of three months to eighteen years. Among this group, there are currently twenty-five families with children who have growth retardation (out of these, seventeen have been clinically classified as idiopathic short stature, eight as growth hormone deficiency - two of which have dysmorphic features as well). Fourteen consanguineous families have children with other endocrine conditions such as hypogonadism, adrenal diseases and beta-cell disorders.

Tara Hussein Tayeb personally conducted clinical investigations and endocrine testing in affected children and established a clinical diagnosis. The genetic analysis of data obtained will be carried out by whole exome sequencing. This will be followed by studying the functions of a mutated protein on a molecular basis and its cellular functions on cell cultures which will facilitate understanding of protein function and its' link to human disease.

Expected results and Conclusion: The proposed study will strive to prove the hypothesis that, in consanguineous families with apparent phenotypes, it may be possible to successfully elucidate novel mechanisms of growth retardation at the level of the chondrocyte and, in parallel, novel mechanisms in some other endocrine conditions as well. If successful, identifying new genetic causes and pathophysiological mechanisms has the potential to improve diagnosis, personalised management and prognosis.

OUR FIRST CASE OF HUTCHINSON-GILFORD PROGERIA SYNDROME (HGPS) DUE TO A PATHOGENIC LMNA VARIANT C.433G>A (P.GLU145LYS)

Ledjona Toni¹, Petra Dušátková¹, Dana Novotná², Dana Zemková¹, Štěpánka Průhová¹, Jan Lebl¹

¹ Department of Paediatrics, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

² Department of Paediatrics, Faculty of Medicine, Masaryk University and University Hospital, Brno, Czech Republic

Background: Hutchinson-Gilford Progeria Syndrome (HGPS) is an extremely rare condition (estimated incidence 1:4-8 million), caused by mutations in LMNA gene, which leads to premature aging. The most common mutation found is c.1824C>T (p.Gly608=). Median life expectancy is shortened to 13 years due to vascular complications such as stroke or myocardial infarction. We present below the history of a child born with another pathogenic LMNA variant who has been described so far in one single patient.

Methods: Clinical, auxological, biochemical and genetic testing in a patient with suspected HGPS syndrome.

Results: We identified a pathogenic variant of LMNA gene c.433G>A (p.Glu145Lys), which is known to disable lamins to form dimers and higher structures in a male patient who was referred to our clinic for failure to thrive by the age of 18 months. His phenotype became suggestive of progeria when 2.5 years old, with partial hair loss, visible veins on his forehead, pinched nose and small recessed jaw. His first ischemic complication manifested via transient hemiparesis at age 4.2 years. When 7 years old, he presented with seizures and unconsciousness due to a massive haemorrhagic stroke which led to a fatal outcome.

Conclusion: This is a case report of our first child with progeria. Unfortunately no therapy is available for patients born with a pathogenic variant LMNA c.433G>A (p.Glu145Lys).

DETERMINATION OF SOME BIOLOGICAL MARKERS OF OBESITY IN CHILDREN WITH CARDIOVASCULAR RISK

Martina Medved¹, Laura Ojsteršek¹, Nataša Marčun Varda^{1,2}

¹ Faculty of Medicine, University of Maribor, Taborska 8, 2000 Maribor, Slovenia

² Department of Paediatrics, University Medical Centre Maribor, Ljubljanska 5, 2000 Maribor, Slovenia

Background: The aim of our study was to evaluate some biological markers of obesity, namely leptin, ghrelin and adiponectin, as potential early cardiovascular risk markers in particular groups of children with cardiovascular risk and correlate them with body mass index (BMI), a measure of obesity.

Methods: 337 children, adolescents and young adults, aged between 8 months and 22 years, who were treated at the Department of Paediatrics, University Medical Centre Maribor, were included in the study. They were divided into four groups: obese children with hypertension, normal weight children with hypertension, children with hyperlipidaemia and a control group of healthy children. Some clinical and biochemical parameters as well as biological markers of obesity, leptin, ghrelin and adiponectin, have been measured according to standard procedures.

Results: All three markers of obesity correlated with BMI, for ghrelin and adiponectin the correlation was negative and for leptin positive. In the obese hypertensive patients, in comparison to controls, all three markers were significantly different, in the group of children with hyperlipidaemia the difference in both leptin and ghrelin was found, and in normal weight hypertensive patients only the difference in ghrelin was noted.

Conclusion: In research groups, significant differences in investigated biological markers of obesity have been found indicating that they might be useful markers for identifying groups of patients that are at cardiovascular risk, with ghrelin being the most promising. In addition, significant correlation of all of them with BMI was found.

EXAMINATION OF CELLULAR PROCOAGULANT FUNCTION BY THROMBIN GENERATION TEST

Renáta Hudák^{1,2}, Ildikó Beke Debreceni², Gabriella Gál Szabó², Zsuzsanna Hevessy², Péter Antal-Szalmás², János Kappelmayer²

¹ Department of Pediatrics, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

² Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Background: Hemostasis screening tests are capable for screening for most coagulation factor deficiencies. However, these assays are unsuitable for detecting an inappropriately enhanced clotting. This can only be achieved by looking at the central serine protease of the coagulation system, the thrombin. In acute myeloid leukemias there is an increased chance to develop thrombotic disorders. I provide an example where thrombin generation test (TGT) was used to determine the procoagulant function of cell lines. We hypothesized, that in addition to leukemic promyelocytes, monocytic leukemia cells may also have a higher procoagulant activity.

Methods: Fibrin formation was assessed by a one-stage clotting assay using a magnetic coagulometer. The TGT of magnetically isolated normal human monocytes (negative control), intact leukemic cells and their isolated microparticles was performed by a fluorimetric assay. Phosphatidylserine (PS)-expression of leukemic cells and microparticle number determinations were carried out by flow cytometry.

Results: All cell lines displayed a significant procoagulant potential compared to isolated normal human monocytes. In the TGT test, the mean of Lagtime and Time to Peak parameters were significantly shorter in leukemic cells (3.9-4.7 and 9.9-10.3 minutes) compared to monocytes (14.9 and 26.5 minutes). The mean of Peak thrombin in various monocytic leukemia cell lines was 112.1-132.9 nM versus 75.1 nM in monocytes, however, no significant difference was observed in the ETP parameter. Factor VII deficient plasma abolished all procoagulant activity, while factor XII deficient plasma did not affect the speed of fibrin formation and thrombin generation, but modulated the amount of thrombin. Factor XI deficient plasma affected the Time to Peak values in one leukemic cell line and also attenuated peak thrombin. Leukemia cell derived microparticles from all three cell lines exerted a procoagulant effect by significantly shortening the Lagtime in TGT, there was a non-significant difference in case of ETP parameter.

Conclusions: All investigated monocytic leukemia cell lines exhibited significant thrombin generation. This phenomenon was achieved by the procoagulants on the surface of leukemic cells as well as by their microparticles.

CLINICAL AND BIOLOGICAL IMPACT OF FACTOR XIII A EXPRESSION IN LEUKEMIC LYMPHOBLASTS

Katalin Gyurina¹, Bettina Kárai², István Szegedi¹, Zsuzsanna Hevessy², Beáta Scholtz³, János Kappelmayer², Csongor Kiss¹

¹ Department of Pediatric Hematology-Oncology, Institute of Pediatrics, University of Debrecen, Hungary,

² Department of Laboratory Medicine, University of Debrecen, Hungary,

³ Center of Clinical Genomics, Institute of Biochemistry and Molecular Biology, University of Debrecen, Hungary

Background: Using multiparameter flow cytometry (FC) leukemic B-cell progenitor (BCP) lymphoblasts were recently identified as a novel expression site of coagulation factor XIII subunit A (Kiss F. et al. Thomb Hemost, 2006.). Retrospectively we identified that event free survival (EFS) and overall survival (OS) were significantly better in patients with FXIII A-positive acute lymphoblastic leukemia (ALL) than in patients with FXIII A-negative ALL. There was a significant correlation between the FXIII A-negative group and the B-others genetic subgroup (Kárai B. et al. Pathol Oncol Res. 2018).

Methods: We analyzed gene expression data, obtained from Affymetrix Human Genome U133A Array, for 405 samples were retrieved from public Gene Expression Omnibus (GEO) repository (series GSE13351, GSE13425 and GSE47051). Gene expression profiles of children with ALL were analyzed using GeneSpring v12.6 software and Ingenuity Upstream Analysis software.

Results: Investigating the GEP databases, patients were separated into two groups: one expressing *F13a1* gene with high intensity and the second one expressing *F13a1* with at least 2 logs less intensity than the high intensity group (low intensity group). Low *F13a1* expression level was prevalent among "B-other" samples, high *F13a1* expression level was associated with t(1;19) genetic subgroup of childhood ALL. We found 11 genes that were characteristically downregulated in the low *F13a1* expression group in each of the three databases, and 59 dysregulated genes according to two of the three databases. According to pathway analysis, we identified a network of genes (IL7R, RAG1/2, DNTT, NHC II) participating in B cell development that were downregulated in the *F13a1* low intensity group. We identified two chromosome loci, 19p13.3 and 16q22 where 11 and 2 genes were dysregulated within the low *F13a1* expression group. Using ingenuity upstream analysis software, we predicted genetic changes in 3 genes, NUPR1, TCF3 and IKZF1 in association with low intensity *F13a1* expression. The NUPR1 upstream regulator may correspond chemoresistance. TCF3 is involved in several chromosomal translocations including t(1;19) with PBX1 as partner, t(19;19) with TFPT as partner and t(12;19), with ZNF384 as partner. We identified 5 genes (EBF1, IKZF1, TCF3, LEF1 and RAG1) which correlate with the B-other genetic subgroup.

Conclusion: FXIII A expression may define a new subgroup of childhood ALL, with a partial overlap between FXIII A-negative and the recently defined BCR-ABL1-like groups. FXIII A expression by FC may help to select those cases which require a more sophisticated genetic diagnosis so as to determine the exact prognosis for applying the optimal risk-tailored therapy. In addition, FXIII A may become a useful marker for Day 15 MRD detection.

Acknowledgements: This study was supported by an OTKA K-108885 grant. Assistance of Giuseppe Basso, Silvia Bressolin and Geertruy te Kronnie from Department of Women's and Children's Health, University of Padova, Italy is appreciated.

ROLE OF MICRORNAS AND HISTONE DEACETYLASE ENZYMES IN THE PATHOGENESIS OF ADULT HEMATOLOGICAL MALIGNANCIES

Zsuzsanna Gaál^{1, 2}, László Rejtő³, Ferenc Erdődi⁴, Bálint László Bálint⁵, Éva Oláh¹, Gábor Veres¹, László Csernoch²

¹ Institute of Pediatrics, University of Debrecen

² Department of Physiology, University of Debrecen

³ Institute of Internal Medicine, University of Debrecen

⁴ Department of Medical Chemistry, University of Debrecen

⁵ Department of Biochemistry and Molecular Biology, University of Debrecen

Changes in the DNA methylation pattern, the histone code, the expression levels of microRNAs and characteristic metabolic alterations referred to as the Warburg effect are all important etiologic factors of the pathogenesis of hematological malignant diseases.

Expression levels of microRNAs and histone deacetylase enzymes were examined by Western blot analysis and reverse transcription with quantitative polymerase chain reaction in the bone marrow specimens of adult patients (n=45) with malignant hematological diseases, principally acute myeloid leukemia. Our major aims were to elucidate the role of miR-378*, miR-23b, miR-26a, SIRT6 and HDAC4 in the pathogenesis of the disease, and to investigate the relationship between the expression levels of NAD⁺-dependent and NAD⁺-independent histone deacetylase enzymes as well.

We observed strong positive correlation between the expression levels of SIRT6 and HDAC4 proteins. The expression levels of Warburg effect related miR-378*, miR-23b and miR-26a positively correlated with each other, with the level of miR-125b and the oncogenic miR-155, while negative correlation was found with the expression level of the tumorsuppressor miR-124. Positive correlation was detected between the expression levels of SIRT6 mRNA and miR-26a, moreover, the level of HDAC4 mRNA also showed positive correlation with the expression of miR-378*, miR-23b, miR-26a, miR-125b and miR-155. The expression level of both SIRT6 and HDAC4 mRNA negatively correlated with the level of miR-124. Comparison of the gene expression levels between the different subgroups of AML revealed statistically significant differences. The expression levels of some genes also showed statistically significant correlation with the white blood cell count determined at the time of the diagnosis.

These results indicate the synergistic pathogenetic role of NAD⁺-dependent and NAD⁺-independent histone deacetylase enzymes, confirm the role of the Warburg effect related miR-378*, miR-23b and miR-26a in the pathogenesis of hematological malignant diseases, and imply the potential oncogenic property of SIRT6 and HDAC4.

Epigenetic and metabolic changes are reversible etiologic factors of leukemia, developing in early stages of the disease, therefore the comprehensive evaluation of genetic, epigenetic and metabolic alterations may lead to more complex prognostic stratification and contribute to personalized treatment in a significant manner, leading to improved therapeutic results.

ALTERATIONS IN BONE MINERAL METABOLISM IN SURVIVORS OF CHILDHOOD CANCER

Alžbeta Lencséssová¹, Ľubica Tichá¹, Daniela Sejnová², Zdenko Killinger³, Juraj Payer³, Ľudmila Podracká¹

¹ Department of Paediatrics, Faculty of Medicine at Comenius University and the National Institute of Children's Diseases, Bratislava, Slovakia

² Department of Paediatric Haematology and Oncology, Faculty of Medicine at Comenius University and the National Institute of Children's Diseases, Bratislava, Slovakia

³ 5th Department of Internal Medicine, Faculty of Medicine at Comenius University Bratislava, Slovakia

Background: The number of survivors of childhood cancer is increasing and is estimated to be 80%. However, in these children are recognized many possible late effects resulting from treatment or the cancer itself. One of the most important complications is osteoporosis and increased risk of fracture during and after cancer treatment. Cancer itself, chemotherapeutic, radiotherapy, malnutrition and decreased physical activity during treatment cause bone mineral deficit.

The aim of this work was to investigate the alteration of bone mineral metabolism in survivors of childhood cancer.

Methods: A group of 65 survivors of childhood cancer aged 11.1 ± 4.0 (mean $\pm$ standard deviation) years of which 41 with hemoblastosis (acute lymphoblastic leukemia), 11 with lymphoma and 13 children with solitary tumor. We focused on laboratory parameters of bone metabolism and bone mineral density (BMD). Dual x-ray absorptiometry (DXA) has been used to assess the BMD and trabecular bone score (TBS).

Results: 7 children (10 %) had impaired linear bone growth that resulted in a body height deficiency of -2.0SD. The mean serum and urinary Ca, P and ALP values were within the reference range. The vitamin D levels (31.4 ± 11 ng/l) reached the lower limit of the standard. Bone mineral density measured by DXA was less than -2SD for age and sex in 32% (21 patients). Up to 21 survivors from the cohort (33 %) had $TBS \leq 1.3$, indicating microarchitectural deterioration of bone tissue. Although the length of cytotoxic treatment was significantly longer in ALL patients compared to solitary tumors (22,15 vs. 11,20, $p < 0,001$), TBS values did not differ in both groups (1,32 vs. 1,31, $p = 0,52$).

Conclusion: Survivors of childhood and adolescent cancers are at risk for low bone mass. Significant bone changes can be present at the early stage of the oncological process or may have a negative longitudinal effect on bone in adulthood. Thus, it is important to monitor bone health in these patients and bone densitometry should be part of the monitoring of the long-term consequences of the oncological process on the organism.

PREVENTABLE AND UNPREVENTABLE RISK FACTORS OF RETINOPATHY OF PREMATURITY

Nina Lenhartová, Katarína Matašová, Zora Lasabová, Andrea Mendelová, Mirko Zibolen

Clinic of Neonatology JLF UK and UNM in cooperation with Department of Molecular Biology JLF UK Martin

Background: With rapidly improving care for extremely preterm newborns ROP remains as the leading cause of vision impairment in children in developed countries and an ever-present threat at every NICU. Studying its pathogenesis has enabled us to recognize its risk factors and develop preventive strategies to counter them. In recent years, great focus in research has been put on mitigating the post-partum IGF-I drop in preterm newborns with a model of high calorie aggressive nutrition, repeatedly proven as safe and effective, and easily employable as a preventive measure. There are however certain risk factors at play, which we are unable to prevent, only predict. The current line of ROP research is starting to focus more on the presence of variations in the genome, which might be responsible for rapid progression of ROP into its severe stages, despite our best efforts to minimize its risk. As of now, several SNPs in genes connected to retinal angiogenesis have been proposed, and tested on several populations, as ROP appears to present itself with a degree of racial and ethnic variability.

Methods: We have collected genetic material from samples of venous blood of extremely preterm neonates born at our department, with the inclusion criteria being GA of <32 weeks and BW <1500 grams. Using DNA sequencing we tested the presence and possible association of certain SNPs with the development of ROP.

Results: We have detected the presence of certain SNPs in the population of extremely preterm newborns from the Northern Slovakia region. More extensive research of these polymorphisms in Slovak population is still needed. Furthermore, the incidence of ROP at our department has dropped significantly with the implementation of several preventive measures in the last 10 years.

Conclusion: ROP is a clinical unit with complicated pathogenesis, and fully elucidating its every component is crucial in battling this disease, where prevention has proven to be of enormous importance. The possible genetic risk, despite being something we are unable to prevent, might someday in the future become a way to predict the onset of ROP in selected risk patients, putting them first in line for more detailed screening.

VAGUS NERVE STIMULATION IN CHILDREN - WHEN TIME MATTERS

Gonzalo Alonso Ramos Rivera¹, Tomáš Foltán¹, Lucia Švecová¹, Miriam Kolníková¹, Martin Novotný², Juraj Šteňo², Pavol Sýkora¹

¹ Children's University Hospital, Department of Pediatric Neurology, Bratislava, Slovakia

² University Hospital Bratislava, Faculty of Medicine, Comenius University, Department of Neurosurgery, Bratislava, Slovakia

Background: To compare the influence of timing of implantation on the effectivity of Vagus Nerve Stimulation (VNS) in the treatment of epilepsy in patients younger than 18 years at the time of implantation.

Methods: We retrospectively evaluated 22 patients followed-up in the Department of Pediatric Neurology of Children's University Hospital in Bratislava, Slovakia. We assessed the influence of timing of VNS implantation (in terms of duration of epilepsy until implantation) on its effectivity. The analysis was performed in the whole cohort, in patients with pathological findings in the brain MRI, and in patients with normal brain MRI, 6 months after the implantation. VNS was considered as effective if reduction of seizures reached $\geq 50\%$.

For statistical analysis we used t test, with significant p value $< 0,05$.

Results: Of the total cohort, VNS was effective in 8 patients and ineffective in 14. The mean duration of epilepsy until VNS implantation was 5,51 years (range: 3,38-11,3; SD: 2,75) in the first subgroup and 8,99 (range: 3,8-16,9; SD: 4,19) in the second (p = 0,0497).

VNS was effective in 3 patients with pathological MRI findings and ineffective in 7. The mean duration of epilepsy until VNS implantation was 7,18 (range: 4,63-11,3; SD: 3,6) and 9,15 years (range: 3,05-16,9; SD: 4,83) respectively (p = 0,5494). In the group with normal MRI, VNS was effective in 5 patients and ineffective in 7. The mean duration of epilepsy until VNS implantation was 4,52 years (range: 3,5-7,82; SD: 1,86) in the first cases and 8,83 (range: 3,08-15,08; SD: 1,86) in the second ones (p = 0,0435).

The mean age at VNS implantation was 10,6 years (range: 3,8-17,9; SD: 4,3).

Conclusion: Early implantation of VNS (in terms of duration of epilepsy) seems to be related to a better outcome, specially in cases without lesions in the brain MRI.

7-DEHYDROCHOLESTEROL MODIFIES THE OPERATION OF KV1.3 CHANNELS IN T CELLS ISOLATED FROM SMITH-LEMLI-OPITZ SYNDROME PATIENTS

Andras Balajthy¹, Zoltan Denes Petho², Gabriella P. Szabo¹, Sandor Somodi³, Zoltan Varga¹, Gabor Veres¹, Gyorgy Panyi², Peter Hajdu²

¹ Department of Pediatrics, University of Debrecen

² Department of Biophysics and Cell Biology, University of Debrecen

³ Department of Internal Medicine, University of Debrecen

Background: In vitro manipulation of membrane sterol level has an impact on the regulation of ion channels, however, a comprehensive study is lacking to confirm the physiological or pathophysiological significance of these experiment. Smith-Lemli-Opitz syndrome (SLOS) is characterized by a reduced or abolished activity of 7-dehydrocholesterol (7DHC) reductase, which leads to the elevation of the 7DHC in the tissues and blood. We utilized T cells isolated from SLOS patients to address the question if in vivo altered membrane sterol composition impairs the operation of Kv1.3, the predominant voltage-gated ion channel of T cells, and if altered Kv1.3 function is reflected in impaired mitogenic responses of SLOS T lymphocytes.

Methods: T lymphocytes were isolated from the peripheral blood of healthy volunteers (age-matched controls) and patients with SLO. 7DHC elevation in T lymphocytes membrane was achieved upon treatment with cyclodextrin/7DHC complex. The biophysical characteristics of the Kv1.3 ion channel were studied using the patch-clamp technique in whole-cell configuration.

Proliferation rate of lymphocytes was assessed with CFSE-dilution assay upon anti-CD3/anti-CD28 stimulation.

Results: Using whole-cell patch-clamp technique we showed that the activation kinetics of Kv1.3 is slower and the midpoint of the voltage-dependence of steady-state activation is shifted to depolarized potentials in SLOS T cells as compared to age-matched controls. Similar changes in the kinetic and equilibrium parameters of Kv1.3 gating were detected in control T cells loaded with 7DHC. Upon removal of putative sterol binding sites of Kv1.3 the channel become insensitive to 7DHC loading. Functional assays revealed that modified operation of Kv1.3 in the SLOS T cells is associated with impaired proliferation rate and a defect in the early steps of Kv1.3- and Ca²⁺-dependent activation process in CD3+ cells.

Conclusion: Our conclusion is that the function of Kv1.3 is modified in SLOS via a direct coupling to the 7DHC in the cell membrane. We propose that this ion channel-sterol interaction reveals a molecular mechanism that may contribute to the pathophysiological conditions in SLOS and lead to the most prominent neurological and cardiovascular symptoms via influencing the physiological function of ion channels.

CONNECTIONS OF PACAP SIGNALLING PATHWAYS IN ALZHEIMER DISEASE KIDNEY OF MICE

Helga Perényi¹, Barbara Hinnah¹, Gabriella Horváth², Róza Zákány¹, Dóra Reglődi³, Gábor Veres², Tamás Juhász¹

¹ University of Debrecen, Faculty of Medicine, Department of Anatomy, Histology and Embryology

² University of Debrecen, Department of Pediatrics

³ University of Pécs, Faculty of Medicine, Department of Anatomy, PTE-MTA, „Lendület PACAP research group”

Alzheimer disease is a degenerative processes in the central nervous system, which also effects on several peripheral organ function (such as kidneys, testicles, pancreas) inducing formation pathological disorders. PACAP (pituitary adenylate cyclase-activating polypeptide) is a small neuropeptide, containing 38 amino acids. It has preventive role in different illnesses and plays an important role in reducing the manifestation of Alzheimer disease in animal models. Our hypothesis was that PACAP signalling crosstalk with BMP signalization has some disorders which may effect on kidney of mice suffering in Alzheimer disease. Framelim is a dietary supplement, containing lysed probiotics, vitamins and fatty acids, which may have a positive effect on the pathological processes. Additionally, active movement may also have role in the manifestation of Alzheimer.

Western blot, RT-PCR and immunohistochemistry were performed to compare the presence of certain elements of PACAP signalling pathway in mice suffering Alzheimer's. We fed the genetically modified and wild type animals with Framelim and active movement was also applied. We demonstrated that protein expression of PAC1 receptor increased in Alzheimer's mice and in Framelim treated group but reduced in active movement. In all experimental groups elevated expression of VPAC1 receptor was detected. PKA protein expression in Alzheimer's disease mice reduced, but no significant alterations were detected in active movement therapy or Framelim. BMPR1, BMP4 and Smad1 protein expressions increased in the kidneys of Alzheimer's mice, but

they were reduced by Framelim and active movement. Furthermore, active phosphorylated form of CREB and the expression of collagen type IV elevated Alzheimer's animals; moreover, during addition of Framelim further elevation was detected. On the contrary active movement therapy significantly reduced the expression of collagen type IV but did not alter the CREB expression.

Our results suggest that PACAP signalization is affected in kidneys and results a basement membrane thickening in Alzheimer disease by the increased production of collagen type IV. Furthermore, the active movement can normalize the signalling disorders which suggest its positive function on PACAP signalling. Although the precise effects of the interventions needs further investigation.

Supported by ÚNKP-2, Ministry of Human Capacities.

CAN PROBIOTIC SUPPLEMENTATION CHANGE CYTOKINE RESPONSE OF CHILDREN WITH URINARY TRACT INFECTION DURING ANTIBIOTIC THERAPY?

Katarina Meštrović Popović¹, Tomaž Langerholc², Nataša Marčun Varda³

¹ General Hospital Celje, Department of Paediatrics, Celje, Slovenia

² University of Maribor, Faculty of Agriculture and Life Sciences, Maribor, Slovenia

³ University Medical Centre Maribor, Division of Paediatrics, Maribor, Slovenia

Background: Urinary tract infections (UTIs) are among the most common bacterial infections in infants and children. Predicting which children with UTI will develop more intense inflammatory response (and possible long term sequelae) remains difficult. UTI leads to both local and systemic inflammatory response in which cytokines play an important role. It is known that probiotics act immunomodulatory. The aim of this study was to compare concentrations of some cytokines in serum and urine of two groups of children with UTI at the beginning of antibiotic treatment and at the end of it. One treated group was added probiotic and the other placebo, next to antibiotic therapy. This study was part of a prospective, double blind, randomized, placebo controlled trial about influence of probiotic supplementation to the antibiotic therapy during UTI.

Methods: In duration of 9-months trial 30 children with UTI were enrolled. 14 were given probiotics (A) and 16 were given placebo (B) in addition to standard antibiotic treatment. Concentrations of cytokines IL-2, 4, 6, 8, 10, 12p70, 17 and TNF- α in serum and IL-6, 8, 10 and 12p70 in urine were determined at the beginning of the antibiotic treatment + probiotic (A)/placebo (B) supplementation and after cessation of antibiotic therapy. Interleukins in blood and urine samples were determined by the Enzyme Linked Immunosorbent Assay (ELISA) tests were performed after manufacturer recommendations: Ready-SET-Go!®, Affymetrix eBioscience, 10255 Science Centre Drive, San Diego, California 92121. *Statistical analysis* was performed with IBM SPSS Statistics version 24 software. To compare results of both groups and determine p values we used ANOVA and t-test.

Results: At the beginning of antibiotic treatment mean values of cytokines *in serum* were (pg/ml): IL-2 in group A 1,67 (+/- 1,97) and in group B 1,67 (+/- 3,61), IL-4 in group A 1,41 (+/- 1,06) and in group B 0,92 (+/- 0,78), IL-6 in group A 18,11 (+/- 26,42) and in group B 19,42 (+/- 14,21), IL-8 in group A 41,34 (+/- 52,33) and in group B 34,24 (+/- 49,58), IL-10 in group A 22,35 (+/- 16,74) and in group B 13,07 (+/- 9,80), IL-12 in group A 3,16 (+/- 3,65) and in group B 9,98 (+/- 21,36), TNF- α in group A 9,18 (+/- 8,25)

and in group B 26,88 (+/- 85,73), IL-17 in group A 3,96 (+/- 4,21) and in group B 4,28 (11,94); **in urine:** IL-6 in group A 248,42 (+/- 429,00) and in group B 25,36 (+/- 38,31), IL-8 in group A 424,58 (+/- 302,08) and in group B 210,42 (+/- 214,71), IL-10 in group A 52,93 (+/- 44,82) and in group B 2,53 (4,77), IL-12 in group A 1,15 (+/- 1,03) and in group B 1,23 (+/- 0,89).

At the end of antibiotic treatment mean values of cytokines **in serum** were (pg/ml): IL-2 in group A 0,91 (+/- 1,04) and in group B 1,74 (+/- 3,24), IL-4 in group A 1,50 (+/- 0,88) and in group B 3,48 (+/- 7,32), IL-6 in group A 1,92 (+/- 1,02) and in group B 3,22 (+/- 4,68), IL-8 in group A 18,13 (+/- 25,67) and in group B 13,66 (+/- 8,87), IL-10 in group A 2,82 (+/- 2,24) and in group B 14,22 (+/- 23,69), IL-12 in group A 3,10 (+/- 3,79) and in group B 6,66 (+/- 12,86), TNF- α in group A 9,17 (+/- 7,88) and in group B 24,69 (+/- 70,55), IL-17 in group A 3,49 (+/- 3,88) and in group B 7,99 (+/- 21,64); **in urine:** IL-6 in group A 4,33 (+/- 12,48) and in group B 2,80 (+/- 5,61), IL-8 in group A 60,51 (+/- 189,03) and in group B 13,64 (+/- 25,73), IL-10 in group A 1,89 (+/- 2,76) and in group B 0,73 (+/- 0,52), IL-12 in group A 1,57 (+/- 1,10) and in group B 1,44 (+/- 1,56).

Differences in values of interleukins IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-17 and TNF- α in blood and IL-6, IL-8, IL-10 and IL-12p70 in urine at the admission and the end of antibiotic treatment were not significant among groups of children on probiotic (A) and placebo (B) supplementation.

Conclusion: Although we measured many pro- and anti-inflammatory cytokines in blood and urine of children treated with antibiotics for UTI, no significant differences between probiotic and placebo supplemented groups appeared. Elevated pro-inflammatory cytokines in serum and urine have been connected to late kidney complications (e.g. scars), but there is too little knowledge about the importance of early changes in inflammatory response with immunomodulation. Clinically, we have proven better response and recovery of children on antibiotic plus probiotic treatment of UTIs– significant shorter hospitalization and antibiotic treatment. Cytokine response did not show similar effect, possibly because complicated immune response to UTI is very individual and host-pathogen interaction dependent. We know that innate immunity plays a crucial role in acute phase of UTI. Well-designed, larger studies on bigger groups of children are needed to show the overall importance of cytokines in UTI.

RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM INHIBITORS AMELIORATE DIABETIC RENAL AND CARDIOVASCULAR COMPLICATIONS

Agnes Molnar^{1,4}, Sandor Koszegi¹, Lilla Lenart¹, Judit Hodrea¹, Dora B. Balogh^{1,4}, Edgar Szkibinszkij^{1,2}, Laszlo Wagner², Adam Vannay³, Attila J. Szabo⁴, Andrea Fekete^{1,4}

¹ 1MTA-SE „Lendület” Diabetes Research Group, Budapest, Hungary

² SE Department of Transplantation and Surgery, Budapest, Hungary

³ MTA-SE Pediatrics and Nephrology Research Group, Budapest, Hungary

⁴ SE 1st Department of Pediatrics, Budapest, Hungary

Background: Diabetic kidney disease (DKD) is the leading cause of end stage renal disease in adults. In DKD the increased activation of renin-angiotensin-aldosterone system (RAAS) via the elevated angiotensin II and aldosterone contributes to renal fibrosis. With the progression, the risk of cardiovascular (CV) disease development increases. Although RAAS inhibitors are the gold

standard therapy in DKD, their antifibrotic and CV protective potential beside their antihypertensive effect has not been tested yet.

Methods: After five weeks of streptozocin (65 mg/bwkg, i.p.) induced diabetes male, adult Wistar rats were treated for two weeks p.o. with ramipril, losartan, spironolactone or eplerenone (10µg - 20mg - 50mg - 50 mg /bwkg/day, resp.). Vehicle-treated diabetic (D) and healthy animals were controls (n=8/group). Heart rate, blood pressure, pulse wave velocity (PWV) and aortic intima-media thickness were measured to assess CV function and arterial stiffness. Renal and metabolic parameters were evaluated. Tubulo-interstitial fibrosis, fibronectin, collagen and α smooth muscle actin (αSMA) levels were estimated. Fibrotic marker levels were also measured from the kidney by PCR.

Results: In diabetic rats the declined renal function was ameliorated by RAAS blockers. Non of the treatments changed the blood pressure. Eplerenon prevented aortic intima-media thickening and Losartan decreased PWV in diabetic rats. The interstitial fibrosis, the amount of fibronectin, collagen and αSMA were decreased by all RAAS inhibitors. Elevated renal PDGF, CTGF, MMP2 and TIMP1 levels were decreased by RAAS blockers. Aldosterone antagonists showed the most robust effect in improving GFR (D: 1.3±0.1 mL/min/100g vs LOS: 1.9±0.1 mL/min/100g, EPL: 1.9±0.1 mL/min/100g, p<0.05 resp.), decreasing fibronectin accumulation (1.5 - 2.1 fold decrease in LOS and EPL vs D resp.) as well as minimizing PDGF and CTGF production (D vs LOS and EPL, p<0.05 resp.).

Conclusion: Diabetes-induced growth factor production enhances the development of renal fibrosis. RAAS blockers decrease the production of profibrotic factors, by this ameliorate the process of renal fibrosis. Without changing the blood pressure, RAAS blockers also improve CV function by preventing arterial wall thickening and arterial stiffness increment. All these findings suggest a new therapeutic potential of RAAS blockers, preferentially aldosterone antagonists in the treatment of renal fibrosis.

Grants: LP008/2017, OTKA-K112629-K116928-FK124491-NN-11460, VKE-2017-00006, EEMOFAKT-2017

SEX DIFFERENCES AND SEX HORMONES IN KIDNEY FUNCTIONS OF AGING RATS

Emese Domonkos¹, Mária Kačmárová¹, Veronika Borbélyová¹, Marianna Gyurászová¹, Alexandra Gaál Kovalčíková¹, Daniela Ostatníková², Július Hodosy^{1,2,3}, Peter Celec^{1,3,4}

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia

² Institute of Physiology, Faculty of Medicine, Comenius University, Sasinkova 2, 813 72 Bratislava, Slovakia

³ Institute of Pathophysiology, Faculty of Medicine, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia

⁴ Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia

Background: Sex differences have been described in kidney functions that might be attributable to the renoprotective effects of estrogens in females, or even to the damaging effect of testosterone in males. Numerous animal experiments brought contradictory results indicating a protective or harmful effect of short-term testosterone depletion in male rodents on renal functions. However,

the consequences of long-term hypogonadism in aging males are unknown. The aim of this study was to investigate sex differences in kidney functions in middle-aged Lewis rats and to find out whether they are attributed to endogenous testosterone produced in males.

Methods: We examined the role of estrogens by inhibiting their production by letrozole in intact animals, and by supplementing estradiol to castrated males. Kidney functions were evaluated assessing creatinine clearance and proteinuria in aging rats of both sexes, as well as in aging males, gonadectomized (GDX) prior to puberty.

Results: Neither sex differences nor any effects of castration were observed on creatinine clearance. At 12 and 15 months of age, males showed higher proteinuria by 72% and 53% than females, respectively. In comparison to intact males, long-term hypogonadism in 12-month old GDX males, but not in 15-month old, resulted in lower proteinuria (by 66%), similar to female rats.

Conclusion: Letrozole did not affect proteinuria either in females or in males. Estradiol-treated GDX males showed higher proteinuria than intact males. Our results confirm sex differences in proteinuria in aging rats, which could be caused by endogenous sex hormone production in males and may be influenced by estradiol supplementation.

APDS - RARE PRIMARY IMMUNODEFICIENCY (CASE)

Ivana Trochanová¹, Zuzana Kuková¹, Andre Šoltýsová², Jaroslava Melicharová¹, Peter Čížnár¹

¹ *Pediatric department, National Institute for Children's diseases, Bratislava*

² *Department of Molecular Biology, Faculty of Natural Sciences Comenius University Bratislava*

Syndrome of the activated subunit PI3-kinase has recently been identified as a new primary immune deficiency. The cause is a heterozygous "gain-of-function" mutation, resulting in increased kinase activity and related changes in the lymphoid array of cells. PI3 kinases affect a variety of cellular processes such as mitosis, cell survival, proliferation, signaling, vesiculation organization, degranulation, cytoskeletal regrouping. In the presentation, we describe the case of an 11 year old boy who was followed for hypogammaglobulinaemia, splenomegaly and lymphadenopathy of an unclear cause. Immunological examination confirmed combined immunodeficiency. The B immunophenotype has been shown to be deficient in antibody production, with hyper-IgM syndrome. The population of transient B cells was expanded and the number of memory B cells was reduced. In the T immunophenotype, the CD4⁺ cell population was reduced, and more than 80% of the T lymphocytes had the character of activated cells, a picture of immunosenescence. Whole exome sequencing identified the previously described delta (p110d) catalytic subunit mutation resulting in a change in functionality of the protein in which glutamic acid at position 1021 replaced lysine (E1021K). In addition to immunosuppressive treatment with immunoglobulins, treatment with a PI3 kinase pathway - mTOR inhibitor, rapamycin (sirolimus), was initiated in the patient. Clinical trials are currently underway with new selective inhibitors that have promising results and allow postponement of hematopoietic stem cell transplantation as the only causal therapy to date.

SIM1 AND MC4R GENE MUTATIONS IN CHILDREN WITH AN EARLY-ONSET SEVERE OBESITY

Daniela Stanikova^{1,2}, Marek Buzga³, Martina Skopkova², Martina Surova², Barbara Ukropcova^{2,4}, Lubica Ticha¹, Denisa Lobotková¹, Miroslava Petrasova⁵, Lucie Piskorová⁶, Jan Bozensky⁶, Jozef Ukropec², Ivona Zavacka⁸, Iwar Klimes², Juraj Stanik^{1,2}, Daniela Gasperikova²

¹ Department of Pediatrics, Medical Faculty of Comenius University, National Institute for Children's Diseases, Bratislava, Slovakia

² Diabgene Laboratory, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

³ Department of Physiology and Pathophysiology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic

⁴ Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

⁵ Department of Pediatrics, Medical Faculty of Safarik University, Kosice, Slovakia

⁶ Department of Pediatrics, Vitkovice Hospital, Ostrava, Czech Republic

⁷ Department of Biomedical Sciences, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic

Background: Mutations in the gene for melanocortin receptor 4 (MC4R) and mutations of the hypothalamic transcription factor single-minded 1 (SIM1) have been shown as a cause of early-onset severe obesity. We aimed to search for the MC4R and SIM1 mutations in children and adolescents with an early-onset severe obesity.

Methods: The MC4R and SIM1 genes were sequenced in 268 and 126 obese children, and adolescents (2-18 years of age), respectively, and in 41 adult lean controls of Slovak and Moravian origin. Inclusion criteria for the children and adolescent were the body mass index standard deviation score higher than 2 SD, and the obesity onset at less than 5 years of age for SIM1 project and less than 10 years for the MC4R analysis.

Results: We have identified two different previously described heterozygous loss of function MC4R variants (i.e. p.Ser19Alafs*34, p.Ser127Leu) in 2 obese probands, and in one obese (p.Ser19Alafs*34), and one lean (p.Ser127Leu) adult family relatives. The prevalence of loss-of-function MC4R variants in obese Slovak children was 0.7 %, what is one of the lowest frequencies in Europe. Furthermore, we have identified one novel heterozygous variant p.D134N and 7 previously described variants in the SIM1 gene. The novel variant was predicted to be pathogenic by 7 in silico software analyses and is located at the highly conserved position of the SIM1 protein. The p.D134N variant was found in 18 years old female proband (BMI 44.2 kg/m²; +7.5 SD), and in 3 obese family members. Regardless of early onset severe obesity, the proband and her brother did not fulfil the criteria of metabolic syndrome. Moreover, the variant carriers had significantly lower (p=0.03) preference of high sugar foods compared to the obese controls.

Conclusions: Both MC4R and SIM1 mutations are a rare cause of early-onset severe obesity in Slovak and Moravian children.

Supported by: ITMS 26240220087

IDENTIFICATION OF NOVEL GENE VARIANTS INVOLVED IN HNF1A-MODY DIABETES

Terézia Valkovičová¹, Martina Škopková¹, Lucia Valentínová¹, Miloslava Hučková¹, Iwar Klimeš¹, Juraj Staník^{1,2}, Daniela Gašperíková¹

¹ Institute of Experimental Endocrinology, Biomedical Research Center of SAS, Bratislava, Slovakia

² Department of Pediatrics, Faculty of Medicine, Bratislava, Slovakia

Background: Mutations in the HNF1A gene (Hepatocyte Nuclear Factor 1 α) are the second most common cause of monogenic diabetes in Slovakia. The HNF1A-MODY patients are sensitive to sulfonylurea (SU) treatment and therefore can benefit from switching from insulinotherapy to SU derivatives. Thanks to next generation sequencing not only more individuals are analyzed, but also higher number of novel HNF1A gene variants is identified. However it is challenging to decide whether the variant is pathogenic or benign.

The aim of the study was a) to perform DNA analysis of the HNF1A gene and b) to identify and classify new HNF1A gene variants in patients with clinical suspicion of monogenic diabetes.

Methods: We analyzed 515 individuals (364 probands and 151 family members) identified within Slovak nation-wide screening programme realized between the years 2003-2017 in our laboratory. The HNF1A gene was analyzed by Sanger sequencing and multiparallel panel sequencing approaches.

Results: HNF1A-MODY diabetes was confirmed in 92 individuals (44 probands and 48 family members), which represent 18 % of all tested individuals. We have found 31 different HNF1A gene variants, including 14 novel unpublished variants. Based on ACMG guidelines (Richards et al., Genetics Medicine 2015) the novel variants were classified as follows: 5 pathogenic, 4 likely pathogenic and 5 with unknown significance.

Conclusion: Significant fraction, 45 % of identified HNF1A gene variants were novel and 35 % of them were variants with unknown significance. For determination the variant pathogenicity, the exact phenotypic and genetic analysis of the whole family is important. The tight cooperation of diabetologists and DNA laboratory is necessary for correct interpretation of novel gene variants. This can lead to proper clinical management of HNF1A-MODY patients.

Supported by APVV187-12, VEGA 1/0211/18

COMPREHENSIVE DEVELOPMENT OF THE SKIN, ORAL AND GUT MICROBIOME IN EXTREME LOW BIRTH WEIGHT INFANTS DURING THE FIRST TWO WEEKS OF LIFE

Lukas Wisgrill¹, David Seki^{1,2}, Christos Zioutis², Angelika Berger¹, David Berry²

¹ Division of Neonatology, Pediatric Intensive Care and Neuropediatrics, Department of Pediatrics and Adolescent Medicine, Medical University of Vienna

² Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna

Background: The human intestine is home to a complex microbial ecosystem important to development, nutrition and health. In recent years, novel tools have been developed to catalogue the phylogenetic and genetic diversity of the gut microbiota leading to intriguing insights into changes in the microbiome associated with many factors such as diet, time and health state. The symbiosis of microbes and humans starts with the beginning of life and many studies revealed different prominent factors shaping our “first hours” microbiome. Extreme low birth weight infants (ELBWI) are high-risk patients needing invasive and supportive care. Dysbiosis in neonates and children is often interwoven with higher risk for later childhood diseases. The aim of the present study is to characterize the comprehensive development of gut, skin and oral microbiome in ELBWI.

Methods: Oral and chest skin swabs as well as stool samples of 15 ELBWI were taken on postnatal day 1, 3, 7 and 14 for 16s rRNA gene-targeted amplicon sequencing using Illumina MiSeq technology. Microbial communities were analyzed for changes in taxonomic profiles as well as alpha/beta diversity metrics with respect to time after birth as well as clinical parameters.

Results: The developing skin, gut and oral microbiomes of ELBWI showed a tendency for distinct community composition from one another, but with some overlap. PCoA clustering displayed three distinct clusters of community types which could not be attributed solely to body sites. The three types were marked by high abundance of either *Escherichia/Shigella*, *Staphylococcus* or *Lactobacillus*. Day of life and the inter-individual sampling type had an impact on the microbiome of each body site. Spontaneous birth did not affect the community composition during the first days of life.

Conclusion: In the first two weeks of life, the microbial communities on different body sites of ELBWI were only partially distinct, indicating that adult-type skin, oral, and gut communities are not completely established in the first two weeks of life. However, there was a tendency for skin samples to be abundant in *Staphylococcus*, which is characteristic of adult skin. Interestingly, most samples could be classified into being one of three “types” by composition, analogous to the human gut enterotypes. Further analysis is necessary to determine what factors influence community type and if this has clinical implications.

