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BOOK OF ABSTRACTS

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GENOMICS AS A BASIS FOR PRECISION MEDICINE

Sonja Pavlovic

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

Background: Although medicine always aimed to be personalized, true implementation of personalized medicine in health care practice has started recently. Fascinating progress of molecular genetics has strongly contributed to this great achievement of modern medicine. Personalized medicine, also known as genome-based medicine and precision medicine, uses the knowledge of molecular basis of the disease in order to individualize treatment for each patient.

Methods: Development of novel powerful high-throughput technologies has enabled better insight into "oms" landscape of many diseases, resulting in application of precision medicine approaches in their treatment.

Results: There are four cornerstones of modern precision medicine: "omics"-based diagnostics, pharmacogenomics, specific molecular targeted, gene and cellular therapy and predictive genomics. One of the most important successes of precision medicine is a discovery of novel diagnostic molecular markers. Furthermore, numerous newly discovered molecular markers have contributed to more precise classification of patients in distinct prognostic groups, leading to specific, more successful treatment protocols. Development of pharmacogenomics platforms and application of molecular–targeted therapy have led to the individualization of therapy, tailored to genetic profile of a disease in each patient. The development of gene therapies which can cure or prevent a disease by targeting disease-causing molecular defect has confirmed that the precision medicine has responded successfully to a great challenge. Additionally, cellular and tissue therapies have opened new possibilities for personalized treatment of many patients. Growing knowledge in predictive genomics leads to the preventive medicine, the most important goal of modern medicine.

Conclusion: There is no doubt that we are getting closer to full implementation of precision medicine in every day clinical practice.

THE ROLE OF NEUTROPHIL EXTRACELLULAR TRAPS IN THE ANIMAL MODEL CRYSTAL-INDUCED CHRONIC KIDNEY DISEASE

Alexandra Gaál Kovalčíková[1], Peter Celec[2], Ľudmila Podracká[1]

[1] Department of Paediatrics, National Institute of Children's diseases and Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovakia
[2] Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Background: The formation of neutrophil extracellular traps (NETs) is a potent "weapon" against pathogens. Except for the antimicrobial function of NETs, their excessive formation promotes tissue damage and coagulation playing an important role in different pathologies including chronic kidney diseases (CKD). However, whether the formation of NETs is involved in the pathology of crystal-induced CKD is still unclear. Our study aimed to find out whether mice deficient in peptidyl arginine deiminase type 4 (PAD4), an enzyme crucial for chromatin decondensation, will be protected from severe kidney damage in crystal-induced CKD.

Methods: In the study were used 17 wild-type (WT) and 15 PAD4-deficient mice (PAD4−/− KO). To induce CKD, mice were intraperitoneally injected with adenine hydrochloride hydrate for 2 weeks. Control mice received saline in the same manner. Markers of NETs formation including extracellular DNA (ecDNA), MPO, NE and NGAL were analyzed in plasma and urine. Blood and urine were collected at the baseline, and after 7 and 14 days of adenine administration. Concentrations of ecDNA were assessed using fluorescent method. Concentrations of MPO, NE and NGAL were assessed using commercial kits.

Results: Serum creatinine was significantly increased on day 3rd in PAD4−/− KO and on day 7th in WT mice confirming successful induction of CKD. Total plasma ecDNA was increased on day 7th by 88% and 250% in WT and PAD−/− KO mice, respectively compared to corresponding control groups. Similarly, urinary ecDNA in WT and PAD−/− KO on day 7th was 4-times and 8-times higher compared to controls. Further, plasma concentrations of MPO, NE and NGAL in WT and PAD4−/− KO mice were significantly increased on day 7th, together with their trend to increase in the urine. Moreover, there were positive mutual correlations between the main structural components of NETs – ecDNA and antimicrobial proteins in plasma and urine.

Conclusion: Increased concentrations of NETs markers including ecDNA, MPO, NE and NGAL in mice with adenine nephropathy suggest neutrophil activation and subsequent NETosis. However, PAD−/− KO mice with diminished NETosis seem not to be protected from severe kidney damage. This suggests that either NETs formation is not directly involved in the adenine-induced CKD or PAD4 is not the only crucial enzyme for chromatin decondensation. Further studies should elucidate the role of other NETs inhibitors in the patomechanisms of adenine-induced CKD. This work was supported by the Grant Agency of Ministry of Education, Science, Research and Sport of the Slovak Republic No. VEGA 1/0212/22.

FLUVOXAMINE, A SIGMA-1 RECEPTOR AGONIST, IS A NEW AND INNOVATIVE THERAPY FOR GLAUCOMA

Timea Medveczki[1], Judit Hodrea[1], Minh N. Tran[1], Anna Takacsi-Nagy[2], Akos Toth[1], Tamas Lakat[1], Balint Szokol[3], Illes Kovacs[4], Gyorgy Torok[5], Attila J. Szabo[1], Andrea Fekete[1]

[1] Pediatric Center, MTA Center of Excellence, Semmelweis University, Budapest, Hungary
[2] PannonPharma Pharmaceutical Ltd
[3] Vichem Chemie Research Ltd
[4] Department of Ophthalmology, Semmelweis University, Budapest, Hungary
[5] Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

Background: Glaucoma is the leading cause of permanent vision loss. The primary risk factor for this condition is elevated intraocular pressure (IOP), which can occur as a result of abnormal resistance to the outflow of aqueous humor, partially due to the fibrosis of the trabecular meshwork (TM). We recently reported that Sigma-1 receptor (S1R) agonist fluvoxamine (FLU) effectively reduces fibrotic processes in TM cells. To further demonstrate the applicability of the S1R as a novel anti-glaucoma drug target, in this in vivo study we investigated the tolerability, IOP-lowering and antifibrotic effects of a FLU containing eye drops in a mouse model of glaucoma.

Methods: Tolerability and toxicity investigations of a novel formulation of FLU containing eye drops, were performed on C57BL/6J wild-type (WT) mice using fluorescein test and slit lamp. IOP increase was induced in 2-3 month old male C57BL/6J WT and S1R knockout (S1R-/-) mice, by periocular injection of dexamethasone acetate (Dex) for four weeks. Treatment groups were: (a) Control; vehicle injection and vehicle eye drops, (b) Glaucomatous; Dex injection and vehicle eye drops, (c) FLU treated glaucomatous; Dex injection and FLU eye drops. Eye drops were applied bilaterally for two weeks, twice daily, starting two weeks after the first injection. Weekly measurements of IOP were performed using ICare Tonolab device. Upon completion of the experiment, the animals were euthanized, and the anterior segments were collected. Tissues were either immediately utilized for S1R, fibronectin and αSMA immunofluorescent labeling or frozen for additional investigations.

Results: FLU eye drops are non-toxic and non-irritating. Dex elevates IOP by 14.21% in the WT group and by 15.13% in the S1R-/- group. More importantly, two weeks of FLU therapy reduced IOP by 11.59% in WT mice, but had no effect in S1R-/- animals. Interestingly, in S1R-/- mice there was a further rise in IOP (6.02%) even after being treated with FLU. We have confirmed the presence of S1R in the mouse TM region. Additionally, the immunohistochemically labeling revealed an elevated amount of extracellular matrix proteins, αSMA and fibronectin in the anterior segment of glaucomatous mice. While FLU therapy decreased the ECM protein levels.

Conclusion: A novel non-toxic FLU eye drop with high tolerability has been formulated. FLU is effective in reducing elevated IOP as well as the fibrosis components in the anterior segment. Therefore, S1R agonists could be new treatment options to lower IOP and for other fibrosis-associated eye disorders.

PARK7 AS A NEW THERAPEUTIC TARGET IN PULMONARY FIBROSIS

Domonkos Pap, Apor Veres-Székely, Beáta Szebeni, Csenge Szász, Péter Bokrossy, Réka Zrufkó, Attila J. Szabó, Ádám Vannay

Pediatric Center, MTA Center of Excellence, Semmelweis University, Budapest, Hungary
HUN-REN–SU Pediatrics and Nephrology Research Group, Budapest, Hungary

Background: Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease characterized by excessive scarring, increasing breathlessness, and death within three to four years after diagnosis. The development of IPF is a result of an aberrant, repetitive alveolar epithelial injury, in which oxidative stress plays a dominant role. Parkinson's Diseases 7 (PARK7) molecule is a central mediator of the antioxidant defense mechanisms of various organs, however, its role in IPF and the underlying mechanisms is still unknown. In the present study, we aimed to investigate the role and therapeutic potential of PARK7 in IPF.

Methods: Pulmonary expression of PARK7 was investigated in the bleomycin induced mouse model of lung fibrosis. The effect of pharmacological PARK7 activation with Comp-23 on the reduced and oxidized form of PARK7 and on the synthesis of extracellular matrix (ECM) components, including alpha-1 type I collagen (COL1A1) and fibronectin (FN) and antioxidant genes including glutamate-cysteine ligase catalytic subunit (Gclc) and NAD(P)H quinone oxidoreductase 1 (Nqo1) in the lungs was investigated in vivo. The effect of Comp-23 treatment on oxidative damage (H2O2, bleomycin) induced death of lung alveolar epithelial cells (A549) and fibroblasts was investigated in vitro.

Results: We found that Comp-23 decreased the level of oxidized PARK7 in the fibrotic lung of mice treated with bleomycin. In addition, Comp-23 treatment decreased the bleomycin induced COL1A1, FN, Gclc and Nqo1 expression in the lung. Comp-23 treatment decreased the H2O2 and bleomycin induced cell death of A549 cells and fibroblasts.

Conclusion: Our data suggest that PARK7 protects the lung cells against oxidative damage thereby reducing the pathologic alterations in the lung leading to the development of IPF. Therefore, PARK7 may serve as a potential therapeutic target in the treatment of IPF.

OPTIMIZATION OF SIRIUS RED-BASED MICROPLATE ASSAY TO INVESTIGATE COLLAGEN PRODUCTION IN VITRO

Apor Veres-Szekely[1,2], Csenge Szasz[1], Domonkos Pap[1,2], Beata Szebeni[1,2], Peter Bokrossy[1], Laszlo Orfi[3], Attila J. Szabo[1,2], Adam Vannay[1,2]

[1] Pediatric Center, MTA Center of Excellence, Semmelweis University, Budapest, Hungary
[2] HUN-REN–SU Pediatrics and Nephrology Research Group, Budapest, Hungary
[3] Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary

Background: Tissue fibrosis is characterized by chronic fibroblast activation and consequently excessive accumulation of collagen-rich extracellular matrix. In vitro microplate-based assays are essential to investigate the underlying mechanism and the effect of antifibrotic drugs.

Methods: In this study, in the absence of a gold-standard method, we optimized a simple, cost-effective, Sirius Red-based colorimetric measurement to determine the collagen production of fibroblasts grown on 96-well tissue culture plates.

Results: Based on our findings, the use of a serum-free medium is recommended to avoid aspecific signals, while ascorbate supplementation increases the collagen production of fibroblasts. The cell-associated collagens can be quantified by Sirius Red staining in acidic conditions followed by alkaline elution. Immature collagens can be precipitated from the culture medium by acidic Sirius Red solution, and after subsequent centrifugation and washing steps, their amount can be also measured. Increased attention has been paid to optimizing the assay procedure, including incubation time, temperature, and solution concentrations.

Conclusion: The resulting assay shows high linearity and sensitivity and could serve as a useful tool in fibrosis-related basic research as well as in preclinical drug screening. Funding: This research was founded by the National Research, Development and Innovation Office (NKFIH), K-142728; Semmelweis University, TKP2021-EGA-24, STIA-KFI-2021; Hungarian Research Network, ELKH-POC-2022-024, the New National Excellence Program of the Ministry for Culture and Innovation from the Source of the National Research, Development and Innovation Fund, ÚNKP-23-3-I-SE-36, ÚNKP-23-3-I-SE-42, ÚNKP-23-4-II-SE-29, ÚNKP-23-5-SE-15; Hungarian Academy of Sciences, János Bolyai Research Scholarship.

SIGMA-1 RECEPTOR AGONIST MITIGATES BLEOMYCIN-INDUCED PULMONARY FIBROSIS IN MICE

Akos Roland Toth[1,2], Adam Hosszu[1,2], Tamas Lakat[1,2], Dora Bianka Balogh[1,2], Kata Cseko[3], Judit Hodrea[1,2], Zsuzsanna Helyes[3], Attila J. Szabo[2], Andrea Fekete[1,2]

[1] MTA-SE Lendulet Momentum Diabetes Research Group, Budapest, Hungary
[2] Pediatric Center, MTA Center of Excellence, Semmelweis University, Budapest, Hungary
[3] Department of Pharmacology and Pharmacotherapy, University of Pécs Medical School, Pecs, Hungary

Background: Idiopathic pulmonary fibrosis is associated with a median survival of just 2-3 years after diagnosis. Current therapies ameliorate pulmonary functional decrement, but do not inhibit the progression of fibrosis or reduce mortality. Thus, effective anti-fibrotic therapies are desperately needed. Based on our previous results in other organs, we aimed to investigate whether Sigma-1 receptor (S1R) agonist fluvoxamine (FLU) can ameliorate pulmonary fibrosis.

Methods: Fibrotic processes were induced with TGF-β or PDGF in A549 lung epithelial cells and primary fibroblasts isolated from the lungs of wt and S1R-/- mice. Cells were treated with FLU. Pulmonary fibrosis was induced in wild-type and S1R-/- mice by oropharyngeal bleomycin (BLM) administration. Mice were treated daily with FLU and sacrificed after 21 days.

Results: FLU mitigated α-SMA production and F-actin formation in both A549 cells and primary lung fibroblasts after pro-fibrotic factor induction. The effect of FLU was not obeserved in fibroblasts isolated from S1R-/- mice. In mice, after 21 days pro-fibrotic factor Tgfb expression was unaltered, while Ctgf expression increased in the BLM group, but not in the wt BLM+FLU group. Elevated expressions of ECM components collagen I, collagen III and fibronectin were reduced to control levels in wt BLM+FLU mice. Evaluation of Masson's trichrome-stained sections underlined the massive anti-fibrotic effect of FLU. In vivo MicroCT showed more preserved aerated tissue area in wt BLM+FLU vs. wt BLM and S1R-/- BLM+FLU.

Conclusion: Based on our preclinical data S1R may be a novel, effective drug target in the treatment of pulmonary fibrosis. Grants: LP2021-3/2021; PC2022-8/2022; OTKA- K135398; TKP2021-EGA-24

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF THE PERITONEAL MESOTHELIUM, A MAJOR BARRIER FOR SMALL SOLUTE TRANSPORT

Eszter Levai[1,2], Iva Marinovic[2], Maria Bartosova[2], Rebecca Herzog[3], Michael Hausmann[4], Domonkos Pap[1], Attila J Szabo1, Klaus Katochwill[3], Sotirios Zarogiannis[5], Claus Peter Schmitt[2]

[1] Pediatric Center, Semmelweis University, Budapest, Hungary
[2] Division of Pediatric Nephrology, Center for Pediatric and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany
[3] Christian Doppler Laboratory for Molecular Stress Research in Peritoneal Dialysis, Department of Pediatrics and Adolescent Medicine, Medical University of Wien, Wien, Austria
[4] Kirchhoff Institute for Physics, Heidelberg University, Heidelberg, Germany
5 Department of Physiology, Faculty of Medicine, University of Thessaly, Volos, Greece

Background: Peritoneal dialysis (PD) requires efficient solute transport which is mediated by paracellular and transcellular pores, channels and carriers. While endothelial and interstitial transport have been studied extensively, impact of mesothelial cells remains uncertain.

Methods: To assess the molecular transport machinery in peritoneum, we studied polarized primary (HPMC) and immortalized human peritoneal mesothelial (MeT-5A), microvascular (HCMEC) and umbilical vein endothelial (HUVEC), calculated human peritoneal mesothelial (MSA) and capillary endothelial (BCESA) surface area in 100 tissue samples, and performed molecular transport-related gene profiling, single molecule localization microscopy, and molecular weight dependent transport studies in vitro and in mice.

Results: The healthy human peritoneal MSA was age-dependently 40-70% lower than the BCESA, and remains preserved during initial two years on PD with double-chamber PD fluids. Junction, transmembrane and transcytotic transporters were highly cell-type specifically expressed, with sealing tight junction (TJ), claudin (CLDN)1 being only expressed in mesothelial cells (MC), CLDN5 in endothelial cells (EC). At nanoscale, TJ-anchoring protein Zonula occludens-1 was consistently expressed along the MC membrane, but less abundant and discontinuously present with lower clustering level compared to the EC membrane. Transepithelial electrical resistance (TER), is 3-fold higher across the MC, reflecting lower ionic conductance. Trans-MC time-dependent creatinine, 4- and 10-kDa dextran transport was slower than across the EC monolayer. Removal of the MC layer from sheep peritoneum abolished tissue TER. In mouse, short-term peritoneal lipopolysaccharide (LPS) exposure altered MC, morphology, but not peritoneal MC coverage and CLDN1 and 5 abundance and increased creatinine, 4 and 70 kDa solute uptake.

Conclusion: We provide comprehensive in vitro, ex vivo and in vivo studies on molecular expression patterns and transport functions of the peritoneal MC and capillary EC barrier. Our findings suggest a major barrier function of the mesothelium for molecular size-dependent, transperitoneal solute transport, requiring reconsideration of current transport models of PD.

PRENATAL ORIGIN OF PEDIATRIC B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA: TRACING BACK LEUKEMIA TO BIRTH USING LEUKEMIC CLONE-SPECIFIC IMMUNOGLOBULIN HEAVY CHAIN REARRANGEMENTS

Sonja Pavlović[1], Teodora Karan-Đurašević[1], Nataša Kaćanski[2], Irena Marjanović[1], Nataša Tošić[1], Jovanka Kolarović[2,3], Dragana Janić[4], Vladimir Gašić[1], Bojan Ristivojević[1], Branka Zukić[1]

[1] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[2] Institute for Child and Youth Health Care of Vojvodina, Novi Sad, Serbia
[3] Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
[4] Institute for Oncology and Radiology of Serbia, Belgrade, Serbia

Background: Several lines of evidence strongly support the view that a significant proportion of childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL) originates in utero, with the preleukemic “first hit” occurring during fetal hematopoiesis. In this study we investigated prenatal origin of BCP-ALL using immunoglobulin heavy chain (IGH) rearrangements as a leukemic clone-specific marker for the detection of preleukemic clones at birth.

Methods: This retrospective study was conducted on a cytogenetically and clinically heterogeneous cohort of 24 pediatric BCP-ALL patients. Identification of leukemic clone-specific IGH rearrangements was performed in diagnostic bone marrow samples using polymerase chain reaction (PCR) and Sanger sequencing. Based on the sequences of clonotypic IGH rearrangements, 2 patient-specific primers were designed for each patient and used in semi-nested PCR for the detection of preleukemic clones in patients’ neonatal blood spots (Guthrie cards).

Results: The study cohort enrolled 12 male and 12 female subjects, aged 1 to 9.6 years (median=3.1 years) at diagnosis. The majority of patients (20/24; 83.3%) had common-ALL, three patients (12.5%) had pre-B, and one patient (4.2%) pro-B immunophenotypic subtype. Chromosomal translocations were detected in 9 patients (37.5%); t(12;21) (ETV6/RUNX1) in 7 patients and t(1;19) (TCF3/PBX1) in 2 patients. The analysis of leukemic clone-specific IGH rearrangements’ composition revealed preferential usage of IGHV3, IGHD2 and IGHD3 family genes, IGHJ6 and IGHJ4 genes, and the predominance of unproductive IGH rearrangements (18/27; 66.7%). Leukemic clone-specific IGH rearrangements were detected in neonatal blood spots of 54.2% of patients (13/24). In two cases that had double IGH rearrangements at diagnosis, only one rearrangement was present at birth, while in the third case both clonotypic rearrangements were detected in neonatal blood. Guthrie card-positive findings were significantly more frequent in children ≤5 years of age at diagnosis than in older children (p=0.011). Regarding patients’ characteristics at birth and at diagnosis, Guthrie card-positivity was not associated with sex, birth weight and mother’s age, as well as with white blood cell count, percentage of bone marrow blasts, karyotype, immunophenotype and the presence of ETV6/RUNX1 and TCF3/PBX1 fusion genes.

Conclusion: Our study confirms that a large number of childhood BCP-ALL cases have prenatal origin, regardless of the molecular subtype defined by chromosomal aberrations. Younger age at diagnosis of BCP-ALL in patients with positive findings on Guthrie cards indicates a relatively short latency period during which the "second hit" necessary for the conversion of preleukemic to leukemic clone and the development of clinically overt disease occurs.

POPULATION PHARMACOGENOMICS OF IMMUNOSUPPRESSIVE AND AMINOSALICYLATE THERAPY: POTENTIAL FOR THERAPY OPTIMIZATION IN SERBIAN PAEDIATRIC INFLAMMATORY BOWEL DISEASE PATIENTS

Marina Jelovac, Biljana Stankovic, Đorđe Pavlović, Nikola Kotur, Bojan Ristivojević, Ivana Grubiša, Irena Marjanović, Vladimir Gašić, Sonja Pavlović, Branka Zukić

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

Background: Inflammatory bowel disease (IBD) is a chronic inflammation of gastrointestinal tract (GIT) represented by two types of the disease – ulcerative colitis where colon is primarily affected and Chron's disease with the entire digestive tract affected. In paediatric patients, IBD symptoms appear to be more severe than in adults. This complex condition is characterized by strong variability in phenotype and prognosis. Also, adverse drug reactions caused by drugs used in therapy of IBD are often more severe in children then adults. We aimed to determine frequencies of variants in pharmacogenes relevant for therapeutic success of immunosuppressants and aminosalicylates administrated to paediatric IBD patients.

Methods: Genomic data of 895 Serbian paediatric patients were obtained by clinical (TruSight One, Illumina) or whole exome (Exome 2.0 Plus, Illumina) sequencing. Pharmacogenomics variants of 6-mercaptopurine, methotrexate, tacrolimus and sulfasalazine drugs response were selected for analysis based on PharmGKB and CPIC databases: CPIC Level Final (TPMT, NUDT15, CYP3A5, G6PD) or CPIC Level Provisional (CYP3A4, ABCB1, SLCO1B1, NAT2).Variant call format files (VCF) were created from FASTQ files using in-house pipeline for alignment and annotation. Calling of star alleles was performed using Stargazer bioinformatics tool and subsequent analysis of frequency differences in Serbian and general European subpopulations were done using 1000 Genomes Project database, Chi square test and R program.

Results: Alleles called in CYP3A5, G6PD and TPMT genes showed no differences comparing to other European populations. Among Serbian paediatric cohort heterozygous diplotype TPMT *1/*3C harbouring only variant rs1142345 C was less frequent than in overall European, Central European, British and Ibearian populations. Significantly different frequencies in Serbian paediatric cohort versus European subpopulations were found for nine haplotypes, one in ABCB1 and NUDT15 genes, two haplotypes in CYP3A4 and NAT2 genes and three haplotypes in SLCO1B1 gene. Four haplotypes were not observed in European population according to CPIC, but were present in our cohort, mainly *10, *16 and *179 in G6PD gene and *10 in NAT2.

Conclusion: Results presented herein show that frequencies of several important pharmacogenomics variants differ in Serbian and other European subpopulations. This could have serious clinical implications and potential more frequent development of adverse drug reactions when treating paediatric IBD patients and other patients in need with standard doses of immunosuppressive therapy. Population-specific pharmacogenomics aspect should be taken into account for therapy optimization in clinical practice, especially for vulnerable patients groups namely paediatric patients.

EXPLORING VARIATION IN ADHESION G PROTEIN-COUPLED RECEPTOR GENES: INSIGHTS FROM GENOMIC DATASETS OF PEDIATRIC RARE DISEASE CASES IN SERBIA

Đorđe Pavlović, Biljana Stanković, Nikola Kotur, Marina Jelovac, Bojan Ristivojević, Irena Marjanović, Vladimir Gašić, Teodora Karan Đurašević, Ivana Grubiša, Branka Zukić, Sonja Pavlović

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

Background: Adhesion G protein-coupled receptors (aGPCRs) represent the second largest family in the GPCR superfamily of receptors. The aGPCR family is comprised by 32+1 members which have been shown to be involved in important physiological processes, such as brain development and immune responses. Their role in pathophysiological processes such as neurological disorders, inflammation, angiogenesis and male infertility has been noted. Also, genetic association of aGPCRs with several diseases has identified them as potential drug targets. Systematic analysis and annotation of aGPCR variants is still lacking. With the emergence of high-throughput technologies and bioinformatic tools to extract this information from genomic data, this objective becomes achievable. We explored the entire aGPCR gene family using data obtained from whole exome sequencing of 113 pediatric cases with various clinical phenotypes. Our aim was to describe the genetic variant repertoire of the coding regions of each gene, in terms of type, damaging effect and affected receptor domains in order to identify variants of potential clinical significance.

Methods: Whole exome sequencing was performed (Illumina DNA Prep with Exome 2.0 Plus Enrichment). Sequencing data was processed using an in-house developed bioinformatics pipeline for germline short variant discovery. For each gene, coding region variants were annotated using the ANNOVAR tool as either synonymous, missense, stop gain, in-frame indel or frameshift indel. Possible impact of the identified variants on the structure and function of proteins was computationally inferred by SIFT and PolyPhen-2.

Results: The majority of variants identified in coding regions of aGPCR genes were either synonymous or missense benign. As anticipated, more deleterious variants were comparatively rare. Genetic variability in each gene was calculated as the number of detected variants per coding nucleotide count. We identified two members of aGPCR family, ADGRE1 and ADGRF3, as being among the genes with the highest genetic variability rate. A detailed look at the ADGRE1 and ADGRF3 genes showed that more common non-synonymous variants are confined to the N-terminal domain.

Conclusion: In this pilot study, we investigated the spectrum of aGPCR genetic variants present in the Serbian pediatric cohort, which is important for defining the aGPCR genomic repertoire in various rare diseases. Also, development of bioinformatics tools capable of automatically and accurately annotating genetic variants is crucial for the field of aGPCRs.

ASSESSMENT OF NEURODEVELOPMENTAL OUTCOME IN INFANTS BORN DURING THE COVID-19 PANDEMIC

Vanda Pal[1], Borbala Lukacs[2], Krisztina Vargane Tari[3], Petra Sztahovits[2], Agnes Kothencz[2], Miklos Szabo[1], Agnes Jermendy[1], Csaba Nador[2], Fanni Pekli-Toth[2]

[1] Pediatric Center, Semmelweis University, Budapest, Hungary
[2] Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary
[3] Peto Andras Faculty, Semmelweis University, Budapest, Hungary

Background: Perinatal morbidity and mortality are low among infants born to SARS-CoV-2 infected mothers, however little is known about their long-term neurodevelopmental outcomes. The COVID-19 pandemic may have led to increased stress levels, anxiety and poor mental health in families, possibly carrying a risk for neurodevelopmental problems. We aimed to examine the effect of maternal SARS-CoV-2 infection on the neurodevelopmental outcomes of the infants born during the COVID-19 pandemic.

Methods: We conducted a case control study between January 2022 and May 2023 at the follow-up clinic of the NICU of the Department of Obstetrics and Gynecology, Ulloi St Division, Semmelweis University, Budapest, Hungary. Infants who were born to SARS-CoV-19 infected mothers (COVID-19 group, n=30) and uninfected mothers (control group, n=31) were examined at 1 year of age using the Bayley Scales of Infant and Toddler Development 3rd edition tool. We used questionnaires to assess duration of breastfeeding (≤6 months vs. >6 months) and maternal education level (no higher education vs. higher education). We excluded preterm infants and participants with missing data in any of the 5 Bayley subtests. Our primary outcomes were the absolute Bayley III test scores. Data are shown as median [interquartile ranges].

Results: Baseline demographic characteristics were similar in the two groups. The Bayley III screening tests were performed at a mean age of 12±2 months. Children of the COVID-19 group scored lower than the control group participants on the following subtests: cognitive (16 [15-17] vs. 18 [16-19] points, respectively; p=0.002), receptive communication (11 [10-12] vs. 12 [10-13] points; p=0.028), expressive communication (13 [11-13] vs. 13 [12-14] points; p=0.030) and fine motor skills (13 [12-14] vs. 14 [13-15] points; p=0.034). We measured no difference in the gross motor skills scores (16 [14.75-17] vs. 17 [14-17], respectively; p=0.342). Duration of breastfeeding and maternal education level did not differ significantly between the two groups.

Conclusion: We found significantly lower cognitive, communication and fine motor skill scores but not gross motor skill scores among infants born to SARS-CoV-2 positive mothers compared to controls. Early targeted interventions may ameliorate these impairments and pave the way for long-term follow-up of this vulnerable population.

SACROCOCCYGEAL TERATOMA: SINGLE-CENTER EXPERIENCE (2005-2020)

Martin Jouza, Anna Jouzova, Petr Jabandziev

University Hospital Brno, Brno, Czech Republic

Background: Sacrococcygeal teratoma (SCT) is the most common congenital tumor. The incidence of malignant types is rare but increases with late detection or in case of relapse. Prenatal diagnosis is based on ultrasound examination and magnetic resonance imaging (MRI). The primary therapeutic modality is complete surgical resection of the tumor.

Methods: Retrospective analysis of fifteen cases of sacrococcygeal teratoma identified and treated at the Department of Obstetrics and Gynecology and the Department of Pediatrics, University Hospital Brno, between 2005 and 2020. The following criteria were evaluated: gestational week of the primary diagnosis, ultrasound findings, pregnancy management, delivery mode, correlation of prenatal ultrasound description with postnatal findings in the newborn, as well as the occurrence of early and late complications in newborns and children.

Results: Out of fifteen cases, six cases (40%) were indicated for pregnancy termination based on ultrasound findings, the parent's decision, and an estimation of an adverse pregnancy outcome. In nine cases (60%), the pregnancy continued and was ended by delivery. In one case, there was an early postnatal death of a newborn after birth in the 25th week of gestation. In eight cases, live fetuses were born in which the tumor was surgically removed between day 1 and 14 months after birth. There was a strong correlation between the description made by prenatal ultrasound diagnosis and related severe complications in newborns. Concerning the early complications, there was only one infection in the surgical wound (12.5%).

Conclusion: Sacrococcygeal teratoma is one of the sporadic congenital malformations. A detailed prenatal ultrasound examination is critical to estimate the pregnancy prognosis. In cases where early surgical treatment is provided, the incidence of severe complications and long-term consequences in children is very low.

WHEN THE HEART SKIPS A BEAT: ELECTROLYTE DISTURBANCES CAUSED BY ENTERITIS – CASE REPORT

Bertalan Horváth, Eszter Vojcek, Péter Tóth-Heyn, Attila Szabó

Semmelweis University, Budapest, Hungary

Introduction: Treating electrolyte disturbances can be challenging in modern medicine. Occasionally, life threatening conditions may also develop. Here we discuss a case of a serious electrolyte disturbance associated with a rare disease called intestinal failure (IF).

Case description: We present a 7-year-old Turkish patient with a known background of IF. He presented with signs of dehydration caused by enteritis and impaired consciousness with the inability to move his limbs and head. On admission he had typical physical and ECG signs of hypokalaemia, supported by laboratory findings (1.5 mM). Severe acidosis was also present (pH: 7.1) Later he developed severe hypophosphatemia (0,27 mM) and hypoglycaemia (3.1 mM). The symptoms ceased with adequate therapy. Several hours later he presented physical and laboratory signs of hypocalcaemia with painful muscle cramps. The symptoms improved with cautious calcium supplementation. Later laboratory findings revealed severe hypomagnesemia that was treated with electrolyte infusion, and the symptoms did not reappear afterwards. History taking from this patient was challenging due to language barriers, nevertheless detailed history with the aid of a Turkish interpreter revealed that the patient had an underlying absorption problem called IF aggravated by enteritis.

Discussion: The NADPH-oxidase mutation leading to damaged intestinal epithelium is a rare cause of intestinal failure. This condition causes difficulties in the absorption from the intestinal tract. Our patient had a Broviac catheter through which he received total parenteral nutrition overnight. However, in this disease the homeostasis of the body can easily be unbalanced by an infection. In this case, the patient's life-threatening electrolyte loss developed on the base of IF aggravated by enteritis. Nevertheless, the diagnosis was especially challenging due to the fact that several severe symptoms co-occurred and also, due to language barriers.

CORD BLOOD LEVELS OF C-PEPTIDE AND GLYCEMIA IN GESTATIONAL DIABETES MELLITUS

Rana Ibrahim, Kateřina Krylová, Jaroslav Stráník, David Neumann

Charles University Faculty of Medicine in Hradec Kralove, Hradec Kralove, Czech Republic

Background: Gestational Diabetes Mellitus (GDM) is among the most common pregnancy associated complications worldwide. Maternal hyperglycaemia during pregnancy appears to have a strong continuous association with negative foetal and neonatal effects such as increased birth weight, neonatal body fat percent, perinatal complications including birth trauma, increased risk of primary caesarean delivery, and clinically defined neonatal hypoglycaemia. The long-term effect of GDM on the offsprings is however still unclear. Several studies demonstrated an adverse effect on neurodevelopment of the infants of GDM mothers. In the perinatal phase of this study we aim to assess the cord blood levels of C-peptide and glycemia in GDM and healthy controls in relation with subsequent results during a year-long follow-up.

Methods: A prospective observational study including 45 mothers took place between August 2023 to March 2024. The cohort includes mothers with high-risk GDM, defined as GDM managed by medications including insulin and or oral antidiabetic drugs (n=15: GDM insulin n=6, GDM insulin+PAD n=3, GDM PAD n=6), mothers with low-risk diet-controlled GDM (n=15) and healthy controls (n=15). Glucose measurement took place using the hexokinase method (COBAS f00 Roche analyser, Mannheim, Germany) and C-peptide via a microparticle-based chemiluminescent immunoassay method (Architect, Abbott, Chicago, USA). GraphPad Prism 6.07 was used for statistical evaluation.

Results: The level of C-peptide was highest in the high-risk GDM group (mean: 421,9; SD: 157,8), followed by the low-risk GDM group (mean: 353,8; SD: 120,2) as compared with the control group (mean: 318,5; SD: 107,5). The level of glycemia was only slightly elevated in the high-risk GDM group (mean: 4,97; SD: 1,15) than the low-risk GDM group (mean: 4,35; SD: 1,06) than the control group (mean: 4,32; SD: 1,0).

Conclusion: The cord blood levels of c-peptide and glycemia were highest in high-risk GDM followed by low-risk GDM as compared with healthy controls. We plan to further follow-up the infants to determine whether these results may be correlated with worse neurodevelopment outcome at 12-months of age as assessed by the Ages and Stages Questionnaire-3, a thorough paediatric examination, a logopedic examination as well as hemoglobin A1C levels in the infants at one-year of age compared to maternal levels measured in the third trimester of pregnancy. We hypothesise that cord levels of c-peptide and glycemia may serve as useful markers for assessing the severity of long-term adverse effects of GDM.

TRANSIENT ANTITHYROID AUTOANTIBODY ELEVATION IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

Eszter Muzslay, Eszter Hámory, Péter Tóth-Heyn, Vivien Herczeg, Andrea Luczay

Pediatric Center Semmelweis University, Budapest, Hungary

Background: Autoimmune comorbidities, such as thyroiditis and celiac disease, are more frequent in children with type 1 diabetes mellitus (T1DM) than in the average population. There is literature data about a phenomenon observed by children with T1DM called transient anti-tissue transglutaminase elevation. There is no literature about transitional antithyroid antibody elevation by children with T1DM. We hypothesize that the same phenomenon can be observed in case of antithyroid autoantibodies by children with T1DM.

Methods: Our retrospective study included patients with T1DM treated at the Pediatric Center, Semmelweis University. 524 children who had been followed in our center from the T1DM diagnosis were included in our analysis. In cases where no ultrasound abnormality was found later, we examined the appearance of autoantibody positivity over time, the degree of increase, the type of antibody, and possible normalization. Descriptive statistical methods were used.

Results: In the examined population of 524 patients, 112 (21.4%) children had an increase in their antithyroid autoantibodies, of which ultrasound-proven thyroiditis (AIT) was diagnosed in 69 children. In patients who did not develop AIT in addition to diabetes (455 people), normalization occurred in 18 cases (4%). We did not experience transient positivity above 10-fold increased values, and the antithyreoid peroxidase (ATPO)-type elevation was more frequent than the antithyroglobulin (ATG)-type (in 16 vs. 2 cases).

Conclusion: Negative ultrasound, mild elevation of autoantibodies, and ATPO involvement raise the possibility of a transient elevation of thyroid autoantibodies. Therefore, performing repeated autoantibody measurements may be worthwhile in these cases to reduce the healthcare system expenses and the mental burden on the patient.

PREDICTIVE MODEL OF REPEATED EPISODES OF FEBRILE NEUTROPENIA IN CHILDREN WITH CANCER

Silvije Šegulja[1], Jelena Roganović[2]

[1] Faculty of Health Studies, Rijeka, Croatia
[2] Children's Hospital Zagreb, Zagreb, Croatia

Background: Febrile neutropenia (FN) is a frequent complication of chemotherapy in children with malignant diseases and a significant cause of morbidity and mortality. Early diagnosis of bacteremia and rapid therapeutic intervention are crucial for the outcome. Therefore, it is important to identify patients with increased risk for severe infections requiring hospitalization and early initiation of antibiotic therapy. Research on this topic in the pediatric population is still rare and hampered by the heterogeneous characteristics of the underlying disease and the effects of chemotherapy. Several risk factors for severe infections and negative outcome have been identified, but the comprehensive guidelines based on evidence and validated predictive algorithms are still lacking. The objective of study was to identify risk factors for a repeated episode of FN in of children with cancer and create a predictive model for a repeated episode of FN with adequate specificity and sensitivity.

Methods: A retrospective study was conducted in 45 children (25 males and 20 females) with cancer (19 hematological neoplasms and 26 solid tumors), median age 5 years (range 3.5 – 13), who were treated at the Division of Hematology and Oncology, Department of Pediatrics, Clinical Hospital Center Rijeka. The inclusion criterion was at least one episode of FN. The correlation of the repeated episodes of FN and its severity with relevant clinical and laboratory parameters was analyzed by logistic regression.

Results: One hundred eighty-two episodes of FN were documented. Thirty-eight (84%) patients had recurrent FN, of which twenty-five (56%) had severe FN. Significant predictive factors for recurrent FN episode are duration of first FN episode ≥9 days and red blood cell count ≤3.0×10^12/L. The predictive model derived from these two factors has an accuracy of 87% (95% confidence interval [CI] 73%-94%), a sensitivity of 82% (95% CI 53%-97%), and a specificity of 88% (95% CI 79%-93%).

Conclusion: The risk for recurrent FN and subsequent severe complications emphasize the need to focus scientific research in the direction of predicting repeated episodes of FN. In our research, length of first FN episode and anemia are significantly associated with risk for recurrent FN. These predictors are useful in identification of high-risk patients who require prompt intervention.

MANAGING REFRACTORY AND RELAPSED HODGKIN LYMPHOMA – OUR 5-YEAR EXPERIENCE

Nuša Matijašić Stjepović, Izabela Kranjčec, Domagoj Buljan, Maja Pavlović, Martina Slukan

Children's Hospital Zagreb, Zagreb, Croatia

Background: Pediatric Hodgkin lymphoma (HL) has excellent prognosis; majority of patients are cured with first line treatment strategies with failure rates of approximately 15-20% even in advanced stage.

Methods: A retrospective study was conducted on children diagnosed with HL from January 1st, 2019, to December 31st, 2023, treated at Children's Hospital Zagreb. Epidemiological and clinical data (age, sex, histology, stage, protocol) were gathered from patients' electronic medical records. In refractory/relapsed (R/R) settings additional information (time to relapse, salvage, maintenance treatment, response to therapy) were recorded. Descriptive statistical analysis was performed.

Results: Nineteen patients (52.6% female, median age 14.6 years) were treated for HL at our Department during the 5-year period. The predominant histological subtype of classic HL was nodular sclerosis (83.3%). Patients were mainly diagnosed in advanced stage (III and IV - 66.7%), having B symptoms (66.7%), and treated according to EuroNet-PHL trial. One male patient was switched to ABVD regimen due to cyclophosphamide allergy. Radiotherapy was conducted in only two cases. Three patients (16.7%) experienced relapse - two girls (patients A and B) had early relapse, while one boy (patient C) had refractory disease. Initially, the girls had advanced, and the boy localized disease; all showed excellent therapeutic response on early assessment. Patients A and B were diagnosed with relapse on routine check-ups (A – elevated sedimentation rate and ferritin as first sign; B – suspicious lymph node on CT scan). Patient C developed new cervical mass during treatment. Patients B and C were given IGEV (ifosfamide, gemcitabine, vinorelbine, prednisolone), followed by autologous hematopoietic stem cell transplant (auto-HSCT) with BEAM conditioning. Patient A received 6 GDP cycles (gemcitabine, dexamethasone, cisplatin), but regardless of a good response to it, experienced a second relapse treated with BBV (bendamustine and brentuximab vedotin), auto-HSCT (BEAM) and BV maintenance (6 post auto-HSCT cycles). In our survey only one male patient had nodular lymphocyte predominant HL (nodular lymphocyte predominant B-cell lymphoma according to ICC 2022), localized form, but received chemotherapy due to residual mass. He experienced early relapse and achieved complete remission upon 4 R-CHOP cycles (rituximab, cyclophosphamide, doxorubicin, prednisone).

Conclusion: All four R/R patients being currently in remission supports the fact of an achievable cure, even though optimal salvage treatment is yet not defined due to the lack of randomized trials. Recommendations, such as those from the EuroNet Pediatric Hodgkin Lymphoma Group, have recently eased the diagnostic and therapeutic approach in pediatric R/R HL patients.

INTEGRATIVE TRANSCRIPTOMIC PROFILING OF THE WILMS TUMOR

Simona Ivančan

University Children's Hospital, Ljubljana, Slovenia

Background: The Wilms tumor is the most common kidney cancer in children. This study characterized complete miRNA and mRNA profiles using a comprehensive NGS sequencing approach to identify miRNAs as transcriptome regulators involved in the Wilms tumor. By analyzing two independent groups of kidney samples with matched controls, we identified 43 miRNAs that are independently differentially expressed in the Wilms tumor irrespective of tumor histological type. Our results provide new insights into the role of transcriptomics in the Wilms tumor and identify universal miRNAs as potential biomarkers for early detection and targets for prevention and treatment of the Wilms tumor.

Methods: Our aim was to identify differentially expressed miRNAs in the WT compared to samples from healthy kidneys (HK). In the second part, we confirmed these results by broadening the study to include the examination and comparison of WT and HK formaldehyde-fixed paraffin-embedded tissue samples (FFPE). In the third part, we specified the impact of differentially expressed miRNAs on mRNA expression in nephroblastoma tissues. We used in silico analysis to predict the correlative effect of miRNAs on mRNA expression and evaluated the results by analyzing the mRNA expression in renal tissue. Using next-generation sequencing we analyzed miRNA profiles of 74 kidney samples, which were divided into two independent groups of fresh frozen tissue and formalin-fixed paraffin-embedded tissue. Subsequent mRNA expression profiling, and pathway analysis were implemented to define the interplay and potential involvement of miRNAs and mRNA in Wilms tumor.

Results: Comparative analysis, irrespective of post-dissection tissue processing, revealed 41 differentially expressed miRNAs, with 27 miRNAs having decreased expression and 14 miRNAs having increased expression in the Wilms tumor tissue compared to healthy kidney tissue. Among global mRNA transcriptomic profile differences, cross-sectional analysis suggested a limited list of genes potentially regulated by differentially expressed miRNAs in the Wilms tumor.

Conclusion: Genetics, epigenetics such as miRNAs, and proteins involved in miRNA cellular processing play an important role in the development of the WT. In addition to the regulatory function of miRNAs, these molecules are also being studied as biological markers for noninvasive primary diagnostic tools , rapid and cost-effective PCR detection. Our study identified 27 miRNAs with decreased expression and 14 miRNAs with increased expression in WT tissue. Additional evaluation of suggested WT miRNAs will emphasize their potential as non-invasive biomarkers and their therapeutic role, preserving the affected kidney and tumor pathogenesis in individuals with WT germinal genetic variants.

PANHYPOPITUITARISM CAUSED BY A SUPRASELLAR GERMINOMA: A CASE REPORT

Mia Radošević[1], Lea Šarić[1], Silvije Šegulja[2], Ana Đorđević[3], Jelena Roganović[4]

[1] Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[2] Faculty of Health Sciences, University of Rijeka, Rijeka, Croatia
[3] Jadran-Galenski Laboratorij d.d.
[4] Children's Hospital Zagreb, Zagreb, Croatia

Introduction: Suprasellar germinomas are rare intracranial tumors frequently associated with permanent endocrine disorders. We present the clinical picture, treatment, and complications of suprasellar germinoma at pediatric age which, besides being life-threatening, has lifelong endocrinological consequences.

Case description: A 12-year-old female patient was presented having had intensive headaches for three weeks and visual disturbances for six months. An ophthalmological examination revealed bilateral papilledema and a marked loss of vision. Emergency brain magnetic resonance imaging (MRI) showed a suprasellar tumor, involving the infundibulum and the optic chiasm, extending to the third ventricle. Laboratory tests confirmed decreased levels of thyroxine, cortisol, gonadotropins, and insulin-like growth factor 1. Maximal tumor reduction was performed, and immunohistopathology established the diagnosis of suprasellar germinoma. MRI of the spine and cerebrospinal fluid cytology confirmed the localized disease. Adjuvant chemotherapy and radiotherapy were performed according to the SIOP CNS GCT II protocol. A post-treatment MRI showed no residual tumor, but pituitary function had not recovered. Three and a half years after the end of the treatment, the patient is in a complete remission, requiring hormonal replacement therapy, continuous education, and psychological support.

Discussion: Suprasellar germinomas are rare tumors that may be associated with hypopituitarism at diagnosis and after therapy. We report the presentation, diagnosis, treatment, and complications of a suprasellar germinoma in a 12-year-old patient. The tumor caused visual impairment, headaches, and hypopituitarism. The patient was treated surgically, followed by adjuvant chemotherapy and radiotherapy. Post-treatment panhypopituitarism required hormonal replacement therapy. The case underscores the complexity of managing these rare tumors and the importance of a multidisciplinary approach. The findings contribute to the understanding of the long-term consequences and holistic lifelong care of pediatric patients with suprasellar germinomas.

TRANSIENT ERYTHROBLASTOPENIA OF CHILDHOOD AND COVID-19 INFECTION: CASE REPORT

Dora Šimić Crnjac[1], Jelena Roganović[2]

[1] Dom zdravlja Zagreb – Centar, Zagreb, Croatia
[2] Children's Hospital Zagreb, Zagreb, Croatia

Introduction: Transient erythroblastopenia of childhood (TEC) is an acquired self-limiting disorder, characterized by anemia and reticulocytopenia due to temporary suppression of erythropoiesis in a previously healthy child. We report a case of simultaneous TEC and Covid 19 infection, and describe the impact of SARS-CoV-2 on the course and outcome of the disease.

Case description: A two-and-a-half-year-old girl was referred to the hospital emergency department due to severe anemia. On admission, she was in good general condition, asymptomatic, with pale skin and mucous membranes, and no other abnormalities. Previous and family history were negative. The findings confirmed isolated severe normocytic normochromic anemia and intercurrent COVID-19 infection. The course was complicated with associated transient neutropenia and thrombocytopenia. Bone marrow aspiration excluded marrow infiltration, revealing accelerated normoblastic erythropoiesis. Spontaneous recovery was observed.

Discussion: The diagnosis of TEC is often delayed due to the gradual onset of the disease. In our patient, SARS-CoV-2 infection was associated with leukopenia, neutropenia, and mild thrombocytopenia. Due to pancytopenia, bone marrow aspiration was performed to exclude leukemic infiltration.

Conclusion: TEC should be included in the differential diagnosis of anemia in a previously healthy young child, aiming to avoid unnecessary diagnostic and therapeutic interventions. Covid 19 infection does not affect a favorable outcome of the disease.

THE INFLUENCE OF CYTOTOXIC DRUGS ON THE IMMUNOPHENOTYPE OF BLAST CELLS IN PAEDIATRIC B PRECURSOR ACUTE LYMPHOBLASTIC LEUKAEMIA

Tomaž Prelog[1], Simon Buček[2], Andreja Brožič[2], Jakob Peterlin[3], Marko Kavčič[1], Janez Jazbec[1], Maša Omerzel[4], Boštjam Markeljc[4], Tanja Jesenko4, Veronika Kloboves Prevodnik[2]

[1] Department of Haemato-Oncology, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia
[2] Department of Cytopathology, Institute of Oncology Ljubljana, Ljubljana, Slovenia
[3] Institute for Biostatistics and Medical Informatics, University of Ljubljana, Ljubljana, Slovenia
[4] Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia

Background: Flow cytometry plays is important in the diagnosis of acute lymphoblastic leukaemia (ALL) and when antigen-specific immunotherapy is indicated. We have investigated the effects of prednisolone, vincristine, daunorubicin, asparaginase and methotrexate on the antigen ex-pression on blast cells that could influence the planning of antigen-specific therapy as well as risk-based treatment assignment.

Methods: Patients aged ≤ 17 years with de novo B-cell ALL (B-ALL) were enrolled in the study. Blast cells were isolated and exposed in vitro to 5 individual cytotoxic drugs in logarithmically increasing concentrations. Then, the expression of CD10, CD19, CD20, CD27, CD34, CD45, CD58, CD66c and CD137 antigens was determined by quantitative flow cytometry.

Results: Cytotoxic drugs caused dose-dependent or dose-independent modulation of antigen expression. Daunorubicin caused a dose-dependent down-modulation of CD10, CD19, CD34, CD45 and CD58 and an up-modulation of CD137. Vincristine caused a dose-dependent down-modulation of CD19 and CD58 and an up-modulation of CD45. Daunorubicin also caused dose-independent down-modulation of CD27 and prednisolone down-modulation of CD10, CD19, CD27, CD34 and CD58. Down-modulation of CD20 was detected only in relation to the specific dose of daunorubicin.

Conclusion: Our results have shown that cytotoxic drugs can alter the expression of antigens that are important for immunotherapy. Importantly, daunorubicin, prednisolone and vincristine caused down-modulation of CD19 and CD58, suggesting that these drugs are better avoided during bridging therapy prior to bispecific antibodies or CAR-T cell therapy. In addition, immuno-phenotypic changes on blast cells induced by different drugs could also influence risk-based treatment assignment.

CAN NUDT15 BE PHARMACOGENETIC OR PHARMACOTRANSRIPTOMIC MARKER FOR 6-MERCAPTOPURINE IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN SERBIA

Bojan Ristivojevic[1], Nikola Kotur[1], Natasa Tosic[1], Biljana Stankovic[1], Vladimir Gasic[1], Djordje Pavlovic[1], Marina Jelovac[1], Goran Milosevic[2], Ivana Grubisa[1], Sonja Pavlovic[1], Branka Zukic[1]

[1] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[2] University Children's Hospital, Belgrade, Serbia

Background: 6-mercaptopurine is a drug used in therapeutic protocol for the treatment of children with acute lymphoblastic leukemia (ALL), mostly during the phase of maintenance therapy. The side effects related to this drug could be partly attributed to pharmacogenetic variants in the TPMT gene. Recently, the NUDT15 gene has been recognized as a new gene of interest for the optimization of 6-mercaptopurine therapy. The association of side effects in children with variants in NUDT15 is well established in Asian populations, yet the relevance of this pharmacogene in European populations remains largely unexplored. The aim of this study was to identify pharmacogenetic variants in the coding and neighboring regions of the NUDT15 gene and analyze if the expression levels of the NUDT15 gene can be of use as a pharmacotranscriptomic marker, to predict the occurrence of side effects of 6-mercaptopurine during the maintenance therapy in children with ALL of Serbian origin.

Methods: The genotyping of coding and neighboring regions of the NUDT15 gene was performed using PCR and Sanger sequencing based technology in 48 children with ALL. NUDT15 expression was analyzed in mononuclear cells of 24 children with ALL before the start of the therapy and 6 healthy individuals by qRT-PCR. The association of genotyping and expression analysis data with surrogate markers of toxicity was assessed using adequate statistical methodology.

Results: This study showed the presence of 5 variants in the NUDT15 gene in the study group (rs61746486, rs79687000, rs45465203, rs41284205, and rs61973267). However, none of the identified variants has shown an effect on the expression or function of the NUDT15 protein. This study showed no statistically significant association between the expression of NUDT15 in mononuclear cells at diagnosis and the surrogate markers of side effects (number of episodes of leukopenia ($p=0.821$), number of weeks without therapy ($p=0.507$), number of weeks with lower dose ($p=0.434$) and average doses ($p=0.374$)) of 6-mercaptopurine during the maintenance therapy.

Conclusion: The data obtained in this study do not support the hypothesis that NUDT15 can be used as a pharmacogenetic or pharmacotranscriptomic marker in predicting the side effects of 6-mercaptopurine therapy in children with ALL in Serbia. In the future, more comprehensive methods on a larger study sample should be performed.

GENETIC ALTERATIONS IN A CONSECUTIVE CHILDHOOD B-ALL COHORT TREATED ON ALL IC-BFM 2009 PROTOCOL IN SLOVENIA

Klementina Črepinšek, Janez Jazbec, Marko Kavčič, Tomaž Prelog, Tine Tesovnik, Barbara Jenko Bizjan, Robert Šket, Jernej Kovač, Maruša Debeljak

University Medical Centre Ljubljana, Ljubljana, Slovenia

Background: In this study, our aim was to comprehensively identify distinct genetic subtypes among patients within our B-ALL cohort using RNA sequencing. Our focus was on characterizing genetic changes, analyzing associated clinical outcomes, and demonstrating the efficacy and reliability of RNA sequencing in a clinical context.

Methods: We conducted our investigation on a consecutive cohort of 99 B-ALL patients treated at the Children's Hospital of the University Medical Centre Ljubljana following the ALL IC-BFM 2009 protocol. Due to limited material, we were unable to analyze two patients. Leveraging RNA sequencing data, we performed gene expression analysis, fusion gene detection, and variant identification. Subtype classification was executed using the R-based tool MD-ALL. To ensure the robustness of our bioinformatic pipeline, we performed multiple subsampling iterations for each sample and reanalyzed the data.

Results: Among the 97 patients included in our analysis, 74 exhibited recurrent genetic abnormalities, while 23 were classified as "B-other". RNA sequencing allowed us to confirm recurrent genetic abnormalities in 71 patients. Notably, we identified additional aberrations in three patients previously categorized as hyperdiploid (two patients) and hypodiploid (one patient), subsequently classifying them as IKZF1 N159Y, Ph-like, and DUX4, respectively. Within the "B-other" group, we successfully pinpointed underlying genetic abnormalities in 21 patients, including three hyperdiploid, seven PAX5alt, three Ph-like, three DUX4, two ZNF384, one IKZF1 N159Y, one PAX5 P80R, and one iAMP21. Despite rigorous analysis, two patients remained unclassified after RNA sequencing. Our results underscored the high repeatability of our approach, as all samples consistently fell into the same genetic subtype across subsampled analyses. Patients in the hyperdiploid and ETV6::RUNX1 groups showed significantly better survival, while those in iAMP, KMT2A, hypodiploid, Ph-positive and Ph-like groups showed significantly poorer outcomes.

Conclusion: Through RNA sequencing, we successfully confirmed or identified genetic aberrations contributing to leukemia development in 96% of our entire cohort, showcasing the reliability of our analysis pipeline. The integration of RNA sequencing into routine diagnostics stands to offer substantial advantages in managing B-ALL patients in our institution, facilitating expedited and accurate diagnostics for all individuals.

GERMLINE VARIANTS IN CANCER PREDISPOSITION GENES IN PEDIATRIC PATIENTS WITH CENTRAL NERVOUS SYSTEM TUMORS

Irena Marjanovic[1], Aleksa Jovanovic[2], Dragana Janic[2], Teodora Karan-Djurasevic[1], Natasa Tosic[1], Ivana Grubisa[1], Nikola Kotur[1], Biljana Stankovic[1], Jovana Komazec[1], Branka Zukic[1], Sonja Pavlovic[1]

[1] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[2] Pediatric Oncology Department, National Cancer Research Center, Belgrade, Serbia

Background: Central nervous system (CNS) tumors comprise around 20% of childhood malignancies. Germline variants in cancer predisposition genes (CPGs) are found in approximately 10% of pediatric patients with CNS tumors. This study aimed to characterize variants in CPGs in pediatric patients with CNS tumors and correlate these findings with clinically relevant data.

Methods: Genomic DNA was isolated from the peripheral blood of 51 pediatric patients and further analyzed by the next-generation sequencing approach. Bioinformatic analysis was done using an "in-house" gene list panel, which included 144 genes related to pediatric brain tumors, and the gene list panel Neoplasm (HP:0002664).

Results: High-grade gliomas were the most prevalent tumor type comprising almost one-third of all cases, followed by medulloblastomas and low-grade gliomas, ependymomas, atypical teratoid rhabdoid tumors and choroid plexus tumors. Our study found that 27% of pediatric patients with CNS tumors have a germline variant in some of the known CPGs, like ALK, APC, CHEK2, ELP1, MLH1, MSH2, NF1, NF2 and TP53. . Three novel variants were detected in the ELP1 gene, 3 in the ALK gene and 1 in the MSH2 gene. Germline variants in the ELP1 gene have been associated with pilocytic astrocytoma for the first time. This study represents the first comprehensive evaluation of germline variants in pediatric patients with CNS tumors in the Western Balkans region.

Conclusion: Our results indicate the necessity of genomic research to reveal the genetic basis of pediatric CNS tumors, as well as to define targets for the application and development of innovative therapeutics that form the basis of the upcoming era of personalized medicine.

FEBRILE NEUTROPENIA IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: HAS BACTERIAL LANDSCAPE CHANGED OVER THE YEARS?

Izabela Kranjčec[1], Nada Rajačić[1], Vinka Knezović[2], Hana Dručak[1], Rebeka Barešić[3], Maja Pavlović[1], Nuša Matijašić Stjepović[1], Domagoj Buljan[1]

[1] Children's Hospital Zagreb, Zagreb, Croatia
[2] Dom zdravlja Zagreb Centar, Zagreb, Croatia
[3] Dječja bolnica Srebrnjak, Zagreb, Croatia

Background: Febrile neutropenia (FN) is a frequent adverse event observed in pediatric acute lymphoblastic leukemia (ALL) cases, accompanied by substantial morbidity and mortality. Prevention, early recognition and prompt intervention are of upmost importance, yet emerging multidrug-resistant pathogens and opportunistic microorganisms represent a worrisome issue.

Methods: A retrospective survey on febrile neutropenia episodes in all children diagnosed with acute lymphoblastic leukemia (ALL) at the Children's Hospital Zagreb in 2018 and 2022 was performed. Epidemiological (sex, age) and clinical (protocol, risk group, FN episodes, blood cultures results, antibiotics prescription and resistance, outcome) data were retrieved from patients' medical records. Descriptive analysis was carried out.

Results: In total, in eighteen children (61.1% male, median age 4 years) treated at the site according to the ALL-IC BFM 2009 protocol (50% as intermediate, 38.9% as high, and 11.1% as standard risk group) 81 episode of FN was recorded (median 4 FN/patient, range 1-11 FN/patient). The majority of FN attacks occurred during consolidation (33.3%), followed by early intensification (27%) and re-induction (19.8%). In one-quarter of FN episodes bacterial infection was identified, Pseudomonas aeruginosa and Escherichia coli were the most common causative agents (23.8%) isolated from blood cultures, and tazobactam/piperacillin the most frequently used antibiotic. Three children (16.7%) died due to sepsis. When comparing the year 2018 and 2022, no remarkable difference was observed regarding the number of patients (10 vs. 8), median age (4.5 vs. 3.5 years), mean number of FN attacks per patient (4.5), treatment phase with greatest number of FN episodes (consolidation), or frequency of positive blood cultures (26.7% vs. 25%). MRSE and Pseudomonas aeruginosa were the most prevalent isolated bacteria in blood cultures in year 2018 and 2022, respectively. The antibiotics susceptibility of Pseudomonas changed over the years, bacteria in 2022 showing resistance to multiple antibiotics, including rather new drugs (ceftazidime/avibactam, ceftolozane/tazobactam).

Conclusion: The results of our study show that, although the burden of febrile neutropenia in pediatric ALL cases has not changed significantly over the years, the shift from Gram-positive to Gram-negative pathogen is evident, and battling multiple-resistant bacteria in children with hematological malignancy is a growing challenge.

HEPATIC SINUSOIDAL OBSTRUCTION SYNDROME AS A COMPLICATION OF A PEDIATRIC SOLID TUMOUR TREATMENT – A SINGLE-CENTRE EXPERIENCE

Nada Rajacic, Jasminka Stepan Giljević

Children's Hospital Zagreb, Zagreb, Croatia

Background: Hepatic sinusoidal obstruction syndrome (SOS) is a form of hepatic injury with distinctive clinical and laboratory characteristics occurring especially after exposure to cytotoxic agents. Its occurrence in the allogeneic bone marrow transplant setting is well known. However, it is essential to recognise this complication during pediatric solid tumour chemotherapy, including high-dosage chemotherapy with autologous stem-cell rescue. Hepatic SOS in children is more prevalent than in adults, more frequently late-onset in the transplant setting and anicteric. Defibrotide usage for severe SOS is associated with better treatment results in the pediatric population.

Methods: We conducted a retrospective survey of 2014-2023 hospital records. Descriptively, we analysed the epidemiological and clinical data on patients suffering from malignant solid tumours and developing hepatic SOS.

Results: Nine solid tumour patients developed hepatic SOS. The average age at diagnosis is 4.1 years, and the median is 3.4 years, with equal sex distribution. Five patients received vincristine and actinomycin-D for Wilms tumour and one for nephroblastomatosis. The left kidney was affected in three, and the right kidney in two cases. The nephroblastomatosis case was bilateral. One patient received the same medications due to paratesticular rhabdomyosarcoma. Hepatic SOS occurred before surgical tumour removal in only two cases. Two patients (Ewing sarcoma, high-risk neuroblastoma) received high-dosage chemotherapy with busulphan and melphalan, followed by autologous hematopoietic stem cell rescue. All patients had hepatomegaly, ascites, significant weight gain and thrombocytopenia. Only two patients had hyperbilirubinemia. Abdominal ultrasound confirmed the diagnosis in all except one patient who had undergone a liver biopsy due to the suspicion of metastatic liver disease. Medical care included strict fluid balance, diuretics, and abundant supportive therapy. Three patients needed abdominal or thoracic drainage. Defibrotide has been available at our institution since 2020; four patients have received it, one of whom developed a severe bleeding complication. When defibrotide was unavailable, we used low-molecular-weight heparin and acetylcysteine. In the later course, two patients continued receiving full doses of vincristine and actinomycin-D after exhibiting tolerance to half the dose of the culprit drugs. Two patients who had undergone high-dosage chemotherapy continued their non-cytotoxic treatment. Five patients underwent therapy suspension or modification. All the patients are in remission, without long-term hepatic sequelae.

Conclusion: Hepatic SOS is a severe complication of pediatric solid tumour chemotherapy application, especially vincristine and actinomycin-D. Defibrotide is readily available nowadays in European countries, and modification of oncological therapy is sometimes necessary.

SELUMETINIB THERAPY IN NF1 PATIENTS WITH PROGRESSIVE PLEXIFORM NEUROFIBROMA

Domagoj Buljan[1], Hana Drucak[2], Filip Jadrijevic Cvrlje[1], Nada Rajacic[1], Jasminka Stepan Giljevic[1]

[1] Department of Oncology and Hematology "Mladen Čepulić", Zagreb, Croatia
[2] Children's Hospital Zagreb, Zagreb, Croatia

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder of the central and peripheral nervous system caused by mutations in the NF1 gene and sporadic mutations in more than 50% of patients. Optic gliomas and progressive plexiform neurofibromas may develop in specific individuals. In plexiform neurofibroma, radiotherapy is contraindicated due to potential malignant alterations, chemotherapy is inefficient, and surgery carries a significant risk of operative complications and disease recurrence. Selumetinib, a selective MEK1/2 inhibitor, has recently been used to treat symptomatic children aged three years and above who have inoperable plexiform neurofibromas.

Methods: Investigations were conducted on the indication for the use of selumetinib in children with NF1 and inoperable plexiform neurofibromas aged 3 to 17 who were treated at the Department of Oncology and Haematology “Mladen Čepulić” in Children's Hospital Zagreb. In addition to general patient data, other records included the time from the diagnosis of NF1 to the introduction of the drug, the average length of therapy, dosage, and adverse events. Descriptive statistics were performed.

Results: Selumetinib treatment was administered to 4 patients (M = 2; 50%; F = 2; 50%) who were diagnosed with NF1 and an inoperable plexiform neurofibroma. The mean age at the time of the NF1 diagnosis was 1.8. The mean interval between the diagnosis and the progression of plexiform neurofibroma (treatment indication) was 7.6 years, with a range of 3 to 12 years. Frequent pain, airway compression and dislocation, optic nerve and bulbar compression were the rationales for initiating selumetinib therapy in our cohort. The treatment lasted approximately nine months with a mean dose of 25 mg/m2 twice a day without further disease progression. Headache, stomach pain, diarrhoea, rash and mildly elevated lactate dehydrogenase and creatine kinase were the most commonly reported adverse effects.

Conclusion: With more convincing data in pediatric patients, selumetinib can keep the disease in a "stable state" without further progression, thus improving the quality of life and functional capacity by reducing pain and preventing the mass effect, i.e., compromising airway and breathing or leading to visual impairment. The acceptable safety profile and absence of cumulative toxic effects permit long-term treatment.

MULTISYSTEM LANGERHANS CELL HISTIOCYTOSIS IN A 17-MONTH-OLD BOY – A CASE REPORT

Sonja T. Marinković, Biljana Đurđević-Banjac, Dragana Malcic-Zanic, Jelica Predojevic-Samardzic

Children's Hospital, University Clinical Centre of The Republic of Srpska, Banja Luka, Bosnia and Hercegovina

Introduction: Langerhans cell histiocytosis (LCH) is a neoplasm of pathologic dendritic cells expressing CD1a and CD207 (Langerin) surface antigens, which some consider a reactive immune disorder and others a neoplastic disorder. The estimated incidence is 4 to 5 per million children 0 to 15 years of age each year. The highest incidence rate is observed before 1 year of age.

Case description: A 17-month-old boy presented to the Pediatric clinic with localised bilateral oedema of the feet dorsum. History reveals that he has had severe seborrhoeic dermatitis resistant to treatment since he was 8 months. Clinical examination shows disseminated seborrhoeic changes with excoriations and crusts dominantly on the head, back and inguinal area, accompanied by bilateral retro auricular swelling, granulomatous lesions of the hard palate, hepatosplenomegaly and bilateral non-pitting oedema of the feet dorsum. A full workup including a biopsy of the retro auricular changes confirmed a multisystem Langerhans cell histiocytosis, with craniofacial bone, skin, liver, spleen and haematopoietic involvement. The patient was started on a 6-week course of vinblastin (6mg/m2) and prednisone (40 mg/m2) according to the Histiocyte Society protocol (LCH-I protocol).

Discussion: Clinical presentations of LCH vary from single lesions to life-threatening disseminated disease. The most commonly affected organs are bone, skin, pituitary, liver, spleen, hematopoietic system and lungs. Cutaneous involvement is typically presented as pinpoint erythematous or skin-coloured papules or pustules. In infants, a seborrheic dermatitis-like rash on the scalp often causes LCH to be misdiagnosed as seborrheic dermatitis, while groin involvement can present as treatment-resistant, recurring diaper dermatitis. Additionally, cutaneous disease is the most common manifestation in patients younger than 2 years of age and is typically representative of multisystem disease. Cutaneous lesions are associated with hepatomegaly, splenomegaly, bone damage and lung damage, whilst isolated cutaneous disease accounts for only 2% of total cases. The prognosis depends on the initial presentation and mortality rates may be as high as 50% for the multisystem disease.

Conclusion: In conclusion, the key is to make an early diagnosis. Considering that cutaneous involvement is the most common manifestation of LCH in children and that skin changes are often misdiagnosed as seborrhea or atopic dermatitis resistant to typical treatments, a skin biopsy and further investigations are obliged in order not to miss the diagnosis of this rare disease.

GASTROINTESTINAL COMPLICATIONS IN CHILDREN UNDERGOING ABDOMINAL AND/OR PELVIC RADIOTHERAPY FOR SOLID TUMOURS

Maja Pavlović[1], Nuša Matijašić Stjepović[1], Domagoj Buljan[1], Toni Krnić[2], Izabela Kranjčec[1]

[1] Children's Hospital Zagreb, Zagreb, Croatia
[2] Sestre milosrdnice University Hospital Centre, Zagreb, Croatia

Background: Abdominal and pelvic irradiation is a part of standard multimodality therapy for solid tumours in children. It is associated with various short-term adverse effects that must be identified and managed adequately to avoid or mitigate long-term consequences.

Methods: A retrospective observational study of the most frequent gastrointestinal (GI) toxicities following abdominal and pelvic radiotherapy was conducted at the Children's Hospital Zagreb. Medical records of pediatric patients treated for solid tumours from 2019 to 2024 were analyzed for demographic (age, gender) and clinical (diagnosis, initial presentation/relapse, stage, treatment phase, previous abdominal surgical procedures, cumulative irradiation dose and GI toxicities) data. Gastrointestinal disorders were classified and graded according to the CTCAE 5.0 version (2017).

Results: Fourteen children (9 males, mean 7,3 years, range 4-17) were evaluated. The most common diagnosis was advanced-stage neuroblastoma (42,8 %). Irradiation was performed mainly as a part of initial treatment (71,4 %), followed by abdominal surgery at 78,6 %, with a mean cumulative dose of 29,74 Gy. Nausea and vomiting following diarrhoea of low grade (1-2) were the most frequently reported toxicities (50 % and 35,7 %, respectively). One serious case of enterocolitis (grade 3/4) warranted prolonged multimodal therapy (adhesiolysis, corticosteroids, enteral nutrition, antibiotics) with complete recovery, while all others spontaneously resolved with minimal symptomatic intervention.

Conclusion: This study shows that GI toxicities following radiotherapy are mild and easily manageable in pediatric patients with solid tumours. It considers small patient numbers, varying radiation field sizes, and significant differences in irradiation dosage, which directly correlate to the occurrence and intensity of adverse events.

PSYCHOLOGICAL PRESENTATION OF A GIRL WITH HIGH-RISK ABDOMINAL NEUROBLASTOMA

Sara Lulić Kujundžić, Mateja Dodig, Jasminka Stepan Giljević

Children's Hospital Zagreb, Zagreb, Croatia

Introduction: Psychological treatment of hospitalized children with malignant diseases during active treatment is a demanding and complex process that requires continuous multidisciplinary monitoring of the patient. From a psychological perspective, a child's malignant disease brings an overall change in the child's daily functioning, the absence of previously usual life activities, isolation from the peer and family environment, a change in family dynamics and structure, physical transformation and different stress coping mechanisms. Adaptation to hospital conditions and the child's health situation takes a different period of time for each patient and their environment, and the difficulties they encounter are also individual and depend on numerous factors, including personality traits.

Case description: During her first hospitalization, a four-year-old girl with high-risk abdominal neuroblastoma reacts to health workers with intense fear and refusal, and difficulties are present in the context of selective mutism. It can be seen that the girl is insecurely attached to her mother, the volume of the girl's communication between mother and mother is also reduced (which also expresses difficulties in adapting to the new situation), and the girl exhibits regressive forms of behavior (refusal to walk, finger sucking, lying in the fetal position).

Discussion: With the intervention of a psychologists, the mother's role alongside the child was replaced by the grandmother, and the girl was included in an intensive treatment based on the behavioral activation. After a few days, changes are visible, the girl walks, participates in activities, establishes contact with psychologists and communicates in front of them. During the entire intensive treatment, the girl was involved in continuous psychological support. During the immunotherapy, which includes the use of retinoids, the girl had emotional and motor restlessness and agitation, and in situations of increased intensity of these, promazine was required. During the following cycles of therapy, the girl becomes more adaptable to new situations, has an orderly mood and is cooperative with the staff.

Conclusion: During the treatment, we observed adjustment disorders of the child and mother, hospitalism syndrome, regression and, in the final phase of treatment, symptoms of post-traumatic stress disorder as well as behavioral disturbances related to the introduction of retinoid therapy as part of the immunotherapy maintenance phase. With this presentation, we wanted to highlight the importance of the work of a multidisciplinary team, from monitoring the patient, responding in a timely manner, and simultaneously respecting the mental and physical health of the child.

A NOVEL LIKELY PATHOGENIC SEQUENCE VARIANT IN THE RUNX1 GENE AS THE CAUSE OF CONGENITAL THROMBOCYTOPENIA – CASE REPORT

Marta Despotović[1], Nina Pereza[1], Borut Peterlin[2], Saša Ostojić[1], Barbara Golob[2], Aleš Maver[2], Jelena Roganović[3]

[1] Faculty of Medicine, University of Rijeka, Croatia
[2] Clinical Institute for Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia
[3] Children's Hospital Zagreb, Zagreb, Croatia

Introduction: Heterozygous sequence variants in the RUNX1 (Runt-related Transcription Factor 1) gene are a common genetic cause of thrombocytopenia and/or platelet dysfunction and an increased risk for myelodysplasia and acute myeloid leukemia. The majority of causative variants are substitutions, which rarely occur de novo. We present a patient with congenital thrombocytopenia caused by de novo deletion variant in exon 9 in the RUNX1 gene.

Case description: A one-month-old male infant was hospitalized because of anemia and thrombocytopenia in the course of acute viral infection. During follow-up, he was in a good general condition with occasional bruising on lower extremities after mild trauma. The patient had persistent slightly decreased values of platelets with normal morphology, but with pathological aggregation with adrenaline and adenosine diphosphate. Due to unclear persistent thrombocytopenia, at the age of five, he was referred for genetic testing. Whole-exome sequencing was performed using next-generation sequencing method (Illumina NovaSeq 6000). A heterozygous likely pathogenic frameshift variant, c.1160delG (NM_001754.4), was identified in exon 9. The report of genetic testing in the patient's father, who is a childhood leukemia survivor, was negative, indicating that the variant is likely de novo.

Discussion: In our patient, whole-exome sequencing revealed the presence of a heterozygous deletion of one guanine in exon 9 (c.1160delG), which was classified as a likely pathogenic variant leading to a frameshift in the RUNX1 protein. Pathogenic heterozygous variants in the RUNX1 gene represent an established cause of mild to moderate thrombocytopenia, functional and ultrastructural platelet defects, and predisposition to myelodysplastic syndrome, acute myeloid leukemia, and less frequently acute T-cell lymphoblastic leukemia.

Conclusion: To the best of our knowledge, this is the first report of the heterozygous de novo variant c.1160delG in the RUNX1 gene. Although pathogenic variants in the RUNX1 genes are very rare, persistently low platelet counts of unclear etiology should raise suspicion of an underlying genetic disorder.

APPENDICEAL NEUROENDOCRINE TUMORS IN CHILDREN AND ADOLESCENTS

Neža Salobir[1], Janez Jazbec[2]

[1] Community health Centre Ljubljana, Ljubljana, Slovenia
[2] University Medical Centre Ljubljana, Ljubljana, Slovenia

Background: Neuroendocrine tumors of the appendix (aNET) are slow growing, indolent tumors which have an excellent prognosis and rarely present with regional node metastasis. They represent 0,1 % of malignancies in children. The treatment and follow-up of aNET in children are not standardised. Appendectomy alone is considered curative for most patients, and more aggressive surgical approach is warranted in the cases with incompletly excised tumors or size > 2 cm.

Methods: Over the last 11,5 years 23 children and adolescents were diagnosed with aNET in Slovenia. We examined the operative reports, histopathology reports and clinical notes from follow-up visits for all patients.

Results: All patients had a diagnosis of well-differentiated aNET. No tumor was larger than 2 cm, the average size was 7 mm. Excision margins were free in 21 of 23 patients. In 3 of 23 a righ hemicolectomy was performed, and in 1 residual tumour was detected in a lymph node. None of the 23 patients had any signs of relapse during follow-up (median follow-up of 3 years).

Conclusion: Reported data and our experience showed that no relapse or death occurred in children and adolescents affected by aNET. Appendectomy alone should be considered curative for most patients, and a more aggressive surgical approach is warranted in the cases with incompletely excised tumours. The decision about treatment in the presence of risk factors for advanced disease should be made by a multidisciplinary team.

MALIGNANT TESTICULAR TUMOR IN A SIXTEEN-MONTH OLD BOY: CASE REPORT

Iva Kapetanović[1], Jelena Roganović[2]

[1] Clinical Hospital Centre Rijeka, Rijeka, Croatia
[2] Children`s Hospital Zagreb, Zagreb, Croatia

Introduction: Malignant testicular tumors are extremely rare in children. They typically present as painless palpable masses or enlargement of the testis. We present the clinical presentation, diagnostic evaluation and treatment of endodermal sinus tumor in a 16-month-old boy.

Case description: The patient was presented with painless enlargement of the right testis noticed by his mother over one month. A firm, painless palpable right scrotal mass was identified on physical examination. Laboratory findings showed elevated levels of serum alpha-1-fetoprotein (AFP). Ultrasound and computed tomography (CT) of the testis, pelvis and abdomen confirmed a testicular mass with increased vascularity. A radical right-sided orchiectomy was performed, and the histopathological analysis established the diagnosis of a prepubertal yolk sac tumor (endodermal sinus tumor). The patient was regularly followed-up clinically, with laboratory tests and ultrasound, and is in remission four years after the diagnosis.

Discussion: Testicular tumors in children are rare, with endodermal sinus tumor being the most common malignant tumor in boys before puberty. Clinical presentation typically includes painless testicular swelling. Differential diagnoses include hydrocele, varicocele, inguinal hernia, testicular torsion, and epididymoorchitis. Diagnosis relies on tumor marker measurement, with AFP being highly elevated in approximately 95-100% cases. On ultrasound, endodermal sinus tumor typically appears as a solid intratesticular mass with homogeneous parenchyma and increased vascularity. CT scans of the chest, and magnetic resonance imaging of the abdomen and pelvis are used to assess disease extent. Treatment involves radical orchiectomy for localized disease, with adjuvant chemotherapy reserved for advanced cases. Regular follow-up improves overall prognosis.

Conclusion: Testicular enlargement can raise suspicion of a malignant tumor. Early diagnostic evaluation and appropriate management are crucial for favorable outcome.

SPITZOID MELANOMA – CASE REPORT

Klara Vranešević[1], Martina Atelj[2], Andrea Dekanić[3], Jelena Roganović[4]

[1] Institute of Emergency Medicine of Osijek-Baranja, Osijek, Croatia
[2] Dom zdravlja Zadarske županije, Zadar, Croatia
[3] Department of Pathology and Cytology, Clinical Hospital Center of Rijeka, Rijeka, Croatia
[4] Children`s Hospital Zagreb, Zagreb, Croatia

Introduction: Spitzoid melanoma is a subtype of melanoma that resembles a benign skin lesion called a Spitz nevus, both in clinical and histological features. It commonly presents as a nodule, often reaching 1 cm or more in diameter. It is most often amelanotic and located on the head or limbs. Although rare, melanomas in children pose clinical challenges.

Case description: A 7-year-old female patient presented with a grey skin lesion located on the right thigh. The patients' mother noticed a change in color and shape of the lesion that had appeared 6 months prior. On admission, a solitary, firm, nontender, sessile nodule measuring 5 mm in diameter was seen. The patient underwent excision, and a pathohistological finding was Spitzoid melanoma, pT2a, Clark III, Breslow 1.77 mm. The re-excision of the lesion with wide margins and a sentinel lymph node biopsy was performed, which was negative. Further diagnostic work up revealed no metastasis. The patient was also referred to a genetic testing which revealed a pathogenic heterozygous variant in the CHEK2 gene. In the later course, she was followed-up closely, and is in remission three years after surgery.

Discussion: Spitzoid melanomas can affect children. "ABCDE" criteria, commonly used for clinical melanoma diagnosis, are detected in less than 50% of Spitzoid melanoma cases as they are usually amelanotic. Because of the rareness and a wide range of clinical appearances, they are often unrecognized in children. Spitzoid melanomas can develop de novo or can be related to a preexisting Spitz nevus. Other features supporting a diagnosis include ulceration, asymmetry, deep penetration to the dermis and subcutis, a high degree of cytologic atypia and a high mitotic rate. The treatment of the same guidelines as for other types of melanomas, which is based on Breslow and Clark classification.

Conclusion: Spitzoid melanoma remains one of the most challenging lesions to diagnose in dermatopathology, due to similarities to Spitz nevus. First-line treatment is based on the excision of the tumor with wide surgical margins. Molecular profile and the role of adjuvant therapy have yet to be established.

INVESTIGATING THE GENETIC COMPLEXITY OF NEUTROPENIA IN PEDIATRIC PATIENTS WITH GLYCOGEN STORAGE DISEASE IB: A MODIFIER GENE PERSPECTIVE

Anita Skakic[1], Marina Parezanovic[1], Djordje Pavlovic[1], Nina Stevanovic[1], Marina Andjelkovic[1], Kristel Klaassen[1], Milena Ugrin[1], Jovana Komazec[1], Vesna Spasovski[1], Maja Djordjevic[2], Maja Stojiljkovic[1]

[1] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[2] Mother and Child Healthcare Institute „dr. Vukan Cupic", School of Medicine, University of Belgrade, Belgrade, Serbia

Background: Glycogen Storage Disease Ib (GSD Ib) is a rare metabolic disorder characterized by deficiency of the glucose-6-phosphate translocase (G6PT), leading to impaired glucose homeostasis. A major characteristic of GSD Ib is neutropenia, accompanied by metabolic disturbances. The severity and progression of neutropenia, along with neutrophil dysfunction, have been observed to show variation among individuals sharing the same genotype. Despite GSD Ib being a monogenic disease, extensive research and clinical experience have revealed that the relationship between genotype and phenotype is not straightforward. This study aims to elucidate the role of potential modifier genes in the context of neutropenia in GSD Ib, with a particular focus on five pediatric patients harboring the pathogenic homozygous genetic variant c.1042_1043delCT in the SLC37A4 gene. Notably, this group of patients exhibits a diverse course of neutropenia, with two patients manifesting mild and intermittent neutropenia, while others experience severe and persistent neutropenia.

Methods: Whole genome sequencing was conducted on five subjects from unrelated non-consanguineous families, all presenting with previously identified pathogenic homozygous variant in the SLC37A4 gene. Advanced bioinformatics tools were employed to analyze the genomic data and explore potential associations between genetic variations and the observed clinical variations in neutropenia. We performed parameterized variant filtering, pathogenicity score-based prioritization (based on scores from pathogenicity scoring tools), and functional association to identify the modifier variant(s) (based on functionally annotated ontologies and knowledgebase).

Results: Our study reveals a complex interplay of potential modifier genes in these subjects, providing insights into the phenotypic heterogeneity observed in GSD Ib patients with the specific c.1042_1043delCT variant. The distinct neutropenic courses, with two patients exhibiting mild and intermittent neutropenia, and others with severe and persistent neutropenia, highlight the importance of further investigation into the genetic factors influencing disease presentation.

Conclusion: This research underscores the potential significance of modifier genes, particularly within the context of the identified pathogenic variant in the SLC37A4 gene, in shaping the diverse course of neutropenia in GSD Ib. Understanding potential genetic modifiers can provide valuable insights into the molecular base of the disease and guide future research focused on developing customized therapeutic approaches for the specific neutropenic phenotype.

INHERITED THROMBOPHILIA AND RISK OF THROMBOSIS IN CHILDREN WITH CANCER: A SINGLE-CENTER EXPERIENCE

Ana Đorđević[1], **Blaženka Grahovac**[2], **Lidija Bilić Zulle**[3], **Jelena Roganović**[4]

[1] Jadran-Galenski Laboratorij d.d.
[2] Department of Pathology, Clinical Hospital Centre Rijeka, Rijeka, Croatia
[3] Faculty of Medicine Rijeka, Rijeka, Croatia
[4] Children`s Hospital Zagreb, Zagreb, Croatia

Background: Thrombosis is an increasingly recognized complication of childhood malignancy and its treatment. The incidence and etiology of pediatric cancer-related thrombosis is still not well understood. The aim of this study was to evaluate the prevalence of common prothrombotic genetic conditions in children with cancer, the frequency of thrombosis, and the role of inherited thrombophilia in the development of thrombosis in a pediatric oncology population.

Methods: Forty-seven children (36 treated for hematological malignancies and 11 for solid tumors) with a median age of 8.8. years (range 0.4 – 19.3 years) were included in the study. Genetic polymorphisms of Factor V Leiden (G1691A), prothrombin G20210A, and methylenetetrahydrofolate reductase (MTHFR) C677T were determined by real-time polymerase chain reaction-based DNA analysis.

Results: Four (8.5%) patients were heterozygous for Factor V Leiden, 3 (6.4%) were heterozygous for prothrombin G20210A mutation, and 3 (6.4%) were homozygous for MTHFR C677T mutation. All patients had implanted central venous catheters. Four (8.5%) children had documented thrombosis, three of which were in the upper venous system. Two of the four patients with thrombosis had Factor V Leiden heterozygosity.

Conclusion: Thrombosis is an important complication of childhood cancer. The risk of thrombosis may be increased in patients with Factor V Leiden. In the absence of consensus guidelines, our results support the recommendation for thrombophilia screening in children with cancer.

TREATMENT OF HODGIN LYPMHOMA IN CHILDHOOD: 10-YEARS EXPERIENCE WITH PET BASED PROTOCOLS

Goran Milosevic[1], Predrag Rodic[1], Nada Krstovski[1], Dejan Skoric[1], Nabil Alhayek[2], Jovana Svorcan[1], Jovana Kostadinovic[1], Milos Veljkovic[3], Jelena Lazic[1]

[1] University Childrens Hospital, Belgrade, Serbia
[2] Faculty of Medicine, University of Belgrade, Serbia
[3] Center of Nuclear Medicine, University Clinical Center of Serbia, Belgrade, Serbia

Background: Pediatric Hodgkin Lymphoma is a complex malignancy with excellent survival rates that exceeds overal survival of 90%. The backbone of treatment is chemotherapy adjusted for disease stage and radiotherapy for selected patients. Contemporary large prospective trials aims for reduction/elimination of radiotherapy for good responders in order to minimize both acute and long-term toxicities associated with irradiation. Our study aimed to evaluate the results of PET/CT assessment at interim and end-of-treatment timings and its effect on treatment outcomes.

Methods: The study included 61 pediatric patients diagnosed with Hodgkin lymphoma at our institution in the last ten years. According to treatment protocol, patients were categorized into two groups. First group was treated according to protocols that used conventional CT diagnostics (27 patients), while second group was treated according PET/CT-based protocols (34 patients).

Results: Our results showed that the use of PET/CT in initial evaluation and early-response assessment, reduced the use radiotherapy compared to classical CT evaluation (32% vs. 52%), without effect on treatment outcome. Initial PET/CT evaluation enabled us to diagnose bone involvement in 4 patients, excluding the need for more invasive procedures such as bone marrow biopsy. In 22% of patients in PET/CT group, PET/CT was found to be discordant negative (PET/CT negative and classical CT positive) which allowed us to continue treatment without irradiation of these patients.

Conclusion: Our results confirmed the benefits of the use of PET/CT in guiding treatment decisions, thus reducing the number of children being exposed to radiation therapy and avoiding radiotherapy related toxicities.

SUPEROXIDE DISMUTASE AND INTERLEUKIN-2 RECEPTOR IN PAEDIATRIC PATIENTS WITH CHRONIC KIDNEY DISEASE OR HYPERTENSION

Mirjam Močnik, Sonja Golob Jančič, Martina Filipič, Evgenija Homšak, Mateja Svetej, Nataša Marčun Varda

University Medical Centre Maribor, Maribor, Slovenia

Background: Oxidative stress and systemic inflammation are significant contributors to the development and progression of cardiovascular disease, causing adverse effects on vascular health and atherosclerosis from an early age. Patients with established cardiovascular risk factors commonly exhibit markers indicating heightened oxidative stress and inflammation. Our study sought to assess the levels of interleukin-2 receptor and superoxide dismutase, which could serve as early indicators of cardiovascular damage due to oxidative stress and inflammation in at-risk children.

Methods: 50 paediatric patients with hypertension, 46 paediatric patients with chronic kidney disease and 33 healthy controls were included in the study. In all, anthropometric measurements, body composition, liver damage and kidney function tests along with superoxide dismutase and interleukine-2 receptor levels were determined.

Results: Superoxide dismutase levels did not differ among groups, however, interleukin-2 receptor levels were significantly lower in patients with hypertension ($p<0.001$) and with overweight/obesity presence ($p<0.001$). Interleukin-2 receptor levels also significantly correlated with several anthropometric measurements and body composition parameters as well as with liver damage and kidney function tests, which was not confirmed for superoxide dismutase.

Conclusion: Superoxide dismutase and interleukin-2 receptor are potential markers for cardiovascular risk and atherosclerosis-associated inflammation assessment in children. Superoxide dismutase might have a greater role with obesity and dyslipidemia present. Interleukin-2 receptor has different roles – while its proinflammatory effect has been implied with kidney function markers associations but lacked the increase among patients with chronic kidney disease, IL-2 might be down-regulated in children with obesity with negative correlations with several adiposity measures and associated laboratory results affecting low-grade inflammation in early stages of obesity.

SERUM AND URINE UROMODULIN DETERMINATION IN CHILDREN WITH CHRONIC KIDNEY DISEASE

Sonja Golob Jančič, Mirjam Močnik, Martina Filipič, Evgenija Homšak, Mateja Svetej, Nataša Marčun Varda

University Medical Centre Maribor, Maribor, Slovenia

Background: Uromodulin is a kidney-specific glycoprotein with an immunomodulatory function. It can be measured in urine and serum. The first is associated with glomerular filtration, and the second with predictive value in cardiovascular risk assessment. The aim of this study was to determine urine and serum uromodulin values in children with chronic kidney disease compared to healthy controls. Additionally, serum uromodulin in children with hypertension was measured.

Methods: 45 paediatric patients with chronic kidney disease and 30 healthy controls had urine and serum uromodulin determined. Additionally, 50 hypertensive patients had serum uromodulin measured.

Results: Serum and urine uromodulin correlated positively; however, serum uromodulin did not differ significantly between groups. On the contrary, urine uromodulin was statistically lower in children with chronic kidney disease ($p<0.001$). Urine uromodulin also correlated significantly with systolic and diastolic pressure, liver elastography, kidney function tests, urate and albuminuria.

Conclusion: Urine uromodulin is significantly lower in children with chronic kidney disease. It also showed better correlation with kidney function and blood pressure than serum uromodulin.

EXTRACELLULAR VESICLES ORIGINATED FROM MESENCHYMAL CELLS OF PERITONEAL DIALYSATE REDUCE FIBROSIS

Beáta Szebeni[1,2], Péter Bokrossy[1], Apor Veres-Székely[1,2], Domonkos Pap[1,2], Zoltán Varga[3], Judith Mihály[3], Éva Pállinger[4], Maria Bernáth[1], György Reusz[1], Attila J Szabó[1], Ádám Vannay[1]

[1] Pediatric Center, MTA Center of Excellence, Semmelweis University, Budapest, Hungary
[2] HUN-REN–SU Pediatrics and Nephrology Research Group, Budapest, Hungary
[3] HUN-REN–TTK Biological Nanochemistry Research Group, Institute of Materials and Environmental Chemistry, Budapest, Hungary
[4] Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, Hungary

Background: There are several data about the beneficial effect of cell therapy in different experimental fibrosis models. Using extracellular vesicles (EVs) as an alternative to cell therapy may have benefits, including lower immunogenicity, the ability to cross the blood-brain barrier, and not inducing acute immune rejection.

Methods: Mesenchymal cells (MSCs) were isolated from peritoneal dialysate (PDE) of patients treated with continuous ambulatory peritoneal dialysis (n=3). Extracellular vesicles (EVs) were separated by tangential flow filtration and size exclusion chromatography from the supernatant of the isolated MSCs. EVs were characterized based on their size and particle numbers, surface markers, morphological features and by their more typical cargo proteins. Their importance in the regulation of fibroblast activation was tested using primary peritoneal fibroblast (pFB) isolated from peritoneal biopsy collected during removal of the Tenchoff catheter.

Results: The isolated mesenchymal cells showed CK-18, α-SMA, CD73, CD105 and CD90 positivity and lack of CD34, HLA-DR, CD45 and CD19 markers demonstrated by immunofluorescent staining and RT-PCR. The separated EVs also showed stem cell marker and CK18 positivity, suggesting that their donor cells are the (isolated) MSCs, which had undergone mesothelial mesenchymal transition. EVs successfully internalized into pFBs and reduced their PDGF-B induced proliferation as demonstrated by the MTT assay. Moreover, these EVs decreased the TGF-β induced collagen accumulation and EGF induced migration of these cells as shown by Sirius Red and TAS assays respectively.

Conclusion: Based on the potential antifibrotic properties of these EVs they may have therapeutic significance, however in vivo testing is also necessary to justify this assumption. This research was supported by the National Research, Development and Innovation Office (NKFIH) K-142728, K 131594; and 2020-1-1-2-PIACI-KFI_2020-00021, Semmelweis University, TKP2021-EGA-24, TKP2021-EGA-31, RRF-2.3.1-21- 2022-00003; Hungarian Research Network, ELKH-POC-2022-024; the New National Excellence Program of the Ministry for Culture and Innovation from the Source of the National Research, Development and Innovation Fund, ÚNKP-23-3-I-SE-36, ÚNKP-23-3-I-SE-42, ÚNKP-23-4-II-SE-29, ÚNKP-23-5-SE-15; Hungarian Academy of Sciences, János Bolyai Research Scholarship.

SALUSIN-β IN CHILDREN WITH CHRONIC KIDNEY DISEASE OR HYPERTENSION

Mirjam Močnik, Sonja Golob Jančič, Martina Filipič, Evgenija Homšak, Mateja Svetej, Nataša Marčun Varda

University Medical Centre Maribor, Maribor, Slovenia

Background: Salusins are newly defined endogenous peptides in the atherosclerotic process. Salusin-β has a proatherogenic role. Since the atherosclerosis begins already in childhood, it serves as a potential biomarker in cardiovascular risk assessment. The aim of our study was to determine its levels in children with chronic kidney disease or hypertension compared to healthy controls. Additionally, we assessed its relation to obesity and pulse wave velocity. The latter is a known marker for arterial elasticity determination.

Methods: 96 paediatric patients (46 with chronic kidney disease, 50 with hypertension) and 33 healthy controls were included in this cross-sectional study. In all, anthropometric measurements, pulse wave velocity and serum salusin-β values were determined.

Results: Salusin-β levels were elevated in patients with chronic kidney disease (p=0.014), but not in patients with hypertension compared to healthy controls. All participants were additionally divided according to weight (normal vs. overweight) status, where levels of salusin-β did not differ between both groups. Correlating salusin-β levels with pulse wave velocity showed significant but weak correlation (r=0.211, p=0.020).

Conclusion: Salusin-β levels were elevated in paediatric patients with chronic kidney disease. Additionally, salusin-β levels correlated significantly with pulse wave velocity. Obesity played a smaller role in these correlations, with significant correlations observed only after combining cardiovascular risk factors revealing certain associations between salusin-β levels and some cardiovascular variables, but with inconclusive findings and, in some instances, even contrary to anticipated outcomes.

CHARACTERIZATION OF 16 NOVEL GENETIC VARIANTS IN GENES ASSOCIATED WITH PAEDIATRIC EPILEPSY: IMPLICATIONS FOR TARGETED THERAPEUTIC STRATEGIES

Marina Andjelkovic[1], Kristel Klaassen[1], Anita Skakic[1], Irena Marjanovic[1], Ruzica Kravljanac[2], Maja Djordjevic[2], Biljana Vucetic Tadic[2], Bozica Kecman[2], Sonja Pavlovic[1], Maja Stojiljkovic[1]

[1] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[2] Mother and Child Healthcare Institute „dr. Vukan Cupic", School of Medicine, University of Belgrade, Belgrade, Serbia

Background: Childhood epilepsies are caused by heterogeneous underlying disorders where approximately 40% of the origins of epilepsy can be attributed to genetic factors. The application of next-generation sequencing has revolutionized molecular diagnostics and has enabled identification of disease-causing genes and variants in childhood epilepsies.

Methods: In our study, 55 children with epilepsy of unknown etiology were analyzed combining clinical-exome and whole-exome sequencing.

Results: Molecular genetic cause of epilepsy was identified in 31 patients and the overall diagnostic success rate was 56%. We identified variants in 23 different genes associated with epilepsy that correlate well with the observed phenotype. This includes genes such as ASH1L, CILK1, KCNMA1, RHOBTB2 and SLC13A5, which have only recently been associated with epilepsy. Half (51.6%) of solved patients carried novel variants. These sixteen novel variants were characterized using various in silico algorithms including Phyre2, EzMol, Aminode and MutPred2 for structure prediction. Interestingly, identification of a causative gene directed attention to 15 individuals, including six individuals who carry entirely novel genetic variants, for whom therapeutic options may be available (ALDH7A1, GRIN1, KCNQ2, PNPO, SCN1A and SCN2A).

Conclusion: Described novel variants will contribute to better understanding of the European genetic landscape, while insights on genotype-phenotype correlation will contribute to better understanding of childhood epilepsies around the globe. Given the expansion of molecular-based approaches, each newly identified genetic variant could become a potential therapeutic target.

THE FIRST 5 YEARS OF THE PEDIATRIC HOME VENTILATION PROGRAM (OLP) IN A HUNGARIAN CENTRE.

Dorottya Antics, Eszter Muzslay, János Benjámin Hérák, Zsüliet Bojtár, Lajos Kovács, Dóra Krikovszky, Judit Kelemen, Attila J. Szabó, Dorottya Czövek

Pediatric Center Semmelweis University, Budapest, Hungary

Background: In recent years, the use of long-term home ventilation has undergone major changes and its indications have expanded. With new technologies and devices, the aim is now to reduce hospitalization and improve quality of life. With the development of non-invasive ventilation (NIV), the use of tracheostomy in children has decreased significantly. This presentation aims to describe the first 5 years of the pediatric home ventilation program in the Pediatric Center of Semmelweis University

Methods: According to the guidelines, children diagnosed with daytime manifest respiratory failure or sleep-disordered breathing confirmed by polysomnography who are being cared for in the clinic or referred to the clinic are enrolled. Once the indication has been established and after approval by the College of Anaesthesiology and Intensive Care, the process of inclusion begins. The choice of mask and ventilator type is made according to the child's age, weight, underlying medical condition and ventilator dependency. The machine settings are determined in the clinic and after training, families continue the ventilation of the child in their home. Children are regularly monitored during outpatient follow-up visits.

Results: Until spring 2024, 91 children (53 boys, age 8.4 ± 6.2 years) were enrolled in the program. The youngest child enrolled was 3 weeks old and weighed 2450 g, and the largest patient was 153 kg. Most of the patients were children with muscular dystrophy (47%) and children hypoventilating due to obesity or obesity-related syndromes (Down syndrome, Prader-Willi syndrome) (22%). During the 5 years, 1 child was transitioned to adult care, 2 children were discontinued from NIV therapy (under close monitoring) due to improvement in their condition, and 2 children were excluded from the program due to severe compliance problems. Since the OLP was established, 6 children have died. Most complications were related to mask-wearing and aerophagy. No child had to be excluded from the program due to a ventilation-related complication.

Conclusion: Long-term NIV at home can be safely implemented in Hungary within a well-regulated framework, and allows sick children and their families to live a fuller life. NIV therapy can be effective in a wide range of diseases and can reduce hospitalizations and improve children's quality of life.

CYSTIC FIBROSIS NEONATAL SCREENING IN HUNGARY. A SINGLE CENTER EVALUATION OF THE FIRST TWO YEARS

Andrea Xue, Judit Kincs, Ildikó Szatmári

Pediatric Center, Semmelweis University, Budapest, Hungary

Background: This presentation evaluates the strategy of the cystic fibrosis newborn screening (CFNBS) programme in Hungary based on the results of the first two year of screening. At the Hungarian NBS Centre located at Semmelweis University, Budapest, 92063 neonates were screened for cystic fibrosis between February 2022 and December 2023.

Methods: A combined CFNBS protocol with immunoreactive trypsinogen (IRT) and pancreatitis-associated protein (PAP) measurements were applied IRT/IRT×PAP/IRT with an IRT-dependent safety net (SN).

Results: Out of 92063 newborns 303 were tested screen-positive. 14 cystic fibrosis (CF) and 2 cystic fibrosis-positive inconclusive diagnosis (CFSPID) cases were confirmed from the screen-positive cases, and 3 false-negative cases were diagnosed later. Based on the obtained results, a sensitivity of 82% and a positive predictive value (PPV) of 4.6 % was calculated.

Conclusion: Following the recognition of false-negative cases, the calculation method of the age-dependent cut-off was changed during the first year of screening. In purely biochemical CFNBS protocols, a small protocol change, even after a short period, can have a significant positive impact on the performance. CFNBS should be monitored continuously in order to fine-tune the screening strategy and define the best local practices.

MOLECULAR BASIS OF PHENYLKETONURIA IN SERBIAN PAEDIATRIC COHORT

Kristel Klaassen[1], Maja Đorđević Milošević[2], Božica Kecman[2], Marina Anđelković[1], Anita Skakić[1], Vesna Spasovski[1], Milena Ugrin[1], Jovana Komazec[1], Sonja Pavlović[1], Maja Stojiljković[1]

[1] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[2] Mother and Child Healthcare Institute „dr. Vukan Cupic", School of Medicine, University of Belgrade, Belgrade, Serbia

Background: Phenylketonuria (PKU) is the most frequent inborn disorder of amino acid metabolism caused by variants in human phenylalanine hydroxylase gene (PAH).

Methods: In this study, a total of 109 PKU patients from Serbia were included, who were classified into three phenotypic categories in accordance with pre-treatment plasma phenylalanine level: classic PKU, mild PKU and mild hyperphenylalaninemia. For genetic analyses, we combined Sanger sequencing, MLPA and next generation sequencing to identify disease-causing variants in PAH gene, which were further classified using ACMG classification. Additionally, we used in silico and/or eukaryotic expression studies to assess the effect of novel genetic variants identified in our patients.

Results: Disease-causing variants were identified in 217 of 218 alleles, reaching detection rate of 99.5%. We detected a total of 32 different variants, of which 29 previously described and three novel ones: p.Gln226Lys, p.Pro244His and p.Pro416Leu. In silico and/or eukaryotic expression studies confirmed pathogenic effect of all novel genetic variants. The most frequent variant was p.Leu48Ser (31.2%), followed by p.Arg408Trp (13.8%), p.Ile306Val (9.2%). p.Glu390Gly (5%), p.Pro281Leu (4.6%), and p.Arg261Gln (3.2%). All detected disease-causing variants were classified as pathogenic using ACMG classification.

Conclusion: Our study brings the updated spectrum of molecular genetic data, variant classification and detailed phenotypic characteristics for PKU patients from Serbia. Therefore, our study contributes to better understanding of molecular landscape of PKU in Europe and to general knowledge on genotype–phenotype correlation in PKU.

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