ABSTRACTS OF THE 9TH CROATIAN HUMAN GENETICS CONFERENCE & 2ND CROATIAN PERSONALIZED AND PRECISION MEDICINE CONFERENCE

ORAL PRESENTATIONS

Presentation number: CSHG TM-1 Abstract number: ABS-14-ISABS-2024

SPECTRUM OF GENETIC VARIANTS IN 306 PATIENTS WITH NON- SYNDROMIC HEARING LOSS FROM CROATIA

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A major challenge in the study of congenital non-syndromic hearing loss (NSHL) is extreme genetic heterogeneity, whereby the same or very similar clinical phenotype is the result of pathogenic variants in a significant number of genes. Next-generation sequencing strategies, such as clinical exome sequencing (CES), have significantly contributed to the diagnosis of genetically and clinically heterogeneous conditions, including hereditary hearing loss (HL). Aim: To report the spectrum and frequency of disease- causing variants detected in 306 unrelated patients with childhood-onset mild to profound NSHL referred to Children's Hospital Zagreb for genetic testing in the period between March 2006 and October 2023. Methods: Multiplex ligation–dependent probe amplification method and Sanger sequencing of the coding region of the GJB2 gene were used for the analysis of the GJB2 variants in all subjects. CES was performed in 21 patients negative for GJB2 biallelic variants. Results: Among 234 disease associated GJB2 alleles detected, there were 19 different clinically relevant GJB2 variants, of which 18 were reported as pathogenic/likely pathogenic. The c.35delG was by far the most common variant, accounting for 73.5% of mutated alleles. More than half of the patients (64/110, 58,2%) with biallelic GJB2 variants were 35delG homozygotes. Seventeen non-GJB2 variants were found in 10 genes (TECTA, NOG, SLC26A4, PCDH15, TMPRSS3, USH2A, GATA3, MYO15A, SOX10, COL2A1) in 11 subjects, and five variants (in TECTA, NOG, PCDH15 and SOX10) were novel (29,4%). Conclusion: By targeted GJB2 molecular screening and CES analysis, we were able to elucidate the genetic cause of HL in 121 patients, resulting in an overall diagnostic rate of 39.5%. CES allowed us to distinguish NSHL from the syndromic form of HL in cases where the phenotype was unclear or where symptoms were absent from an early age.

Keywords: Non-syndromic hearing loss, NSHL, GJB2, Clinical exome sequencing, syndromic HL

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THE DIAGNOSTIC JOURNEY OF CHROMATIN REMODELING DISORDERS

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Advances in genome sequencing technologies led to discovering of more than a thousand new genes and variants involved in NDDs (neurodevelopmental disorders) pathogenesis. Chromatin dynamics play key role in various developmental processes and their dysregulation is often linked to abnormal neurodevelopment causing chromatin remodeling disorders (CRDs). Due to their overlapping clinical features, and lack of molecular genetic data knowledge they present significant diagnostic challenge in routine clinical practice. For this study, we analyzed 29 patients from our cohort of NDD-s patients whose initial clinical features and molecular genetic testing results were suggestive on CRDs. Initial diagnostic evaluation included family tree analysis, description of clinical features, routine laboratory and radiological evaluation, hearing, vision, and developmental assessment. Phenotype abnormalities were annotated to specific HPO (Human Phenotype Ontology) terms. Clinical exome sequencing has been performed using Illumina TruSight One Kit. Data interpretation performed according to databases: USC Genome Browser, Decipher, PubMed, ClinVar, Varsome, OMIM. Initial exome sequencing results confirmed pathogenic variants in 12 patients: Charge syndrome (2), Kabuki (2), Coffin-Siris (4), Snijders Blok-Campeau (1), Kleefstra (1) and CHD8 -related disorder (2). In the remaining patients additional evaluation included: parental genetic testing, reanalysis of previously sequencing data, repeated clinical evaluation, reverse phenotyping and additional genetic testing (arrayCGH) confirmed CRDs in three patients: Kabuki (1), Cornelia de Lange (1), and KMT5B (1) related CRDs. Our approach emphasizes the importance of comprehensive approach to the diagnostics of CRDs patients combining clinical and molecular genetic approach. Unsolved patients require additional analysis: epigenetic signature and whole genome sequencing studies and specific expertise from collaborative rare disease studies.

Keywords: chromatin remodelling disorders, neurodevelopmental disorders, comprehensive approach, rare disease studies

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THREE CASES OF FIBRO-DYSPLASIA OSSIFICANS PROGRESSIVE WITH EXTRA LONG-TERM FOLLOW-UP

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Fibrodysplasia ossificans progressiva (FOP) is an ultra-rare and severely disabling genetic disorder. The worldwide prevalence is approximately 1 per 2 million. Heterozygous mutations in ACVR1/ALK2 gene exist in all sporadic and familial cases of FOP. The primary aim of this study is to describe the clinic and management of three children suffering from FOP and followed for nineteen, twenty-three and forty years, respectively. Secondary aim is to provide clinical advice on how to diagnose the condition with special reference to the great toes malformation and give current best therapeutic approaches, including controversial issue of surgery. The three cases characterized with malformed great toes initially followed by progressive loss of mobility for a period from nineteen to forty years. Two of three patients presented here had surgical intervention according to specific indication. Additional attention is given to the natural history of the great toe malformation and stepwise decrease of patients' mobility status. Conventional radiology indicates the diagnosis and RNA/DNA test confirm it. Short and valgus deformity of the great toe combined with progressive heterotopic ossifications in the soft tissue is "almost" pathognomonic for FOP, but the RNA/DNA testing of the ACVR1 gene is strongly recommended to confirm diagnosis. Long-term natural history and the fate of the great toe malformation shows influence on mobility status in patients with FOP. Maintaining of the best possible mobility status is of utmost important goal of conservative supporting treatment and even in selected cases surgical intervention. However, prevention of any trauma, of soft tissue including intramuscular injection is recommended.

Keywords: *Fibrodysplasia ossificans progressiva*, FOP, great toe, surgery, case report

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A RETROSPECTIVE STUDY OF DIAGNOSTIC NEXT GENERATION SEQUENCING AT THE UNIVERSITY OF RIJEKA, FACULTY OF MEDICINE, CROATIA FROM 2018 TO 2023

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Next generation sequencing (NGS) is becoming increasingly implemented in various medical specialties for the purpose of diagnostic genetic testing. Considering the complexity and time-consuming efforts of this method, identifying patients with appropriate indications is an imperative. Therefore, the aim of this study was to determine the number, indications, and results of diagnostic NGS testing performed at the University of Rijeka, Faculty of Medicine, since its implementation. A retrospective study was conducted from years 2018 to 2023 to analyse the indications for patient referral for diagnostic NGS testing from the Clinical Hospital Centre Rijeka to the Faculty of Medicine in Rijeka, as well as subsequent test reports. All NGS tests were performed in collaboration with the Clinical Institute for Genomic Medicine in Ljubljana using Illumina NovaSeq 6000 (Illumina Inc). From April 2018 to December 2023, 355 diagnostic NGS tests were performed, with 15.5 times increase in the number of ordered tests. The most common specialists who referred patients for diagnostic NGS were paediatricians (55%), neurologists (29%), internists (7%), ophthalmologists (3%), gynaecologists (2%), and others (4%). Referral diagnoses were confirmed in 110/355 (31%) of cases, in whom likely pathogenic 34/110 (31%) or pathogenic sequence variants 76/110 (69%) were detected. Variants of uncertain significance were identified in 36/355 (10%) cases. Genetic counselling was provided to 21% of patients with class 3-5 sequence variants (30/146, 21%). Our results show an increase in the number of diagnostic NGS tests from 2018 to 2023, reflecting the raised awareness of clinicians for the need of genomic testing in clinical practice. The prevalence of confirmed referral diagnoses and numbers of genetic counselling might additionally be increased by implementing further efforts in genetic education of clinicians.

Keywords: diagnostic genetic testing, genetic education, genomics, next generation sequencing

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PHARMACOGENOMICS: CHALLENGES AND OPPORTUNITIES

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In the era of precision medicine, pharmacogenomics (PGx), among other "omic" disciplines, has an inevitable role. It helps predict drugs' efficacy, tolerability, and safety and should guide clinical decision- making to optimize therapy outcomes. The genes affecting a drug pharmacologic profile, i.e., those encoding metabolizing enzymes and transporters, as well as drug targets and some proteins associated with safety, are the most often analyzed. According to the highest level of clinical evidence, genotype- guided therapeutic recommendations for various drugs from different therapeutic classes are incorporated into the clinical guidelines, like those published by the Clinical Pharmacogenetics Implementation Consortium. However, a classical approach based on the genotyping pre-defined panel of selected functionally characterized gene variants does not completely predict the inter-individual variability in the drug pharmacokinetics and pharmacodynamics, i.e., its efficacy and safety. Therefore, broader approaches such as next-generation sequencing are needed to identify rare gene variants. It is also time to look into the entire human genome to detect genetic variants in coding and non-coding regions and structurally complex variants. These novel comprehensive tools implementation into the clinical practice is a key to diagnostic and therapeutic outcomes improvement. Another challenge represents understanding and applicability of large amounts of generated information. Artificial intelligence is a potent tool for "big data" analysis because it helps understand the heterogeneity in processes that contribute to the individualized genetic-tailored therapeutics approach, leading to better quality and more cost-effective healthcare. However, these goals are achievable only with continuous education of all those involved in providing patient care to keep pace with the rapidly evolving field of pharmacogenomics.

Keywords: pharmacogenomics, gene polymorphisms, drug-gene interactions, precision medicine

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GENOTYPE AND PHENOTYPE VARIABILITY OF SEVEN PATIENTS WITH GLUCOSE TRANSPORTER TYPE 1 DEFICIENCY SYNDROME

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Glucose transporter type 1 deficiency syndrome (GLUT1-DS) is a rare neurometabolic disease caused by pathogenic mutations in the SLC2A1 gene, resulting in impaired glucose delivery through the blood-brain barrier and insufficient brain energy production. The disease presents with a phenotypic continuum that includes various types of early-onset seizures and complex movement disorders. This retrospective case study included seven patients with GLUT1-DS followed at the Department of Pediatrics, University Hospital Centre Zagreb. The onset of the first symptoms started between 2 months and 5 years of age, with a median of 13 months. Four patients had seizures, one had episodic eye-head movements, and two had paroxysmal dyskinesia as the presenting signs. Two patients with epilepsy later developed dyskinesias. Clinical suspicion of GLUT1-DS was raised in three patients in whom the diagnosis was made after a hypoglycorrhachia finding and confirmed by gene testing subsequently. Four patients were diagnosed through next-generation sequencing (NGS). The delay in diagnosis from the first symptom was 0.5 to 15 years, with a median of 6 years. A ketogenic diet was commenced in all the patients to provide an alternative energy source for the brain, which alleviated symptoms and improved EEG in most of them. At the follow-up, five patients had mild cognitive problems. The best outcome was in two patients diagnosed and treated earlier, which may be biased due to shorter follow-up. GLUT1-DS should be suspected in patients with complex movement disorders, early-onset seizures, and drug-resistant epilepsies. Hypoglycorrhachia is a biochemical hallmark. The diagnosis is made or confirmed by gene testing. More patients are diagnosed through NGS, yet a significant diagnostic delay remains. The ketogenic diet is the only disease-specific treatment that improves patient outcomes.

Keywords: Glucose transporter type 1 deficiency syndrome, epilepsy, paroxysmal dyskinesia, hypoglycorrhachia, next-generation sequencing, ketogenic diet

Presentation number: CSHG TM-7 Abstract number: ABS-123-ISABS-2024

INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FOR PRECISION MEDICINE IN PATIENTS AFFECTED BY INHERITED CARDIAC CHANNELOPATHIES

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Precision Medicine (PM) is an innovative approach that, by relying on large population datasets, patient genetics and characteristics, and advanced technologies, aims at improving risk stratification and at identifying patient-specific management through targeted diagnostic and therapeutic strategies. Cardiac channelopathies are being progressively involved in the evolution brought by PM and some of them are benefiting from these novel approaches, especially the long QT syndrome. We will explore the main layers that should be considered when developing a PM approach for cardiac channelopathies, with a focus on modern in vitro strategies based on patient-specific human-induced pluripotent stem cells. PM is where scientists and clinicians must meet and integrate their expertise to improve medical care innovatively but without losing common sense. In my talk, I will provide the cardiologist's point of view by comparing state- of-the-art techniques and approaches, including revolutionary discoveries, to current practice. This point matters because the new approaches may, or may not, exceed the efficacy and safety of established therapies. Thus, our eagerness to implement the most recent translational strategies must be tempered by an objective assessment to verify whether the PM approaches are indeed making a difference for the patients. PM may shape the diagnosis and treatment of cardiac channelopathies for years to come. Nonetheless, its potential superiority over standard therapies should be constantly monitored and assessed before translating intellectually rewarding new discoveries into clinical practice.

Keywords: Induced pluripotent stem cells, cardiomyocytes, precision medicine, inherited cardiac channelopathies, personalized therapies

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IMPLEMENTING WHOLE GENOME SEQUENCING (WGS) IN CLINICAL PRACTICE

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The integration of whole genome sequencing (WGS) into clinical practice represents a transformative shift in personalized medicine. Unlike the analysis of individual genes, gene panels, and whole exome sequencing (WES), WGS enables the detection of variants in deep intronic and non-coding regions of the genome that have an essential role as regulatory regions of the genome. Additionally, WGS allows for the simultaneous detection of not only SNPs and InDels but also structural variants (SVs) and copy number variants (CNVs). Thus, WGS is becoming the test of choice in diagnosing rare diseases, predicting treatment responses, and guiding therapeutic decisions in clinical practice. The value of WGS lies in its ability to provide not only diagnostic but also pharmacogenetic and nutrigenetic analyses, enabling evidence-based drug dosing and the issuance of nutritional guidance based on the nutrigenetic profile of each patient to prevent the most common multifactorial diseases of today, including obesity, diabetes, and cardiovascular diseases. Moreover, WGS facilitates the identification of novel disease-associated genes involved in the development of cancer, enabling more precise treatment, genetic counseling, and family planning in the case of hereditary cancer syndromes. The implementation of machine learning methods in the clinical interpretation of variants of uncertain significance (VUS) and for molecular classification of specific tumor types now enables personalized and precise oncological treatment that improves the prognosis of patients with cancer. Looking ahead, advancements in WGS technologies, coupled with enhanced data analysis techniques and machine learning models, promise to further optimize its clinical utility, and broaden its accessibility, ultimately revolutionizing the field of modern medicine.

Keywords: whole genome sequencing, personalized medicine, artificial intelligence, machine learning, oncogenomics

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INSIGHTS IN OSTEOSARCOMA EVOLUTION BY SINGLE-CELL RNA SEQUENCING

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Tumor evolution and intratumor cell heterogeneity are deeply intertwined. Each time a tumor cell divides, new alterations of the genome are acquired resulting in tumor heterogeneity. That is a prerequisite for the selection of the fittest cell clones driving the evolution of the tumor resulting in treatment resistance, tumor relapse and development of metastases. To follow tumor evolution, we implemented single cell sequencing and analyzed transcriptome data from 3 osteosarcoma cell culture samples derived from the same patient at the stage of diagnosis, post-chemotherapy and relapse. The 18-year-old male was diagnosed with chondrobastic osteosarcoma (G3) of the femur. After 7 cycles of preoperative chemotherapy according to the EURAMOS protocol, the tumor was resected. This was followed by 12 cycles of postoperative chemotherapy. Due to the recurrence, an additional resection was performed. Tissue samples were seeded in the cell culture dish and passage 3 cells were processed using 10x Genomics technology. Processing raw reads with the Cell Ranger pipeline resulted in more than 92% of reads confidently mapped to the human genome (GRCh38). After quality control with the Seurat package, the cells were annotated using reference-based annotation methods which revealed distinct populations of mesenchymal stromal cells and stromal cells like fibroblasts. At the time of diagnosis, both fibroblast and mesenchymal stromal populations showed higher metabolic activity. Immediately after post-chemotherapy, they induced strong inflammatory, self-renewal pathways, and even apoptotic pathways. After relapse, cells induced self-renewal, hypoxia, epithelial-mesenchymal transition (EMT) signaling but also proliferation pathways.

Keywords: osteosarcoma, single-cell sequencing, tumor heterogeneity, tumor evolution, chemotherapy

Presentation number: CSHG TM-10 Abstract number: ABS-108-ISABS-2024

EXPRESSION PATTERN OF PDE4B, PDE4D AND SFRP5 MARKERS IN THE COLORECTAL CANCER

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Colorectal cancer (CRC) is the most frequently diagnosed malignant disease of the gastrointestinal system and new diagnostic and prognostic markers are needed to elucidate complete tumor profile. We used CRC tumor tissues (Dukes A-D) and adjacent noncancerous tissues of 43 patients. Immunohistochemistry was used to examine the expression of PDE4B, PDE4D and SFRP5 markers. In CRC stages, the distribution of PDE4B positive cells varied, with differing percentages between epithelium and lamina propria. Statistically significant differences were found in the number of PDE4B positive epithelial cells between healthy controls and all CRC stages, as well as between different CRC stages. Similarly, significant differences were observed in the number of PDE4B positive cells in the lamina propria between healthy controls and all CRC stages, as well as between different CRC stages. CRC stage Dukes' C exhibited a significantly higher number of PDE4B positive cells in the lamina propria compared to CRC stage Dukes' B. Significant differences were noted in the number of PDE4D positive epithelial cells between healthy controls and CRC stages Dukes' A, B, and D, as well as between CRC stage Dukes' C and stages A, B, and D. CRC stage Dukes' A had significantly more PDE4D positive cells in the lamina propria compared to stage D. Significant differences were also observed in the number of SFRP5 positive cells in the lamina propria between healthy controls and all CRC stages, as well as between CRC stages Dukes' A and D. While the expression of PDE4D varied across CRC stages, the expression of SFRP5 remained consistently strong in both epithelium and lamina propria, with significant differences noted mainly in the lamina propria. These findings suggest alterations in PDE4B, PDE4D and SFRP5 expression during CRC progression, as well as between different stages of CRC with potential implications for understanding the molecular mechanisms involved in CRC development and progression.

Keywords: colorectal cancer, PDE4B, PDE4D, SFRP5

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IDENTIFICATION OF SKELETAL REMAINS IN CROATIA AND BOSNIA & HERZEGOVINA, INCLUDING THE HOMELAND WAR – A 30-YEAR REVIEW

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Over the past 30 years, forensic experts from Croatia and Bosnia and Herzegovina have embraced advanced technologies and innovations to enable great efficacy and proficiency in the identification of war victims. The wartime events in the countries of former Yugoslavia greatly influenced the application of the selection of DNA analyses as routine tools for the identification of skeletal remains, especially those from mass graves. Initially, the work was challenging because of the sheer magnitude of the events, the technical aspects, and the political aspects. Collaboration with reputable foreign forensic experts helped tremendously in the efforts to start applying DNA analysis routinely, our deep involvement significantly contributed to the further improvement of DNA analysis methods, making DNA identifications increasingly successful. We strove to improve through collaboration and experiences gained, and most importantly being driven by the numerous missing persons' families that had high expectations of us. While the work we did was published in scientific journals, the invaluable information and experience we attained are still significant today. Therefore, in this abstract, we wanted to provide a brief overview of the history and the most significant achievements related to the application of DNA analysis in identifying skeletal remains in situations where standard identification methods were insufficient.

Keywords: DNA identification, skeletal remains, Homeland War, Croatia, Bosnia & Herzegovina

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THE GENETIC SCENARIO OF HISTORICAL HUMAN MIGRATIONS IN SOUTH-EASTERN EUROPE – THREE DECADES OF CROATIAN GENETIC HERITAGE STORY

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The presentation will provide an overview of scientific data on the Croatian genetic heritage accumulated over the past three decades. Studies of of mitochondrial DNA (mtDNA), Y chromosome, and Short Tandem Repeats (STRs) within South-Eastern European populations, with a special focus on Croatia, suggest that the genetic history of these populations fits within the broader genetic landscape of Europe and Eurasia, with high observed levels of genetic diversity. This is a consequence of the region's geographical location, which has positioned it as an ancient crossroads for numerous migrations, different cultures, populations, tribes, and religions over millennia. Many of these elements have contributed segments of their genetic heritage to the contemporary genetic pool of Croatia and the surrounding region. However, certain signals in the genetic landscape of Southeastern Europe are more dominant and several such signals will be mentioned in more detail in the lecture (Neolithisation, Slavic introgression). It is also important to highlight Croatian island populations, where evolutionary forces such as genetic drift and bottleneck effect have significantly influenced the specific patterns of their genetic diversity, placing them in the focus of anthropogenetic research in Croatia.

Keywords: human migrations, genetic heritage, Croatia, Southeastern Europe

POSTER PRESENTATIONS

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AN ASSOCIATION BETWEEN COX-2 POLYMORPHISM AND INCREASED PLASMA HDL CHOLESTEROL LEVELS AFTER CLOZAPINE TREATMENT

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Patients with schizophrenia frequently exhibit abnormal neurotransmitter signaling, attenuated skin flush response to niacin (a water-soluble B vitamin), and are more likely to experience weight gain, lipid disturbances, and glucose dysregulation. Several studies have shown antipsychotic effects of the selective cyclooxygenase-2 (COX-2) inhibitors, such as celecoxib. We previously found an intriguing interaction between functional rs689466 polymorphism (A/G polymorphism) of the COX-2 gene and smoking status on schizophrenia onset. We also found that COX-2 polymorphism influenced skin flush response to niacin, among patients under antipsychotic treatment. Here, we investigated whether antipsychotic treatment was influenced by the COX-2 polymorphism. A total of 186 antipsychotic- naïve first-episode psychosis patients or nonadherent chronic psychosis individuals were genotyped by polymerase chain reaction analysis/restriction fragment length polymorphism. At baseline, and after 8 weeks of antipsychotic treatment, we assessed patients' Positive and Negative Syndrome Scale (PANSS) scores and factors, and metabolic syndrome-related parameters (fasting plasma lipid and glucose levels, and body mass index). In the total patient group, COX-2 polymorphism did not affect PANSS psychopathology or metabolic parameters ($P > 0.05$). However, patients with clozapine treatment positive for the COX-2-G allele (COX-2-GG homozygous and COX-2-AG heterozygous), compared to COX-2-AA homozygous, exhibited significantly higher increases of HDL cholesterol levels ($P < 0.05$). The COX-2 polymorphism accounted for ~9.6% of the HDL cholesterol levels variability. Our results suggest a relatively weak contribution of COX-2 polymorphism to HDL cholesterol level variations within a subgroup of patients with clozapine treatment. Antipsychotic-specific differences in the effect of COX-2 polymorphism may be due to inflammatory properties of clozapine.

Keywords: antipsychotic agents, cyclooxygenase-2, gene, polymorphism, psychosis

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PLA2G4C GENE POLYMORPHISM AND NICOTINE DEPENDENCE AMONG SCHIZOPHRENIA PATIENTS

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By playing a role in signal transduction and membrane phospholipid homeostasis, phospholipases (PLA2s) may influence conditions that are associated with altered dopamine action, such as substance use disorders and schizophrenia. We previously found that rs1549637 polymorphism of the PLA2G4C gene in interaction with polymorphism of the PLA2G6 gene, predicts an elevated schizophrenia risk. We also found that PLA2G4C gene polymorphism influences the clinical expression of schizophrenia, as measured by the Positive and Negative Syndrome Scale (PANSS), in the male patients. Here, we investigated the relationship between the PLA2G4C gene polymorphism and the risk of nicotine dependence in Croatian schizophrenia patients. We also addressed whether interaction between PLA2G4C gene polymorphism and smoking might influence schizophrenia onset and PANSS psychopathology. Genotyping was performed in 260 chronic patients (males/females: 134/126) by polymerase chain reaction analysis/restriction fragment length polymorphism. There were no significant differences in the distribution of PLA2G4C genotypes and alleles according to smoking status and no PLA2G4C genotype-smoking interaction on disease onset ($P > 0.05$). However, we revealed a significant PLA2G4C genotype-smoking interaction that predicted positive symptom severity among female patients ($P < 0.05$). A trend toward lower PANSS positive symptom scores observed among female smokers positive for the PLA2G4C-T allele (PLA2G4C-TT homozygous and PLA2G4C-AT heterozygous) compared to non-smoking females positive for the PLA2G4C-T allele mostly influenced this finding. Our results indicate no PLA2G4C gene polymorphism's effect on the risk of nicotine dependence, but they suggest that PLA2G4C genotype-smoking interaction might be of relevance in clinical psychopathology, in a gender-specific fashion.

Keywords: gene, phospholipase A2, polymorphism, schizophrenia, smoking

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ASSOCIATION BETWEEN THE ACE I/D POLYMORPHISM AND OBESITY AMONG PATIENTS WITH LUNG CANCER

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High body mass index (BMI) has been linked with both a reduced risk of lung cancer and better overall outcome of lung cancer patients. Recent studies have demonstrated a probable association between a functional insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene and obesity risk in the general population. In our previous study in lung cancer patients from a Croatian population, the ACE I/D polymorphism was shown to be associated with squamous cell carcinoma risk. In the present study, we investigated whether the ACE I/D polymorphism influenced obesity risk among patients with lung cancer. Genotyping was performed in 305 patients using polymerase chain reaction analysis. Patients were classified as obese with BMI ≥ 30, overweight (BMI: 25 – 29.9) or of normal body weight (BMI: 18.5 – 24.9). No significant differences were observed in the genotype and allele distributions of ACE polymorphism between different BMI categories in the total patient group or in the subgroup of patients with different types of lung cancer ($P > 0.05$). In subgroup of patients with squamous cell carcinoma, we found significantly different distributions of ACE-I allele carriers (ACE-II homozygous and ID heterozygous) and ACE-I allele non-carriers (ACE-DD homozygous) between BMI categories ($P < 0.05$). An increase in the ACE I allele carriers compared to ACE-I allele non-carriers among overweight (39.4% vs. 21.3%, respectively) and a decrease in the ACE I allele carriers compared to ACE-I allele non-carriers among obese (6.0% vs. 19.1%, respectively) contributed mostly to this finding. Overall BMI values were not significantly associated with ACE polymorphism either in the total patient group or in the subgroup of patients with different types of lung cancer ($P > 0.05$). ACE-I/D polymorphism might be of relevance in determining obesity risk in the subgroup of patients with squamous cell carcinoma.

Keywords: angiotensin converting enzyme, gene, lung cancer, obesity, polymorphism

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ROLE OF IL-6, IL-10 AND TNFα GENE VARIANTS IN PRETERM BIRTH

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The association of gene variants for interleukin 6 (IL-6) (rs1800796), interleukin 10 (IL-10) (rs1800896) and tumor necrosis factor-α (TNFα (rs1800629) with the occurrence of spontaneous preterm birth (PTB) was investigated to determine whether these genetic variants are a risk factor. 199 blood samples from pregnant women who had given birth prematurely and 200 control blood samples (samples from pregnant women with term delivery) were analyzed to determine single nucleotide polymorphisms (SNPs) for IL-6 (rs1800796), IL-10 (rs1800896), and TNFα (rs1800629). DNA extraction was performed on mini-spin columns according to the manufacturer's protocol. The quality and purity of the isolated DNA were tested using a Qubit 3 fluorometer. Genotyping was performed with an ABI PRISM 7500 SDS using TaqMan SNP genotyping assays. The genotypes obtained were analyzed using the 7500 Software v2.3 package. Carriers of the A/A genotype for the rs1800629 of the TNFα gene have 4.81 times greater chance of late- onset PTB compared to carriers of the G/G and A/G genotypes in the recessive inheritance model. The presence of the G/G genotype in the recessive inheritance model compared with the G/A and A/A genotypes for the rs1800896 of the IL-10 gene represents a potentially protective factor, with mothers in the term-birth group having an almost 2-fold lower odds of PTB in general and an almost 10-fold lower odds of early PTB. On the other hand, carriers of the A/G genotype of rs1800896 have a 1.54-fold higher chance of preterm birth in general and a 1.6-fold higher chance of late preterm birth in the super dominant inheritance model compared to the A/A and G/G genotypes in the group of mothers with PTB. In this study, no association was found between PTB and rs1800796 of the IL-6 gene. rs1800629 in mothers was associated with PTB. rs1800896 shows a potentially protective effect for the occurrence of PTB in this study. No association was found between PTB and rs1800796.

Keywords: premature birth, single nucleotide polymorphisms, inflammation, cytokines, interleukins

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MATERNAL ENDOGENOUS AND EXOGENOUS FACTORS AS RISK FACTORS FOR CONGENITAL HEART DEFECTS IN CHILDREN WITH DOWN SYNDROME

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Animal models have shown that maternal DNA methyltransferases (DNMT) are associated with birth defects, including different type of congenital heart defects (CHD). Approximately 50% of patients with Down syndrome (DS) have CHD. This study investigated the influence of maternal DNMT polymorphisms (DNMT1 (rs2228611), DNMT3A (rs1550117), DNMT3B (rs1569686) and DNMT3B (rs2424913)) and exogenous maternal factors (cigarette smoking, alcohol consumption, medication use) on the occurrence of CHD in children with DS. The study included 154 mothers of children with DS, 49% (75/154) mothers had a child with DS and CHD (DS-CHD+ mothers) and 51% (79/154) mothers had child with DS without CHD (DS-CHD- mothers). Atrial septal defect was present in 37%, ventricular septal defects 23%, atrio-ventricular defect in 23% and others CHD in 17%. All participants provided written informed consent prior to participation in the study. Information about CHD was obtained from each child's medical records. Before the sampling, the mothers were asked to complete a questionnaire that asked about demographic data, cigarette smoking, alcohol intake and medication use. Genomic DNA was extracted from peripheral blood leukocytes according to standard protocol. Genotyping was performed by PCR-RFLP. Maternal DNMT polymorphisms were not found to be a risk factor for CHD in DS ($P > 0.05$). Slightly more than half of participants 64% (99/154) completed the questionnaire. The results showed that DS-CHD+ mothers smoked more frequently before pregnancy than mothers with DS child but without CHD ($P = 0.0022$). No correlation was found between the polymorphisms and the other exogenous factor ($P > 0.05$). We found no association of maternal DNMT polymorphisms with CHD in children with DS. However, we found a significant association between cigarette smoking in DS-CHD+ mothers. These results emphasize the need for a multifactorial approach that could resolve in uter

Keywords: Down syndrome, DNMT, cardiac heart defects, maternal polymorphisms, exogenous factors

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THE ROLE OF ACE GENE POLYMORPHISM IN PRETERM BIRTH: INSIGHTS FROM A META-ANALYSIS AND A CASE-CONTROL STUDY IN THE CROATIAN POPULATION

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The ACE gene, encoding the angiotensin-converting enzyme (ACE), plays a crucial role in the regulation of placental circulation and angiogenesis. Impaired ACE activity has been linked to adverse pregnancy outcomes, including preterm birth (PTB). Despite extensive research, the exact cause of PTB remains unclear. Previous studies have suggested a connection between the insertion/deletion (I/D) polymorphism of the ACE gene and PTB, but results have been inconsistent across different populations. Therefore, we conducted a case-control study on the association between the ACE I/D polymorphism and PTB in the Croatian population. Moreover, we conducted a meta-analysis to systematically evaluate the results of previous studies on the association between ACE I/D polymorphism and PTB. In a case-control study, genotyping was performed on a total of 206 subjects, consisting of 121 individuals with PTB and 85 controls with full-term pregnancies. For meta-analysis, PubMed, Scopus and Google Scholar databases were systematically searched using a combination of keywords including "ACE", "angiotensin-converting enzyme", "ACE I/D polymorphism", "preterm birth" and "preterm delivery." The results of the case-control study did not indicate significant differences in the genotype and allele distributions, nor in different genetic models between PTB and control group. Meta-analysis was performed on 6 studies (including our case-control study) involving 601 cases and 787 controls. The significant difference was observed only when comparing II vs DD genotype (P = 0.050; OR = 0.178; 95%CI = 0.516-1.000). The present results suggest that the ACE I/D polymorphism does not emerge as a significant risk factor for PTB. Nevertheless, the observed modest association among homozygous genotypes emphasizes the need for further research to clarify the exact involvement of ACE in the pathogenesis of PTB.

Keywords: angiotensin-converting enzyme; ACE I/D polymorphism; case-control study; meta-analysis; preterm birth

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PAEDIATRIC CASE OF ACERULOPLASMINEMIA: 10.5-YEAR-OLD BOY WITH PERSISTENT MICROCYTIC ANAEMIA AND BIALLELIC LOSS-OF-FUNCTION VARIANTS IN CP GENE

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Aceruloplasminemia (ACP) is a rare adult-onset disorder in which iron accumulates in the brain and other organs, resulting in microcytic anemia, diabetes, retinopathy, liver disease, and progressive neurological symptoms. Cause are biallelic pathogenic variants in CP gene, encoding ceruloplasmin, a metalloprotein involved in maintaining iron homeostasis. Here we present a pediatric case of ACP. A 10.5-year-old boy was referred to us with persistent microcytic anemia since the age of 7 months, elevated liver transaminases, low iron and copper values, and elevated ferritin values. The extensive diagnostic work-up all came back negative. A clinical exome sequencing of proband and his parents was performed using TruSight One Sequencing panel (Illumina, USA). Two compound heterozygous variants in the CP gene were found. The first one is a novel likely pathogenic frameshift indel p.Asp48ThrfsTer37 inherited from his father. The second one is a known pathogenic nonsense variant p.Arg215Ter inherited from his mother. Both detected variants presumably cause nonsense-mediated decay and loss-of function (LOF). Biallelic LOF variants in CP gene are known mechanism of the disease. According to these genetic findings and clinical presentation, our patient is diagnosed with ACP. ACP generally presents in adulthood, from age 25 years to older than 70 years. The most common first symptom is sideropenic anemia, which occurs before the age of 20 years in 80% of patients. Early presentation of the disease, such as in this case, is extremely rare. To our knowledge, there is only one boy whose symptoms started at the age of eight (PMID: 36308763). Prompt diagnosis and therapy are crucial to prevent neurological complications since, once established, they are usually irreversible. Thereby, ACP should be considered if persistent microcytic anemia without apparent cause is present from a very young age.

Keywords: Aceruloplasminemia, ACP, Microcytic anemia, CP gene, Ceruloplasmin

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TNFRSF10A GENE POLYMORPHISM AND INTERFERON- TREATMENT RESPONSE IN MULTIPLE SCLEROSIS PATIENTS

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Interferon-beta (IFN-β) is widely used as a first-line disease-modifying treatment for multiple sclerosis (MS), although 30–50% of MS patients do not respond to this therapy. Identification of genetic variants that predict response to IFN-β may be useful for treatment prognosis. The TNFRSF10A gene encodes a protein that triggers apoptosis and is involved in T cell-mediated autoimmune diseases. The aim of this study was to investigate the influence of TNFRSF10A (rs20576) gene polymorphism on the response to IFN-β treatment in Croatian and Slovenian MS patients. A total of 274 IFN-β–treated MS patients (221 female; 63 male) were genotyped for TNFRSF10A (rs20576) gene polymorphisms using real-time polymerase chain reaction. Patients were diagnosed with relapse-onset MS according to the revised McDonald criteria. Based on the clinical criteria for MS treatment efficacy, they were classified as responders, Rs (N=165) and non-responders, NRs (N=119). Overall, no significant differences were found in the distribution of genotypes or allele frequencies of TNFRSF10A (rs20576) polymorphisms between Rs and NRs patients. However, the frequency of TNFRSF10A GG genotype was statistically significantly higher (p=0.039; OR=0.31; 95%CI 0.11-0.97) in male NRs (72.0%) compared to NRs (47.5%) patients. In addition, we observed a trend towards a higher prevalence of TNFRSF10A heterozygotes in NRs (38.1%) compared to Rs (27.1%) among female patients (p=0.066). Our results suggest that the presence of the TNFRSF10A (rs20576) gene variant may be associated with response to IFN-β treatment in a sex-specific association in MS patients, but further studies with a larger number of patients are needed.

Keywords: TNFRSF10A, gene polymorphism, multiple sclerosis, IFN-β treatment response

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SECOND CASE OF GONADAL MOSAICISM AND A NOVEL NONSENSE NR2F1 GENE VARIANT AS THE CAUSE OF BOSCH-BOONSTRA- SCHAAF OPTIC ATROPHY SYNDROME

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Bosch-Boonstra-Schaaf syndrome is a rare autosomal dominant disease characterized by developmental delay, intellectual disability, and optic atrophy, caused by the loss of function of the NR2F1 gene. To date, approximately a hundred cases have been reported with significant diversity in the remaining clinical features and, although precise genotype-phenotype correlation has not been completely elucidated, milder phenotypes appear to be associated with nonsense mutations. The goal of this report is to present two sisters with Bosch-Boonstra-Schaaf Optic Atrophy Syndrome with a novel nonsense mutation in the NR2F1 gene and gonadal mosaicism as the cause, making it the second such case described in the literature. A 32-year-old female patient (sister I) and her 30-year-old sister (sister II) were referred to the general ophthalmology clinic due to visual impairment. Sister, I have had visual impairment since early school years and sister II since birth. In addition, both sisters exhibited nystagmus, decreased visual acuity, pale optic discs, visual field defects, reduced vitamin levels, and thin optic nerves on MRI. Both sisters reported having intellectual difficulties during primary and secondary school. Whole exome sequencing in both sisters revealed a novel heterozygous nonsense pathogenic variant in the NR2F1 gene (c.169C>T) leading to early truncation of the NR2F1 protein. The variant was not found in the parents, suggesting gonadal mosaicism as the potential cause of Bosch-Boonstra-Schaaf Optic Atrophy Syndrome in both sisters. The novel nonsense NR2F1 gene variant described in two sisters in this case report contributes to the knowledge about genotype-phenotype correlation in Bosch-Boonstra-Schaaf Optic Atrophy Syndrome, supporting the previous findings which associate mild phenotypes with nonsense mutations. In addition, our findings further support gonadal mosaicism as a potential cause of this rare disease.

Keywords: Bosch-Boonstra-Schaaf Optic Atrophy Syndrome, genotype-phenotype correlation, genetic testing, gonadal mosaicism, next-generation sequencing

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SEMAGLUTIDE DIRECTLY AFFECTS VIABILITY OF HEPATOCYTES, STEATOSIS EXTENT AND KEY PROTEINS INVOLVED IN LIPID METABOLISM IN AN IN VITRO MODEL OF MASLD

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is a global health issue with complex pathophysiology. Peroxisome proliferator-activated receptor gamma and alpha (PPARγ, PPARα) and microsomal triglyceride transfer protein (MTTP) play an important role in lipid metabolism. GLP-1 receptor agonists (GLP-1RAs), such as semaglutide, have demonsted a potential as a therapeutic option. In our research we aim to investigate effects of semaglutide in a cell culture model of MASLD. Cell culture model of MASLD was established by incubating Huh-7 cell line with 1 mM oleic acid (OA) for 24 hours. Cells were cotreated with 2 nM, 5 nM and 10 nM of semaglutide. Cell viability was measured using MTS assay. Steatosis was assessed by Oil-Red-O staining and colorimetric quantification. Concentrations of proteins were determined by ELISA kits. Fetal bovine serum and ethanol mixture was used as a solvent and served as a negative control. Cell viability in MASLD model was significantly reduced by 41.58% ($p<0.001$) compared to the negative control. 2 nM, 5 nM, and 10 nM of semaglutide increased cell viability compared to MASLD model by 20.51% ($p<0.03$), 14.42% and 18.45%, respectively. Oil-Red-O showed that 2 nM, 5 nM, and 10 nM of semaglutide reduced lipid content compared to the MASLD model by 6.64% ($p<0,001$), 12.36% ($p<0,001$), and 32.41% ($p<0,001$), respectively. MASLD model showed significantly higher concentrations of PPAR compared to the negative control ($p<0.001$). Compared to the MASLD model, the 2 nM group showed a statistically significant decrease of PPARγ by 29% ($p = 0.04$). An increase of PPARα compared to the negative control was statistically significant in all groups, while only 10 nM group showed a statistically significant decrease ($p = 0.002$). Semaglutide has beneficial cell injury and lipid accumulation reducing properties in an in vitro model of MASLD, as well as complex effects on PPARγ, PPARα, and MTTP.

Keywords: MASLD, GLP1, SEMAGLUTIDE, PPAR, HUH-7

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KETOGENIC DIET CAUSE SEX-SPECIFIC MOLECULAR CHANGE IN SKELETAL MUSCLE

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The ketogenic diet has emerged as a compelling dietary strategy for diverse health conditions, including weight management and metabolic disorders. Emerging evidence suggests that the efficacy and outcomes of the ketogenic diet may vary between sex, prompting a deeper investigation into the complex interplay between sex and metabolic response. In this study, effect of ketogenic diet on sex- specific differences in skeletal muscle metabolic profiles were analyzed. Twelve weeks old, male, and female C57Bl/6N mice were assigned to standard (SD) or ketogenic diet (KD) for 12 weeks. Mice were randomized in four groups (two control groups: male SD (10) and female SD (10) and two experimental groups: male KD (9) and female KD (7). Skeletal muscles sections were placed onto Indium-Tin-Oxide slides and analyzed by MALDI-TOF-MSI using positive mode and 300-700 m/z ratio. Based on principal component analysis (PCA) ketogenic diet had a significant effect on males but not on females who resisted gaining weight. PC1 component explained 44.8 % of variations and PC2 13.7 % of variations between groups. Hydroxyheptadecanoic acid, a long-chain fatty acid, was increased, while 7-methylguanosine 5-triphosphate (m7GTP), a key molecule in mRNA translation, was decreased in experimental males. Those results indicate sex-specific effect of ketogenic diet on mice metabolic profiles and mechanism of protein synthesis. This study was funded by the Faculty of Medicine Osijek, projects: MEFOS-2023-IP11 and MEFOS-2024-IP6, and European Union through the European Regional Development Fund grant agreement No. KK.01.1.1.02.0015, "Research and diagnostics of malignant, infectious and rare metabolic diseases based on MALDI TOF technology.

Keywords: ketogenic diet, sex-specific differences, obesity, skeletal muscle, MALDI-TOF-MSI

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A PATIENT WITH SHAAF-YANG SYNDROME – CASE REPORT

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Schaaf-Yang syndrome (SYS) is a rare neurodevelopmental disorder, affects many parts of the body and is similar to Prader-Willi syndrome. It is usually manifests at birth with muscular hypotonia and distal joint contractures in affected individuals. SYS is inherited in an autosomal dominant, maternally imprinted manner (paternally derived MAGEL2 allele). Case: Patient was born by C-section because of breech position. After birth, dysmorphic face, multiple joint contractures, hypotonia and feeding problems were noticed. Some type of seizures began in infancy and severe developmental delay was noticed. In the age of 17months he had severe complication after cardiac resuscitation due to infective myocarditis. After that, we detected sleep apneas and non-invasive ventilation support was started. In the age of 3 years, growth hormone therapy was started due to short statue, but therapy was stopped after 6 months because of scoliosis progression. Now, he is 4 years old boy and development delay, intellectual disability and autistic disorder is detected. During early infancy we started with genetic evaluation (molecular karyotype, SMA, MD type I and „Arthrogryposis Panel "), initial tests were normal. Finally, clinical exome sequencing detected pathogenic heterozygous mutation in MAGEL2 gene (NM_019066.4) and diagnosis of SYS was established. It is important to consider mutations in the MAGEL2 gene in the evaluation in patients with Prader-Willi-like disease that manifests as developmental delay/intellectual disability, hypotonia, feeding difficulties, and autism spectrum disorder. SYS has some unique features, such as arthrogryposis, which can help discriminate this syndrome from PWS.

Keywords: Schaaf-Yang syndrome, MAGEL2 gene, joint contractures, hypotonia, neurodevelopmental disorder