Abstracts of poster presentations

Medical Genetics

Best practices in translational and personalized medicine

Presentation number: MG 1

GENETIC DIAGNOSTICS LED TO PREVENTIVE ICD IMPLANTATION IN A PATIENT WITH THE BRUGADA SYNDROME FAMILY HISTORY

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The Brugada syndrome (BrS) is a rare but potentially life-threatening heart rhythm disorder with a high incidence of sudden death in patients with structurally normal hearts. The incidence of BrS varies between 1 and 30 per 10000 people. Approximately a quarter of those with BrS have a family member who also has the condition. The affected patients may have episodes of passing out. However, abnormal heart rhythms (such as ventricular fibrillation or polymorphic ventricular tachycardia) may even result in a fatal outcome. It is an autosomal dominant inherited condition most commonly caused by the SCN5A gene. It encodes the cardiac sodium channel. At 17 years of age, our patient had been assigned with the cardiologic diagnosis of right bundle branch block and left anterior hemiblock. The patient's father had suddenly passed away at 36 years of age (undefined etiology, but the family suspects it was heart disease). His father's nephew had suffered from heart arrest at 38 years of age and was implanted with an implantable cardioverter-defibrillator (ICD) after he tested positive for BrS (pathogenic variant SCN5A c.4222G>A (p.Gly1408Arg)). The nephew's children have also tested positive for BrS on genetic testing. Without notice, another close relative (grandson from his grandmother's sister) had passed away at 22 years of age while playing basketball. The patient underwent diagnostic genetic testing that included a panel of 294 pathogenic gene variants that are associated with a risk of pathologic cardiac conditions. The results of the genetic testing confirmed one pathogenic variant of clinical significance in the SCN5A gene (c.4222G>A (p.Gly1408Arg)) that is associated with autosomal dominant BrS, long QT syndrome type 3, dilated cardiomyopathy, and atrial fibrillation. Since the patient's clinical presentation has been asymptomatic for the BrS, the significance of the confirmed SCN5A pathogenic variant is preventive. We recommended measures to the patient to reduce the risk of sudden death due to serious abnormal heart rhythms such as ventricular fibrillation or polymorphic ventricular tachycardia. After a detailed medical examination, the patient was fitted with an implantable cardioverter-defibrillator (ICD) due to the expert's recommendation.

Key words: Brugada syndrome, sudden cardiac death, SCN5A, implantable cardioverter-defibrillator, atrial fibrillation

Presentation number: MG 2

IMPLEMENTATION OF TAILORED PREVENTION INITIATIVES BY IMPROVING KNOWLEDGE ABOUT BREAST CANCER RISK FACTORS

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Accurate calculation and perception of personal breast cancer (BC) risk are critical components of primary and secondary BC prevention. The aim of this study was to examine knowledge of BC risk factors and attitudes toward primary chemoprevention among women at varying BC risk. A cross-sectional, single-site study enrolled 249 Croatian women at average (AR) and high risk (HR) of BC according to the Gail model who underwent mammographic examination. All data were collected by personal interview using a validated questionnaire developed for this study (Cronbach's alfa 0.707). The actual BC risk of each participant was compared with her self-perceived risk. Women who incorrectly estimated their BC risk were additionally divided into two groups: overestimated and underestimated groups; in cases of AR women and HR women incorrectly estimating their BC risk. A total of 249 women; median age 57 years (IQR 47-62 years) were classified into one of 2 risk groups: AR (74%) and HR (26%). 36% of women had a radiologically determined higher breast density. HR women were significantly older (Mann Whitney U test, P<0.001), had more family members with BC (chi-square test, P < 0.001), and first-degree relatives with any cancer (chi-square test, P < 0.001). Among HR women, 72.3% underestimated their BC risk. At AR, 13% of women overestimated risk, whereas 86% correctly estimated their risk. Knowledge of BC risk factors was assessed by 16 questions. Interestingly, the knowledge of higher BD as a BC risk factor was extremely low in both groups, even lower in HR women (34% vs. 38%). There were no significant differences in attitudes toward primary chemoprevention in relation to BC risk. Risk stratification and objective knowledge of true BC risk are key to a personalized approach to BC screening. Women's awareness of BD's impact on BC risk is poor, especially in comparison with literature data after mandatory BD information disclosure in U.S. Our results show that only 28% of HR women correctly assess their own risk. Although most of the Croatian women correctly assessed their BC risk (71% overall), the focus should be on a HR group that mostly underestimated their risk (72%) and seemed to be unrealistically optimistic. The reasons and explanations for this optimistic bias need to be thoroughly explored to improve prevention behavior change.

Key words: breast density, breast cancer, risk-based screening, personalized breast cancer risk assessment

Epigenetics

Presentation number: MG 3

EFFECTS OF SIMULTANEOUS MANIPULATION OF DNA METHYLATION AND HISTONE MODIFICATIONS ON EXPRESSION OF GENES INVOLVED IN EPITHELIAL-MESENCHYMAL TRANSITION USING CRISPR/dCas9 TOOLS

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CRISPR/dCas9 molecular tools have enabled targeted manipulation of the epigenome and therefore, investigation of the direct effect of epigenetic modifications on gene expression. However, the interplay of different epigenetic modifications in the regulation of transcriptional gene activity is still poorly understood. Regarding the vast number of different chromatin modifications, it is not clear how specific modifications play together in the complex process of gene regulation. Herein, we studied the potentially synergistic effect of DNA methylation and different histone modifications on the transcriptional activity of two candidate genes, ZEB1 and SNAI1, coding for the key transcriptional factors regulating the epithelial to mesenchymal transition (EMT). For this purpose, we used the CRISPR/dCas9 system to manipulate DNA methylation, using DNMT3A-dSpCas9 fusion, and different histone modifications using dCas9 fused effector domains: G9a-SET, LSD1-SET domains, and the catalytic domain of KDM5a. In HepG2 cell line, the CpG islands of ZEB1 and SNAI1 were targeted with DNMT3A-dSpCas9 using multiple gRNAs, simultaneously covering the entire islands. The combinatorial use of DNMT3A-dSpCas9 with G9a-dSpCas9 was monitored for 70 days, and a synergistic effect on the expression of both genes was observed, which remained for a prolonged period on SNAI1. None of the effector domains for manipulation of histone modifications could itself increase DNA methylation. However, the G9a-SET domain combined with DNMT3A-dSpCas9 showed a stronger effect on DNA methylation compared to control where the inactive G9a-SET domain was used. A change in the expression of downstream EMT markers E-CAD and CRB3 following epigenetic manipulation was also observed. When DNMT3A-dSpCas9 was combined with LSD1-dSpCas9, no clear effect was observed on either of the two genes, while for the combination with KDM5a-dSpCas9, the effect was short-term. Changes in several epigenetic modifications simultaneously can lead to a synergistic effect on gene expression, depending on the gene locus. Ongoing research will unravel if introducing DNMT3L into cells can bridge DNA methylation and histone modifications.

Key words: CRISPR/dCas9 technology, DNA methylation, histone modifications, epithelial to mesenchymal transition

Presentation number: MG 4

CRISPR/dCas9 MOLECULAR TOOLS REVEAL THE REGULATION OF FUT8, MGAT4A, MGAT4B, MGAT5, MGAT3, AND B4GALT1 GENES BY CpG METHYLATION

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In hepatocellular carcinoma (HCC), as well as in various other cancers, protein glycosylation is altered and as such is associated with tumor proliferation, invasion, metastasis, angiogenesis, and multidrug resistance. Mechanisms are mostly epigenetic. Indeed, aberrant DNA methylation is one of the epigenetic modifications that is highly perturbed in cancer, resulting in changes in transcriptional activity of many key genes, thus leading to characteristic cancer behavior. One group of genes, which might be affected, are glyco-genes coding for glycosyltransferases. The aim of this study is to explore epigenetic regulation of glyco-genes using cutting-edge CRISPR/Cas9 based molecular tools for epigenome editing. In hepatocellular carcinoma model cell line HepG2, we targeted seven candidate glyco-genes using dCas9-DNMT3A and dCas9-TET1 fusions, and subsequently analyzed CpG methylation, transcriptional gene activity, and whole-cell N-glycome as a final phenotype. Transfected cells were collected at two time points (8th and 12th day following transfection). Targeted methylation of selected CpG sites in ST6GAL1, FUT8, MGAT4A, MGAT4B, MGAT5, and B4GALT1 genes induced hypermethylation at these sites (up to 40% on average, depending on the gene), which was followed by a statistically significant change in transcriptional activity of all these genes except ST6GAL1. Targeted demethylation of MGAT3 gene (up to 45% on average) was accompanied by a statistically significant change in its transcription. As a final phenotype, whole-cell protein glycosylation was analyzed and changes in several glycosylation traits in HepG2 glycome were observed. These results suggest that alterations in CpG methylation lead to differential expression of glycosyltransferases, thus leading to aberrant protein glycosylation in HCC.

Key words: epigenome editing, gene regulation, CRISPR/dCas9, DNA methylation, protein glycosylation

Presentation number: MG 5

MANIPULATION OF HNF1A, HNF4A AND FOXA2 USING CRISPR-BASED MOLECULAR TOOLS SUGGEST THEIR ROLE IN REGULATION OF PROTEIN GLYCOSYLATION IN LIVER AND PANCREATIC CELL MODELS

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Transcription factors HNF1A, HNF4A and FOXA1/2/3 regulate developmental and tissue-specific transcriptional gene networks in liver and pancreas. These genes are involved in many metabolic processes as well as in acute inflammation by regulating proteins such as fibrinogen, C-reactive protein and receptor interleukin 1. Alternative glycosylation affects protein structure and function, and aberrant protein glycosylation is observed in inflammation, diabetes and cancer. For instance, abnormal glucose stimulated insulin secretion in diabetes might occur through epigenetic change in HNF1A and FOXA2, resulting in deregulation of MGAT4A, MGAT4B and MGAT5 glycosyltransferases responsible for proper glycosylaton of GLUT receptors on beta cells. In addition, HNF1A is identified by GWAS studies as a master regulator of key fucosyltransferases in liver. In order to investigate possible effects of HNF1A, HNF4A and FOXA2 expression on downstream glycogenes we used CRISPR/dCas9-based tools for manipulation of their transcriptional activity in human model cell lines for liver (HepG2) and pancreas (1.1B4). Following CRISPR-based manipulations, we analysed total protein glycosylation. Promoters of HNF1A, HNF4A and FOXA2 were targeted with KRAB-dCas9 (for silencing) and/or VPR-dCas9 (for activation) using specific sgRNAs. Silencing of HNF1A, HNF4A and FOXA2 in HepG2 cells resulted in downregulation of ST6GAL1 and FUT6 and upregulation of B4GALT1, FUK, FUT3, FUT5, FUT8 and MGAT5A, with concomitant change in glycosylation. Increase of antennary fucosylated, core fucosylated, agalactosylated and asialylated glycans and a decrease in galactosylated and sialylated structures were observed. In 1.1B4 cell line, silencing of HNF1A, HNF4A and FOXA2 led to overexpression of FUT5 and FUT6 and reduced expression of FUK and FUT8 but these changes were not reflected in the glycan profile. The HNF1A and HNF4A activation in 1.1B4 cells resulted in overexpression of FUT3, FUT5 and FUT6 and reduced expression of MGAT4A. This change led to an increase in agalactosylated, asialylated and oligomanose glycans and a decrease of sialylated, galactosylated and core fucosylated glycans. Our results indicate that HNF1A, HNF4A and FOXA2 regulate, at least partly, protein glycosylation in the pancreatic and liver cell models.

Key words: epigenetics, CRISPR/dCas9, glycogenes, protein glycosylation

Presentation number: MG 6

VIRTUAL EPIGENETICS IN FORENSIC: AN EXAMPLE OF METOPIC SUTURE PREVALENCE IN MODERN CROATIAN POPULATION

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Aim was to determine the prevalence of metopic suture in the modern Croatian population. A total of 458 MSCT images were analyzed (250 from the University hospital Split and 208 from the University hospital Zagreb) using OsiriX 12.0 imaging software. The sample consisted of 240 females (median age 67; range 18-93) and 218 males (median age 68; range 20-91). The metopic suture was scored in 3D volume rendering view as absent or present. The persisting metopic suture was scored complete if the suture was aligned nasion to bregma. The incomplete metopic suture was scored as only nasal, only parietal, or both nasal and parietal but not connected. All the analyzed metopic sutures were scored as complete. Of the total population analyzed, persistent metopic suture had 3.1%, 6 of 240 females (2.5%), and 8 of 218 males (3.7%). There is no statistical significance ($\chi 2 = 0.53$, $p=0.47$) between sexes. Frequencies of metopic suture vary across populations, the highest prevalence was reported in the modern Indian population (16%), modern Dutch (11.5%), Italian (10.7%), and the lowest among modern American blacks (2.2%). The relatively small frequency in the Croatian population shows that metopic suture could be a useful forensic tool application in human individualization and identification, and it can be useful in ancestry estimation as well as in positive identification and clinical environment. This study was founded by Croatian Science Foundation UIP-2020-02-7331 “CT for ID”.

Key words: MSCT imaging, virtual databases, metopic suture, population affiliation, Croatia

Presentation number: MG 7

USE OF CRISPR/DCAS9-BASED MODULAR SYSTEM DEMONSTRATES ANTAGONISTIC AND SYNERGISTIC EFFECTS OF EPIGENETIC MANIPULATIONS ON GENE EXPRESSION

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The development of the CRISPR/Cas9 system has enabled a shift from its primary role as a genome-editing tool to its application in targeted alteration of the eukaryotic epigenome. By linking various epigenetic effector domains to the catalytically inactive nuclease Cas9 (dCas9), the active fusions are obtained that can be targeted to a genome site of interest by a specific guide RNA (gRNA) molecule. The effect of epigenetic change on gene expression enables understanding the significance of the specific epigenetic marks in complex gene expression regulation. To further enhance CRISPR/dCas9 system for epigenome editing we have developed a fully modular and upgradeable system where various domains can easily be fused to dCas9: DNMT3A-dCas9 fusion for addition and TET1-dCas9 fusion for removing methyl group to/from CpG dinucleotides, as well as dCas9-VPR and dCas9-KRAB fusions for direct activation and silencing of gene transcriptional activity. We also enabled the use of two Cas9 orthologs from the species Streptococcus pyogenes (SpCas9) and Staphylococcus aureus (SaCas9) for fusion with different effector domains which allowed both, antagonistic and synergistic manipulations at different loci. By further expanding the modular system to increase the number of gRNA molecules, to a maximum of six different ones, we have enabled to target dCas9 fusions to a larger genome region. We were able to confirm that our modular system works efficiently by simultaneous targeting the candidate gene pairs BACH2 – HNF1A and IL6ST – MGAT3 in HEK293 cells with DNMT3A-dSpCas9 and TET1-dSaCas9. Induced methylation and demethylation of the individual genes within the pairs were accompanied by a change in the level of gene transcription. In addition, we were able to demonstrate that changes in methylation and gene expression levels of gene pair HNF1A – MGAT3 have an effect on the glycan phenotype in BG1 cells. Also, by simultaneous targeting the HNF1A locus using TET1-dSaCas9 and VPR-dSpCas9 fusions, we showed a synergistic effect on gene expression which was maintained up to 30 days after transient cell transfection. Furthermore, by upgrading our system, we reduced the off-target effect of dCas9 fusions.

Key words: CRISPR/dCas9 system, Cas9 orthologs, epigenome editing, direct regulation of gene expression, CRISPR/dCas9 off-target effect

Genetic basis of diseases

Presentation number: MG 8

CASE REPORT: A MULTIDISCIPLINARY APPROACH TO THE MANAGEMENT OF RICKETS DISEASE CAUSED BY DE NOVO MUTATION IN THE PHEX GENE

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Rickets disease has been a persistent disease, first described in the Viking populations throughout the centuries. However, the cause of this form of primary Rickets was the lack of vitamin D. There are secondary forms of Rickets in which lie a genetic component. X-linked hypophosphatemia (XLH) is a disorder of renal phosphate wasting caused by pathogenic variants in the phosphate-regulating endopeptidase gene, PHEX - essential to the phosphate homeostasis in the body. Biochemical findings include hypophosphatemia with low to normal circulating 1,25-dihydroxy vitamin D levels, elevated serum alkaline phosphatase activity in children, and normal serum calcium. XLH demonstrates variable expressivity, even within families, and has complete penetrance in both males and females. Symptoms can range from extreme lower limb bowing apparent by the first year of life to isolated short stature in otherwise asymptomatic appearing adults. We present a case of a 30-year-old female patient diagnosed with Rickets disease at the age of 3, which reacted unsuccessfully to treatment with vitamin D. The patient presented to our clinic for orthopedics at St. Catherine Specialty Hospital in 2021 with severe scoliosis and varus deformity of the lower extremities. Upon discussion, the patient's anamnesis revealed a history of hypophosphatemia. Genetic testing was undergone and revealed a mutation in the PHEX gene (c.1646-1G>C) confirming the diagnosis of x-linked hypophosphatemia. The pathogenic variant (c.1646-1G>C) was not previously present in population databases, but algorithms that predict the effect of sequence changes on RNA splicing suggested this novel mutation disrupts the acceptor splice site in intron 15 of the PHEX gene and lead to a loss of protein function. After an extensive conversation with the patient, the decision for operative management was taken. Laboratory workup showed decreased phosphate levels at 0.66 mmol/L and hemoglobin at 85 g/L, while calcium and parathyroid hormone were increased at 2.58 mmol/L and 9.02 pmol/L, respectively. Substitution therapy led to the normalization of laboratory findings. After detailed medical examination, surgery in the form of a corrective osteotomy was agreed upon, which will improve the patient's quality of life.

Key words: Rickets disease, PHEX mutation, hypophosphatemia, RNA splicing

Presentation number: MG 9

CCR5-Δ32 GENE VARIANT IN CELIAC DISEASE

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Celiac disease is a chronic immune-mediated enteropathy of the small intestine that is triggered in genetically susceptible individuals by the consumption of gluten in the diet. Although HLA class II genes are undoubtedly involved in the development of celiac disease, only 40% of the genetic susceptibility to celiac disease can be attributed to them. This suggests that other genes within or outside the HLA region play a role in the pathogenesis of the disease. Chemokines and their receptors are involved in numerous aspects of the immune system and have been studied in various autoimmune diseases. Deletion of 32 bp in the C-C chemokine receptor type 5 (CCR5) gene results in loss of expression of the receptor. The CCR5-Δ32 mutation has already been recognized as a modifying pathogenetic factor in type I diabetes. The inflammatory diseases type I diabetes and celiac disease co-segregate in a population, suggesting a common genetic origin. Therefore, the aim of our study was to determine the possible influence of the CCR5-Δ32 mutation on the predisposition and clinical expression of celiac disease. The study included 175 patients diagnosed with celiac disease according to the revised ESPGHAN criteria and 175 healthy controls matched for age, sex, and place of residence. Polymerase chain reaction was used to genotype the CCR5-Δ32 mutation. The allele frequency of the CCR5-Δ32 mutation was significantly higher ($p=0.028$) in patients than in controls. However, the effects of the CCR5-Δ32 mutation on the clinical expression of the disease did not show significant effects ($p>0.05$). The study showed no statistically significant differences in clinical presentation of the disease according to sex. The presence of the CCR5-Δ32 mutation influences the predisposition to celiac disease in our patients, but further studies with a larger number of patients are needed.

Key words: CCR5-Δ32, gene variant, celiac disease

Presentation number: MG 10

ASSESSMENT OF GENETIC TESTING FOR GILBERT'S SYNDROME IN BOSNIA AND HERZEGOVINA IN THE PERIOD 2014-2022

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Gilbert's syndrome is a genetic, autosomal-recessive liver disorder. It is characterized by periods of elevated levels of unconjugated bilirubin in the blood (hyperbilirubinemia). Hyperbilirubinemia is caused by reduced activity of the enzyme glucuronyltransferase (coded by UGT1A1 gene) which conjugates bilirubin making it soluble in water. Patients with Gilbert's syndrome have 7 TA repeats on both alleles of the UGT1A1 gene (TA7 / TA7) while healthy individuals are homozygous for the TA6 allele (TA6 / TA6). Heterozygotes in most cases do not develop hyperbilirubinemia. Routine genetic testing of Gilbert's syndrome consists of DNA isolation from the blood, PCR amplification and fragment detection on genetic analyzer. From 2014 to the beginning of 2022, 21 people were referred for Gilbert's syndrome genetic testing in our institution. Only one patient was homozygous for the TA6 allele and one was heterozygous, the rest of them were TA7 / TA7 homozygous meaning that the diagnosis was confirmed. The most of them were between 13 and 19 years old, confirming that clinical manifestations usually occur during puberty when the concentration of steroid hormones increases the bilirubin in the blood what indicates referral to genetic testing for Gilbert's syndrome. Also, 12 out of 21 patients were male what could support the fact that Gilbert's syndrome is more common in men than in women. Furthermore, concerning testing for this disease in Bosnia and Herzegovina, number of tested samples per year is growing in only one institution where before the end of the first trimester of 2022, five patients were tested and in previous 7 years 1-4 samples were tested per year. This short overview shows that recognition and consciousness about Gilbert's syndrome is growing what is very important because Gilbert's syndrome is a benign disease that does not require specific treatment and must be distinguished from other disorders of unconjugated hyperbilirubinemia.

Key words: Hyperbilirubinemia, Gilbert's Syndrome, genetic testing, UGT1A1 gene

Presentation number: MG 11

MICRO- AND MACRONUTRIENT PROFILE IN DOWN SYNDROME CHILDREN AND ADOLESCENTS: SYSTEMATIC REVIEW AND META-ANALYSIS

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Down syndrome (DS), an expression of complete or partial trisomy of chromosome 21, is the most common genetic disorder known to date. Nowadays, there is a great deal of clinical interest in the question of whether children with DS benefit from nutritional supplementation to improve their development, cognitive decline, and overall health, especially if started early in childhood. To date, the relevant scientific literature has not been systematically reviewed and organized. Therefore, our aim was to provide a systematic review and meta-analysis of the micro-and macronutrient profile in DS children and adolescents. This study was conducted in accordance with PRISMA guidelines. We identified all relevant case-control studies published by January 1, 2021, by searching the PubMed and Scopus databases for original English-language articles analyzing the micro-and macronutrient profile of individuals with DS. After a comprehensive analysis, 40 studies were included in the qualitative synthesis and 31 in the quantitative synthesis (meta-analysis). MetaAnalysis software, version 3.0 (Biostat, Inc., Englewood, NJ, USA) was employed for the meta-analysis. Significant results ($p \leq 0.05$) were obtained for zinc, selenium, copper, vitamin B12, sodium and calcium. Serum, plasma, and whole blood analyses showed lower zinc levels in DS compared with controls (SMD serum[95%CI]=-2.32[-3.22,-1.41]; SMD plasma[95%CI]=-1.29[-2.26,-0.31]; SMD blood[95%CI]=-1.59[-2.29,-0.89]). Similarly, plasma and blood selenium concentrations were significantly lower in DS (SMD plasma [95%CI]=-1.39[-2.26,-0.51]; SMD blood[95%CI]=-1.86[-2.59,-1.13]. Intraerythrocyte copper and serum B12 were higher in DS (SMD Cu [95%CI]=3.33[2.19,4.46]; SMD B12[95%CI]=0.89[0.01,1.77). Finally, salivary sodium and calcium were slightly elevated (SMD Na [95%CI]=1.06[0.29,1.82]; SMD Ca[95%CI]=0.49[0.16,0.83], whereas blood calcium was lower in DS children/adolescents compared to controls (SMD Ca[95%CI]=-0.77[-1.34,-0.21]). This study provides the first field overview of micro-and macronutrient profiles in DS children and adolescents. Additionally, the evidence-based foundation for future dietary interventions has also been established.

Key words: adolescents, Down syndrome, children, macronutrients, micronutrients

Presentation number: MG 12

OPITZ-KAVEGGIA (FGS1) SYNDROME AND XYY CHROMOSOMOPATHY – IS IT VARIANTS OF UNCERTAIN SIGNIFICANCE (VOUS) REALY VOUS - CASE REPORT

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Opitz-Kaveggia syndrome (FGS1) (OMIM # 305450) is a very rare disorder, the prevalence is unknown, although several hundred cases have been reported. Syndrom caused by mutation in the MED12 gene located at position Xq13 recessive pattern condition that affects many parts of the body. The physical features include hypotonia, facial appearance including small, underdeveloped ears; hypertelorism, macrocephaly prominent forehead; down-slanting palpebral fissures, brain anomaly, seizures and heart defects. We report 4 months old boy, born in a second pregnancy (first pregnancy misscariage) of nonconsanguineous parents, with intrauterine growth retardation, premature birth at 32 weeks of gestation, birth weight 1.3 kg. Noninvasive prenatal testing is normal. Dysmorphic features present from birth macrocephaly, depressed nasal bridge, hypertelorism, gothic palate, microretrognathia, low-set ears, mild contractures of both knees, micropenis. Neonatal period complicated with more comorbidities - respiratory distress syndrome, prolonged mechanical ventilation, bronchopulmonary dysplasia, recurrent infections, thrombotic incidents, heart failure with include multiple muscular ventricular septal defects, atrial septal defect, agenesis of the corpus callosum and bilateral hearing impairment. Karyotyping and chromosomal microarray diagnose sex chromosome aneuploidy XYY sindrom which does not explain such a complex disease. VOUS in the MED12 gene c.3692-7A> G in hemizygous status was identified by whole exome sequencing. Parental testing shows the same variant in the mother. Opitz-Kaveggia syndrome (FGS1) is a very rare X-linked recessive disorder, caused by mutation in the MED12 gene. The MED12 gene provides instructions for making a protein that helps regulate gene activity, and MED12 protein forms part of a large complex that turns genes on and off. The MED12 protein is thought to play an essential role in development both before and after birth. Although the mutations alter the structure of the MED12 protein, it is unclear how they lead to intellectual disability, and the physical features associated with this condition. We think that this is a new pathogenic variant of the MED12 gene because the patient has all the described comorbidities of FSG1.

Key words: Opitz-Kaveggia syndrome, FGS1, XYY chromoosomopathy

Presentation number: MG 13

IL-6 AND IL-8 GENE EXPRESSION IN MALE CHILDREN WITH OBESITY: A POSSIBLY EARLY ATHEROSCLEROTIC INFLAMMATORY PREDICTOR

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Atherosclerosis is one well know complication that can occur during obesity and it might have inflammation processes as important part of its pathology. In some previous study results show that interleukin 8 (IL-8) and interleukin 6 (IL-6) are associated with numerous different inflammatory processes in obese state and contribute to pathogenesis of atherosclerosis and other cardiovascular diseases (CVD). Aim of this study was to analyze if there is a correlation between IL-6 and IL-8 gene expression and other anthropometric and biochemical parameters in subcutaneous adipose tissue (SAT) of healthy male children as an early sign of metabolic dysregulation that can be connected with obesity in early childhood and have impact on later development of atherosclerosis. We determined changes in gene expression of IL-6 and IL-8 in SAT in lean and overweight/obese healthy male children. Tissue samples from SAT fat depots were obtained during surgery from 32 normal weight male children age 5,12±3,21 years, and 22 overweight/obese male children age 5,95±3,24 years, who underwent elective abdominal surgery having hernia repairs or orchidopexies at the Department for Pediatric Surgery of the University Hospital Osijek. Subject were divided in two groups by their BMI Z-score. On SAT samples immunohistochemistry for detection of CD163+ cells was performed and gene expression of IL-6 and IL-8 by quantitative RT-PCR with reference genes for data normalization was measured. Children in the overweight/obese group showed a higher expression of IL-6 ($p=0,019$) and IL-8 ($p<0,001$) in subcutaneous adipose tissue compared to normal weight children. The expression of IL-6 in SAT correlated positively with the number of CD163+ cells in same adipose tissue compartment, as has the expression of IL-8. The expression of IL-8 in SAT also correlated positively with BMI Z-score, triglyceride serum concentration, average surface area of SAT adipocytes and gene expression of COL6a3. Increased gene expression of IL-6 and IL-8 in subcutaneous adipose tissue of male children could indicate a possibility that obesity and chronic low-grade inflammatory processes in subcutaneous adipose tissue during early childhood can contribute to pathogenesis of atherosclerosis in adulthood.

Key words: gene expressions, obesity, male children, atherosclerosis

Presentation number: MG 14

CELL-LINE MODEL UNCOVERS ACTIVITY OF RUNX1 AS A POTENTIAL MODIFIER OF IMMUNOGLOBULIN G GLYCOSYLATION

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Down syndrome (DS) is a condition caused by trisomy 21 that entails numerous symptoms, one of which are premature signs of aging. As revealed by our recent research, these signs of premature aging are also reflected in plasma-derived immunoglobulin G (IgG) glycosylation, which is a well-known marker of biological age (Krištić et al. 2014). We have recently uncovered that individuals with DS show on average a 19-year increase in biological age when compared to chronologically age-matched controls of normal karyotype, however, the mechanism that causes this substantial difference is yet to be discovered. We hypothesize that these differences could be explained by the presence of a third copy of certain chromosome 21 genes, followed by the increased expression of proteins encoded by those genes. One candidate chromosome 21 gene is RUNX1 based on a recent GWAS study which found SNPs around this gene to be associated with human plasma IgG glycosylation (Klarić et al. 2020), and its extra copy has been discovered as an initiator of leukemogenic predisposition in DS (Nižetić and Groet 2012). We here used EBV-immortalized lymphoblastoid cell lines (LCLs) from an individual with DS and their disomic parent and sibling and treated the cells with a chemical inhibitor of the protein encoded by RUNX1. After treatment, the comparison of glycosylation profiles of IgG generated by these LCL cells revealed that inhibition of RUNX1 very significantly affected the glycosylation of IgG from LCLs derived from the disomic controls, yet no significant change in profile was observed in the cell-derived IgG of the person with DS. This finding complies with the aforementioned GWAS results and further implies that RUNX1 could be an important modulator of the general IgG profile in people with a normal number of chromosomes. Other mechanisms may prevail in skewing the glycan profiles in cells derived from people with DS.

Key words: Immunoglobulin G glycosylation, Down syndrome, RUNX1

Presentation number: MG 15

NEXT GENERATION SEQUENCING PANEL CUSTOMIZATION FOR LOSS AND REVERSIBILITY OF SENSE OF SMELL AND TASTE AFTER SARS-COV-2

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Sensory-neural loss of taste and smell can occur as a result of the destruction of neuroepithelium by toxic inflammatory factors or due to genetic factors such as polymorphisms on olfactory or taste receptor genes. Partial or complete loss of smell (anosmia/hyposmia) and taste (hypogeusia/ageusia), with or without distorted perception of smell and taste (dysosmia/dysgeusia), has a broad differential diagnosis. Reversibility of loss of sense of smell and taste is possible in cases of inflammation, and can be sampled by various drugs, disorders and genetic factors. At the beginning of the COVID-19 pandemic, dysosmia and dysgeusia were not considered important symptoms for COVID-19. After the initial onset of the pandemic, several studies have reported taste and odor disorders in patients with SARS CoV-2 infection. Identification of dysosmia and dysgeusia may help in the early detection of SARS CoV-2 infection. There are approximately 400 OR genes (olfactory receptors) that have different pseudogenes, copy number variations, and single nucleotide (SNP) polymorphisms that can alter receptor responses, making the olfactory system and consequent dysosmia susceptible to various influences. In addition, there are 2 main families of genes for taste receptors that can influence dysgeusia, as well as several genes that are in SARS CoV 2 viral fusion cascade. of this study is to design NGS panel which will elucidate main contributor genetic factors to dysosmia and dysgeusia. For the main panel design we used literature references and research on olfactory and taste receptor genes. For 144 genes that was our starting material we identified genome locations, sizes and SNP positions according to hg38 genome build. From that we filtered 69 genes that are main contributors, according to literature, for dysosmia/dysgeusia. After initial research, using DesignStudio by Illumina we checked the coverage which for our panel is 99% with 266 targets and 84,36 kb panel size with final count of 66 genes after we removed duplicates and low coverage target genes. Next step in study is validation and testing of panel to determine effectiveness of variant calling and efficiency of panel. The knowledge is applicable to further research on varying ethiology of loss of smell and taste and potential treatments.

Key words: NGS, dysosmia, dysgeuzia, COVID19

Presentation number: MG 16

NEUROOCULOCARDIOGENITOURINARY SYNDROME (NOCGUS) - CAUSED BY PATHOGENIC VARIANT IN THE WDR37 GENE - CASE REPORT

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Neurooculocardiogenitourinary syndrome (NOCGUS) (OMIM# 618652) is a multisystem disorder described 2019 year, caused by heterozygous mutation in the WDR37 gene on chromosome 10p15, autosomal dominant inherritance. Characterized by neurological impairment with structural brain defects and seizures, poor feeding, poor postnatal growth, ocular anomalies, dysmorphic facial features, and variable skeletal, cardiac and genitourinary defects. Death in infancy may occur. We report a 7 years old boy, born in a second pregnancy (two other childrens is healthy) of nonconsanguineous parents, with intrauterine growth retardation, birth weight 2,5 kg. Dysmorphic features present from birth: hypertelorism, bilateral corneal opacity with keratoconus, gothic palate, depressed nasal bridge, ears lower laid poorly modeled, mild microretrognathia, thorax short “soft” chunky; hyperextensibility of all joints, knee contracture, hypoplastic scrotum, enlarged liver 3 cm, hypotension, impaired reflexes. Complementary treatment: developmental brain anomaly (cerebellar hypoplasia Dandy Walker variant, corpus callosum hypoplasia, cortical atrophy), with early onset of epilepsy; eye anomaly - congenital corneal opacity and keratoconus, congenital heart defect - ventricular septal defect, bicuspid aortic valve and aortic root dilatation. Inherited metabolic diseases are excluded, karyotyping and chromosomal microarray were normal. Pathogenic variant in the WDR37 gene was identified by whole exome sequencing (WES): 10-1126405-T-G; c.385T>G; heterozygous. Boy have profund intelectual dIsabillity (IQ 35) and global developement delay. Neurooculocardiogenitourinary syndrome (NOCGUS) is a very rare autosomal dominant genetic disorder The WDR37 is predicted to encode a 494-amino acid protein of unknown function. Data collected via publicly available databases suggest a broad pattern of expression for Wdr37 in mice with enrichment in ocular and brain tissues. There is no cure for NOCGUS, affected individuals need a team of specialized doctors for treating the various problems, which can occur.

Key words: Neurooculocardiogenitourinary syndrome, coloboma, epilepsy

Presentation number: MG 17

DNMT3B rs2424913 AS A RISK FACTOR FOR CONGENITAL HEART DEFECTS IN DOWN SYNDROME

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Congenital heart defects (CHDs) are the most common type of congenital malformations, present in approximately 40 to 50% of individuals with Down syndrome (DS). The most common CHDs in DS are septal defects. DNA hypomethylation is suggested to be associated with the development of CHDs in DS, particularly with septal defects. The DNA methylation pattern is established and maintained by DNA methyltransferases (DNMTs). The aim of this study was to assess the association between single nucleotide polymorphisms of DNMT genes and CHDs in DS individuals. The study was performed on 249 participants with DS, including 132 DS individuals with CHD (DSCHD+) and 117 DS individuals without CHD (DSCHD-). Genotyping of single nucleotide polymorphisms DNMT1 (rs2228611), DNMT3A (rs1550117), DNMT3B (rs1569686), and DNMT3B (rs2424913) was performed using PCR-RFLP method. Statistical significance was considered at $P<0.05$. The most common congenital cardiac defect among DSCHD+ participants was atrial septal defect (ASD), followed by a ventricular septal defect (VSD) and atrioventricular septal defect (AVSD). Statistically significant higher frequency of the DNMT3B rs2424913 CT and rs2424913 TT genotypes were observed in DSCHD+ group compared to DSCHD- group ($p = 0.032$; $p = 0.011$). Additionally, significance risk for CHD under the dominant genetic model (CC + CT vs TT) for DNMT3B rs2424913 ($p= 0.011$) was demonstrated. DNMT3B rs2424913 TT genotype, as well as the T allele, had a significantly higher frequency in DS individuals with ASD in comparison to DS individuals with other CHDs ($p = 0.028$; $p = 0.018$). Study results suggest that DNMT3B rs2424913 CT and rs2424913 TT genotypes, as well as the dominant genetic model of the same polymorphism, might be a possible predisposing factor for CHD in DS individuals, particularly in the ones with ASD.

Key words: congenital heart defects, DNA methyltransferase, Down syndrome, single nucleotide polymorphism

Presentation number: MG 18

SAUL-WILSON SYNDROME – COMPLEX SKELETAL ABNORMALITIES CAUSED BY PATHOGENIC VARIANT IN THE COG4 GENE - CASE REPORT

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Saul-Wilson syndrome (OMIM # 618150) is a very rare disorder, at least 16 affected individuals have been reported in the scientific literature. Syndrom caused by mutation in the COG4 gene located at position 16q22.1, autosomal dominant inherritance. This gene provides instructions for making proteins known as the conserved oligomeric Golgi (COG) complex. Syndrom characterized by primordial dwarfism and other skeletal abnormalities, average adult height 107 centimeters. We report a 17 years old boy, body weight 19 kg; body height 96 cm; born in a second pregnancy (three other childrens is healthy) of nonconsanguineous parents, with intrauterine growth retardation, birth weight 2,1 kg. Dysmorphic features present from birth like arthrogryposis that disappear with growth, progeroid facial appearance, failure to thrive, lypodistrophy narrow nasal bridge with convex nasal ridge, prominent columella, mild micrognathia, blue sclerae. Skeletal findings include: profound short stature, clubfoot, short distal phalanges of fingers and toes. Flexion contractures of the knee joints, pseudoarthrosis, syringomyelia, thoracic scoliosis, lumbar hyperlordosis, coxa valga, skeletal dysplasia, pectus carinatum. Mild intelectual dIsabillity (IQ 65). Karyotyping and chromosomal microarray were normal. Pathogenic variant in the COG4 gene was identified by whole exome sequencing (WES): NM_015386.3: c.1546G> A (NP_056201.1: p.Gly516Arg), heterozygous. Saul-Wilson syndrome (SWS) is a very rare autosomal dominant genetic disorder The COG4 gene mutations that cause SWS result in production of an abnormal COG4 protein which is part of the COG complex, the transport of proteins between the Golgi apparatus and the endoplasmic reticulum is increased. It is unclear how this change in retrograde transport impairs bone growth and leads to the signs and symptoms of Saul-Wilson syndrome. There is no cure for SWS, affected individuals need a team of specialized doctors for treating the various problems, which can occur.

Key words: Saul-Wilson syndrome, dwarfism, skeletal abnormalities

Presentation number: MG 19

THE CONNECTION BETWEEN LEPTIN RECEPTOR GENE EXPRESSION, SERUM LEPTIN CONCENTRATION AND BODY MASS INDEX IN DIFFERENT MALIGNANT BREAST TUMORS DEPENDING ON LYMPH NODE METASTASES PRESENCE

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Previously we showed (Koprivić et al. Acta clinica Croatica) that obese females have a significantly higher level of leptin, regardless of the malignant breast tumor type. Observed through body mass index, the most significant differences in serum leptin levels are in the luminal B1 group. This study aimed to assess whether there was an association between leptin receptor gene (LEPR) expression and serum leptin concentration with body mass index (BMI), depending on the presence/absence of lymph node metastases. 53 females with malignant breast cancer were divided into two groups depending on the presence or absence of lymph node metastases (21 negatives and 32 positive lymph nodes). According to the St. Gallen Conference on breast cancer, carcinomas are divided into 5 subgroups according to molecular analysis and related to the expression profile of certain genes: Luminal A, Luminal B HER2 negative (LUM B1), Luminal B HER2 positive (LUM B2), HER2 positive and triple-negative subgroup. This division is based on estrogen, progesterone, and the expression of human epithelial growth factor. The positive or negative lymph node was specified with the tumor-node metastasis system classification AJCC (American Joint Committee on Cancer). LEPR gene level was determined by quantitative real-time PCR and serum leptin concentration by ELISA method. Each female was also measured BMI. Correlation analysis was calculated using Pearson's correlation coefficient at 95% confidence of interval (SigmaPlot v11.2, USA). Statistically significant negative correlation was found between serum leptin level and gene LPRT expression (r=-0.451, P=0.0403) and significant positive correlation between BMI and serum leptin level (r=0.771, P=0.0000425) in group of females with absence lymph node metastases. In female malignant breast tumor groups with presence of lymph node metastases only significantly positive correlation was observed between BMI and serum leptin level (r=0.677, P=0.0000207). The association between the LPRT gene and serum leptin levels is more pronounced in malignant breast tumors with the absence of lymph node metastases. Regardless of the presence/absence of lymph node metastases, changes in serum leptin concentration depend on a woman's BMI status.

Key words: breast tumor, leptin, leptin receptor, BMI, lymph node

Presentation number: MG 20

CONGENITAL BILATERAL HOANAL ATRESIA AS THE FIRST SIGN OF MUTATION IN THE CHD7 GENE

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CHARGE syndrome (OMIM # 214800) is a rare autosomal dominant genetic disorder that occurs in approximately 1 in 8,500 to 10,000 newborns, caused by mutation in the CHD7 gene located at position 8q12.2. Syndrom characterized by coloboma, heart defects, choanal atresia, growth retardation, genital abnormalities, and ear abnormalities. We report a 6-month-old boy, born in a second pregnancy (one healthy child) of nonconsanguineous parents, with intrauterine growth retardation, premature birth at 34 weeks of gestation, birth weight 1,5 kg, as a life-threatening, premature, hypotrophic infant with multiple difficulties as part of an underlying congenital disease that may clinically respond to CHARGE syndrome. Dysmorphic features present from birth both nostrils impassable by probe, dolichocephalic head, high forehead, depressed nasal bridge, blepharophimosis, thin upper lip, gothic palate, microretrognathia, generalized hypotonia, laryngotracheomalacia, callosum corpus hypoplasia, ventricular septal heart defect, cryptorchidism. Twice time surgically treated bilateral congenital hoanal atresia Karyotyping is normal. Pathogenic variant in the CHD7 gene was identified by whole exome sequencing (WES): 8-61757422-GT-G; c.4852del; heterozygous. Boy have global developement delay. CHARGE syndrome is a rare autosomal dominant genetic disorder and in hoanal atresia it is necessary to think of CHARGE as the cause The CHD7 gene provides instructions for making a protein that regulates gene expression by a chromatin remodeling. Most mutations in the CHD7 gene lead to the production of an abnormal CHD7 protein that is broken down prematurely. Shortage of this protein is thought to disrupt chromatin remodeling and the regulation of gene expression.

Key words: CHARGE syndrome, choanal atresia, heart defect

Presentation number: MG 21

EXPRESSION PATTERN OF APOPTOTIC INDUCING FACTOR IN THE INNER EAR DEVELOPMENT OF YOTARI (DAB1 -/-) AND WILD TYPE MICE

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DAB1-protein deficiency was investigated on the inner ear development of yotari in comparison to wild-type (wt) mice by expression of apoptotic inducing factor (AIF) at mice embryonic E13.5 and E15.5 in order to examine caspase independent apoptotic pathway. The spatial and temporal immunofluorescence expression pattern of AIF was determined by calculating area percentage covered by positive signal in the epithelium and mesenchyme of cochlear and semicircular ducts. Data were analyzed by t-test and were presented as a mean±SD. AIF expression in the epithelium of cochlear and semicircular duct were significantly higher in wt in comparison to yotari mice. The highest AIF expression was at E15.5 in the epithelium of cochlear duct, but this expression was twofold lower than in wt mice. AIF expression in mesenchyme of the cochlear and semicircular ducts were always statistically higher in wt in comparison to yotari, except for higher AIF expression in the cochlear duct of wt at E15.5 in comparison to yotari mice. Our results emphasize the relevance of AIF during development of vestibular and cochlear functions where they can serve as potential therapeutic targets in impairments of the inner ear.

Key words: apoptotic inducing factor (AIF), inner ear, yotari and wild-type mice, expression

Presentation number: MG 22

INVOLVEMENT OF EPITHELIAL TO MESENCHYMAL TRANSITION FACTORS DURING THE HUMAN EYE EMBRYOGENESIS AND TUMORIGENESIS

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The expression pattern of cytokeratin-8, vimentin, nestin, beta-tubulin, HSP70 and syndecan-1 markers was analyzed in histological sections of 8th week developing and postnatal human eye, in retinoblastoma and different uveal melanomas to establish their involvement in epithelial to mesenchymal transition. Tissue sections were examined by double immunofluorescence to abovementioned markers and the area percentage of positive signals was evaluated quantitatively and semi-quantitatively. The one-way ANOVA followed by Tukey's posthoc test was used for statistical analysis. Vimentin immunoreactivity characterized retinal and/or choroidal cells in healthy and tumorous tissues: expression was lower in developing retina and retinoblastoma, while it was high in epitheloid and spindle melanoma. Beta-tubulin and HSP-70 expression was highest in tumor tissue of retinoblastoma, epitheloid and mixoid uveal melanomas. Cytokeratin-8 was observed only in development and rarely in the choroid of mixoid melanoma. Nestin immunoreactivity was highest in the retinoblastoma and spindle melanoma, and missing in epitheloid melanoma, while sindecan-1 had highest expression in epitheloid and mixoid melanoma. Their differential expression appeared between types of melanomas. The differences in the expression pattern of factors involved in epithelial to mesenchymal transition correlate with the origin and stage of cell differentiation of tissue samples. The balanced expression pattern is required for both human eye development and eye tumorigenesis. Therefore, understanding of their involvement and interplay is important for possible new therapeutical targets based on epithelial to mesenchymal transition underlined in developmental eye plasticity and neoplasm.

Key words: human eye, embryogenesis, tumorigenesis, epithelial to mesenchymal transition

Individualized (personalized) medicine

Presentation number: MG 23

TWO RARE CASES OF CEREBROTENDINOUS XANTHOMATOSIS IN THE SAME FAMILY CAUSED BY AN INTRONIC MUTATION IN CYP27A1

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Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive lipid storage disease associated with abnormally high blood cholestanol levels. Sterol 27-hydroxylase enzyme deficiency leads to abnormal metabolization of cholesterol and diverts the metabolic pathway in the direction of reduced production of chenodeoxycholic acid and an increased formation of 25-hydroxylated bile alcohols and cholestanol. CTX patients commonly have infantile-onset diarrhea, childhood-onset cataract, adult-onset progressive neurologic symptoms, and adolescent to young adult-onset tendon xanthomas. Cholestanol accumulates in the brain, tendons, eyes, and blood vessels, causing a range of clinical manifestations, including ataxia, epilepsy, dementia, and even parkinsonism. We present a case of two brothers aged 34 and 38 with a similar clinical picture that includes ataxia, cognitive deficit, congenital cataract, and diarrhea since birth. The older brother also experienced grand mal seizures. Parents are healthy and are not in consanguinity. Moreover, other family members are without neuromuscular diseases and known heredity in the family. Patients came to the St. Catherine Specialty Hospital to perform genetic testing and counseling due to a suspicion of a genetic disorder. Next-generation sequencing of 444 genes associated with various neurological and neuromuscular disorders with clinical examination, radiological and biochemical analysis established a diagnosis of autosomal recessive cerebrotendinous xanthomatosis caused by a homozygous form of variant c.1184+1G>A in both brothers. This mutation affects a donor splice site in intron 6 of the CYP27A1 gene. Studies have shown that disruption of this splice site results in the skipping of 89 nucleotides of exon six and introduces a premature termination codon which results in mRNA nonsense-mediated decay. However, replacement therapy with chenodeoxycholic acid may prevent clinical deterioration. Early treatment in symptomatic patients was shown to stop progression and, in some cases, reduction of pre-existing neurological deficits. Patients were referred to systematic clinical monitoring and treatment under the supervision of the clinical medical team at St. Catherine Specialty Hospital.

Key words: sterol 27-hydroxylase, cholestanol, metabolic disease, CYP27A1, chenodeoxycholic acid

Presentation number: MG 24

QUANTITATIVE ANALYSIS OF MIRNA IN SUDDEN CARDIAC DEATH TISSUE AND BLOOD SAMPLES

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Cardiovascular diseases are the leading cause of death worldwide. Currently, microRNAs (miRNAs) are very promising biomarkers in various cardiovascular diseases. MiRNAs are short non-coding RNAs, which regulate a vast variety of biological processes. They bind to their target mRNA at the 3'UTR and can either inhibit translation or initiate their degradation. The focus of the current study was to investigate the expression of different miRNAs in heart tissue and whole blood samples from sudden cardiac death (SCD) in comparison to control hearts. The expression of different miRNAs in heart tissue and whole blood of SCD and control cases was assessed via q-RT-PCR in accordance with the MIQE guidelines. SCD group was divided in cases with structural changes (myocardial infarction, MI) and cases without visible changes. Statistical evaluation of significance and receiver operating characteristic (ROC) analysis was performed via R Studio. An upregulation of miR-1, miR-26a and miR-133a in tissue of SCD samples without structural changes compared to MI and controls were found. Furthermore, miR-1 and miR-133a were upregulated in MI whole blood. Receiver operating characteristics show a better diagnostic accuracy of miR-1 and miR-133a in whole blood than in tissue. MiR-1 and miR-133a were among the first miRNAs to be discovered in mammals, but their physiological purpose still remains unclear. The results of this study reveal that whole blood is better suited for quantitative expression analysis of these miRNAs. Especially the muscle specific miRNAs (MyomiRs) miR-1 and miR-133a showed expression differences in tissue and whole blood of MI and SCD. The higher expressions of these miRNAs in whole blood after MI may point to necrosis of the heart tissue and therefore could be a useful degradation marker for acute MI. There are still challenges that need to be addressed to establish these new biomarkers. Nevertheless, this study may be the basis for further clinical and basic research in the field of blood-based miRNA-profiling in sudden cardiac death.

Key words: MicroRNA, biomarkers, sudden cardiac death, myocardial infarction

Presentation number: MG 25

TREATMENT OF PULMONARY SARCOIDOSIS USING ALLOGENIC BONE MARROW-DERIVED MESENCHYMAL STEM CELL THERAPY IS SAFE: A CASE REPORT

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In the 21rst century mesenchymal stem cells (MSC) are used from a variety of sources whether adipose derived, placental or bone marrow. These mesenchymal stem cells have a proven potent immunomodulator effect both in vitro and in vivo. The growing field of personalized medicine allowed for the introduction of stem cell therapy in the treatment of systemic and localized diseases. We report a case of a 67-year-old male patient, first diagnosed with sarcoidosis in 2005, that responded well clinically and biologically to ImmunoARTTM (developed by Educell Ltd., member of Medical Biobank Swiss Institute SA (MBSI)) allogenic, HLA-incompatible and non-related bone marrow-derived MSCs in a dose of 106/kg. The patient presented to St. Catherine Specialty Hospital in 2021 with an exacerbation of respiratory symptoms. After a clinical and radiological examination with laboratory workup, radiological findings were consistent with pulmonary sarcoidosis, while laboratory work revealed increased leucocytes at 14.2 g/L, CRP at 51.2 mg/L and lymphocytes at 4.25 g/L. The patient was then administered with intravenous application of MSCs on three occasions in the out-patient clinic. MSC doses were prepared from a young, healthy donor who agreed to donate bone marrow for allogeneic treatment and who was negative for viral markers (HBs Ag, HBc Ab, HCV Ab, HIV 1-2 Ab, TPHA, HBV NAT, HCV NAT, HIV NAT) according to EU legislation. Cells were prepared in a controlled and verified laboratory for “Hospital exemption” cell preparation in the cleanroom facility in safety cabinet class A and expressed CD105, CD 73 and CD 90 but lacked the expression of CD45 and CD34. Over the course of MSC therapy, the patient showed clinical and biological with a decrease in inflammatory parameters. Laboratory values were assessed at days 2, 5 and 7. On day 7, leucocytes were 11.2 g/L, lymphocytes 4.0 g/L and CRP 5.1 mg/L. In the short follow-up period the patient felt subjectively better, without any side effects to the MSC therapy. Unfortunately, the patient dropped out from follow up, therefore the prolonged effects of this therapy were not able to be assessed. Therefore, systemic MSC therapy presents an opportunity for treatment of sarcoidosis that needs to be further researched.

Key words: Mesenchymal stem cells, allogenic MSC, sarcoidosis

Infectious diseases

Presentation number: MG 26

ANTIVIRAL POTENTIAL OF TRADITIONAL INDIAN HERBAL MEDICINE AGAINST SARS-COV-2: AN IN SILICO STUDY

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Indian Traditional System of Medicine (ITSM) is one of the oldest traditional medicine systems classified into five different traditional approaches named Ayurveda, Yoga, Unani, Siddha, and Homoeopathy. Number of studies showed that people rely on traditional medicine as a support against SARS-CoV-2 and other respiratory viruses. The antiviral potential of herbal preparations is proven experimentally and explained by its competitive binding affinity and inhibition of viral attachment and replication. Main protease of SARS-CoV-2 (Mpro) (PDB ID: 6Y84) that is responsible for virus replication and gene expression is a drug target for many antiviral drugs. We analysed binding affinity of selected compounds that are extracted from plants used in ITSM, to Mpro using in silico tool AutoDock Vina 1.1.2. Selected compounds were acanthoside, acetovanillone, apigenin, astragalin, cucurbitacin B, curcumin, kaempferol, luteolin-7-rutinoside, malic acid, marmin, myricetin, myrtenol, pektolinarin, quercetin, rutin, somniferone, syrigaresinol, and violanthin. Acetoside and remdesivir were used as positive controls of binding affinity for Mpro. Results highest affinities (rmsd l.b. 0.000; rmsd u.b. 0.000) were observed for somniferone (-11.2), then for luteolin (-11.0), rutin (-10.3), violanthin (-10.2), and cucurbitacin B (-10.0), all expressed in kcal/mol. After visualization via PyMOL 2.4., cucurbitacin B, luteolin, somniferone and positive control remdesivir had similar binding interaction of SARS-CoV-2 main protease (Mpro) close to position of Lys5, residue that is one of the previously explored regulatory sites for various molecular interactions. Our findings suggest that selected compounds of Indian herbal medicines represent potential inhibitors against SARS-CoV-2 Mpro, but further investigation of the mechanisms of action as well as the potential side effects are needed for final confirmation of inhibitory functionality of these compounds.

Key words: molecular docking, SARS-CoV-2, Indian Traditional System of Medicine, main protease

Presentation number: MG 27

RT PCR AND GALACTOMANNAN FROM BAL AND BLOOD SAMPLES IN DIAGNOSING INVASIVE ASPERGILLOSIS

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Invasive aspergillosis (IA) is most frequent mould infection with high mortality in the immunocompromised patients. Spores of Aspergillus can be inhaled and can cause infection in immunosuppressed patients. Risk of IA correlates with duration and severity of neutropenia. Mycological cultures are positive for Aspergillus spp. in at most 26% of the invasive aspergillosis cases. Due to low sensitivity of the mycological cultures are diagnosed indirectly by the detection of galactomannan (GM) from blood samples or bronchoalveolar lavage (BAL). The aim of this study was to compare results obtained from BAL and blood samples routinely tested on GM with results obtained with PCR from same samples. The medical ethics committee of University Hospital Centre Zagreb approved the study. In this study 23 samples from hospitalized patients previously tested for GM (Platella Aspergillus Ag, Bio-Rad, France) and stored on -20oC were tested with AsperGeniuSpecies multiplex real-time PCR assay (PathoNostics, Maastricht, The Netherlands) on Rotor Gene Q. 12 previously tested blood samples (6 positive and 6 negative) and 11 bronchoalveolar fluids previously tested on galactomannan were tested with Aspergillus RT PCR. The optimal cycle threshold cut-off value for the Aspergillus species PCR was <38. Among 6 GM positive blood samples previously tested, 2 of them where positive also on RT-PCR for Aspergillus spp. and the rest of them were negative. Very interesting one of the blood samples positive on GM with high values (3,39 ODI) was negative on Aspergillus PCR and that high values are usually connected with poor prognosis for neutropenic patients. 11 Bal samples previously tested on GM where enrolled in the study with ODI bellow 1 (1 is cut-off for GM from BAL) where tested with Aspergillus PCR. Two of them with GM ODI 0,1 and 0,6 where positive on Aspergillus RT PCR with Ct values 25,75 and 26,15, respectively. Combination of GM testing and RT-PCR for Aspergillus spp. could enhance specificity and sensitivity of the mycological diagnostics and help in introducing early and appropriate therapy in order to improve the outcome of the patients.

Key words: Invasive Aspergillosis, galactomannan, RT PCR, molecular biology

Molecular diagnostics: current technology and applications

Presentation number: MG 28

KNOWLEDGE AND ATTITUDES ON GENETIC TESTING IN CROATIAN POPULATION: A PRELIMINARY STUDY

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Aim was to investigate the knowledge and attitudes about genetic testing in the Croatian general population and determine the factors affecting their willingness to participate in different types of genetic testing. We conducted a cross-sectional online survey from 6 to 14 March 2022 on a sample of 215 adults (72.1% females, median age = 31). The survey questionnaire included general demographic data, data on blood donation, and diagnosis of severe diseases in relatives. We included questions regarding knowledge on genetic testing (e.g., the meaning and availability) and created two scales measuring participants' attitudes toward genetic testing. The first scale examined their attitudes and willingness to participate in genetic testing, while the second one explored fear related to involvement in such activities. Participants demonstrated on average relatively positive attitudes and higher levels of willingness to participate in genetic testing (total score = 32.05/40; 95%CI 31.03-32.96) and moderate levels of fear related to involvement in such activities (total score = 10.94/20; 95%CI 10.35-11.53). Participants' sex ($P = 0.048$), religion ($P = 0.047$), and reported knowledge ($P < 0.001$) significantly contributed to their attitudes and willingness to participate in such activities, while their fear of involvement was related to political affiliation ($P = 0.012$). Croatian population shows openness toward genetic research and testing that can be affected by their sex, religion, knowledge, and political affiliation. To obtain better insight into those factors, future studies should be conducted on a larger and more comprehensive sample.

Key words: genetic testing, attitude, knowledge, Croatian population, personalized medicine

Presentation number: MG 29

FREQUENCY OF HLA DQ2.5 AND HLA DQ8 GENOTYPE IN CLINICAL PATIENTS WITH IBD SYMPTOMS OVER FOUR-YEAR PERIOD IN POPULATION OF BOSNIA AND HERZEGOVINA

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Celiac disease is an autoimmune disorder affecting the inflammation of the small intestine, triggered by the gluten consumption. Predisposition is determined by the HLA II DQA and DQB genes. Symptoms can be very unpleasant for individuals, but since they may disappear with gluten-free diet, an early celiac diagnosis is very important. Aim of this study is to determine the distribution of HLA genotype frequency in representative samples of Bosnian-Herzegovinian population with IBD symptoms. For this study a total of 170 patients with clinical IBD symptoms which were tested for HLA-DQ2.5 or HLA-DQ8 genotype, were observed. Clinical data were collected from medical histories of patients. All patients underwent HLA genotyping in ALEA Genetic Center from 2018 to the end of March, 2022. Buccal swabs or blood samples were used as the DNA source. For the testing, Celiaclear (molGENTIX SL, Spain) reagents were used according to manufacturer's instructions. To detect HLA genotype, fragmental analysis was performed on the SeqStudio Genetic Analyzer. This study showed that there was a strong association between results of HLA-DQ2.5/DQ8 molecular testing and clinical manifestations of patients. This molecular analysis was recommended to patients based on their clinical features. Patients mainly reported: abdominal pain, diarrhea, bloating, gas, constipation, fatigue, reduced appetite and weight loss. Correlation of these symptoms with celiac disease was confirmed by HLA molecular typing, where results themselves coincided with clinical manifestations for most of the patients. During the study, it was noticed that the HLA-DQ2.5 was more frequent genotype in the population of Bosnia and Herzegovina over four year period. Clinical manifestation can significantly indicate the type of molecular analysis. HLA molecular typing for celiac disease is an important parameter for the discrimination of individuals genetically susceptible to celiac disease.

Key words: celiac disease, HLA genotype, molecular testing, IBD symptoms

Presentation number: MG 30

CHALLENGES IN OBTAINING HIGH-QUALITY DATA FROM A CUSTOM-MADE PANEL FOR THE NEXT GENERATION SEQUENCING (NGS) USING ION TORRENT GENESTUDIOTM S5 PLATFORM

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Aim was the sequencing procedure optimization of a total of 16 human genes and their regulatory regions from 60 COVID-19 patients from the General Hospital of Tešanj, Bosnia and Herzegovina. Selected genes were found to be potentially associated with differential immunological COVID-19 response according to previously published data. Methods: DNA isolation from whole blood was performed using QIAamp® DNA Mini Kit, as per the manufacturer's instructions. Next Generation Sequencing was conducted on Ion Torrent GeneStudioTM S5 platform. Library preparation was done using Ion AmpliSeqTM Library Kit Plus and was optimized for low-quality DNA. In addition, three samples were subjected to clinical exome sequencing using the TruSight One Sequencing Panel (Illumina, San Diego, CA). NGS was optimized through separating two primer pools, increasing the number of PCR cycles, and decreasing the annealing temperature for the primer pool that showed poorer amplification results. Primer pool 1 obtained results for all of the 60 patients, while pool 2 obtained results for a total of 48 patients, including three clinical exome sequences. The analysis was not limited by the quality of collected samples and DNA, but by the quality of custom-made primers from pool 2. Separating the two primer pools allowed for complete results when it comes to primer pool 1 and partial completion of results with primer pool 2.

Key words: COVID-19, next-generation sequencing, Ion Torrent GeneStudioTM S5, immunological response

Presentation number: MG 31

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) MOLECULAR TESTING IN HISTOLOGY AND CYTOLOGY SPECIMENS

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Lung cancer is a leading cause of cancer related deaths in the world. Adenocarcinoma is one of the most commonly diagnosed subtypes of non-small cell lung cancer. Nowadays, as medicine advances, treatment is based on the molecular characteristics of certain types of cancer. The epidermal growth factor receptor (EGFR) belongs to the ErbB family of receptor tyrosine kinases. EGFR sequencing at the Clinical Hospital Dubrava is performed using the Idylla™ EGFR mutation test on the Biocartis Idylla system. It is an in vitro polymerase chain reaction (PCR) test that is the most commonly used method for detecting EGFR mutations and is key evidence for investigating possible gene therapy in patients with non-small cell lung cancer. Idylla™ EGFR mutation test is a diagnostic test for qualitative detection of exon 18 (G719A/C/S), exon 21 (L858R, L861Q), exon 20 mutations (T790M, S7681), exon 19 deletion and exon 20 insertions. From September 2020 until March 2022, a total of 89 samples for EGFR mutation were tested. The diagnosis of lung adenocarcinoma was made by histological and cytological examination. There were 59 histological sections (66.3%) and 30 cytological smears (33.7%). The mutation was found in 23 (25.8%) samples, 11 (47.8%) in histological and 12 (52.2%) in cytological smears. The most common mutation was deletion in exon 19. The second most common mutation is L858R on 4 histological samples. The L861Q mutation was confirmed in 2 metastatic adenocarcinomas on cytological smears. The insertion in exon 20 was confirmed on 1 cytological smear, as well as the T790M mutation and the deletion in exon 19. The G719A/C/S mutation was confirmed in 2 cytological samples, while the L858R and L861Q mutations were confirmed in 2 cytological smears. Biocartis Idylla™ is simple system for rapid detection of relevant mutations. Due to the high sensitivity, the system gave positive results already at 20% share of tumor cells. With fast handling and results ready in 150 minutes, this method accelerated the analysis and the possibility of applying targeted therapy. Lung adenocarcinoma patiens with detected EGFR mutations are treated with tyrosine kinase inhibitors erlotinib, gefitinib and crizotinib. A bigger issue is becoming resistance to these drugs, so the priority is the development of new target drugs.

Key words: adenocarcinoma, EGFR, histology, citology

Presentation number: MG 32

SARS-COV-2 VIRUS: COMPARISON OF MANUAL AND AUTOMATIZED RNA EXTRACTION TECHNIQUES FROM NASOPHARYNGEAL SAMPLES

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SARS-CoV-2 virus caused the COVID-19 pandemic and the fast spread of SARS-CoV-2 (COVID-19) has created the need for fast diagnostic testing. Reliable protocols for viral RNA extraction and amplification are crucial for the detection of SARS-CoV-2 virus. The aim of this study was to compare seven RNA extraction techniques, of which two were automatized RNA extraction techniques and five manual RNA extraction techniques. The detection of extracted RNA was validated by LabGunTM COVID-19 PCR Kit (Ref CV9017B) from LabGenomics Co., Ltd targeting RdRp gene, based on their Ct value. Thirty clinical nasopharyngeal samples were collected in ALEA Genetic Center for the comparison of different techniques for RNA extraction. Twenty four nasopharyngeal samples were positive samples and six negative samples were used as a negative extraction control. The Bio-Rad CFX96 Touch Real-Time PCR Detection System with the LabGunTM COVID-19 Assay was used as a molecular detection technique for SARS-CoV-2. T-test was used for the comparison of different extraction techniques. Values with $p<0.05$ were considered statistically significant. Results show that most of these techniques meet the basic requirements for RNA extraction. Two extraction techniques have been chosen with optimal results in all of the parameters (cost effectiveness, RNA yield and RT-PCR results).

Key words: SARS-CoV-2 virus, RNA extraction, Real-time PCR

Presentation number: MG 33

COMPARATIVE ANALYSIS OF SARS-COV-2 DETECTION KITS

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SARS-CoV-2 is a coronavirus that causes a respiratory disease, COVID-19. For COVID-19 testing, real-time PCR is considered gold standard and therefore many commercial SARS-Cov-2 detection kits are available. Rapid and accurate diagnostic tests are essential for controlling the COVID-19 pandemic. Aim of the study is to determine diagnostic values of 10 different commercially available SARS-CoV-2 detection kits, based on their Ct value. For this study thirty clinical nasopharyngeal samples were collected in ALEA Genetic Center. Twenty four of them were positive, while six were negative and used as a negative control. Positive samples were selected based on the day when first symptoms appeared. RNA was extracted using the same extraction method for all samples. For amplification and comparison of detection kits, the same RT- PCR instrument was used. Accuracy, sensitivity, specificity and Cohen's kappa coefficient were estimated to evaluate diagnostic values of the tested kits. This study showed that all kits showed 100% specificity. Accuracy, sensitivity and kappa coefficient varied among examined assays. Based on clinical features, LabGunTM COVID-19 Assay by LabGenomics proved to be the most sensitive, the most accurate and most specific. Therefore, this assay was used as a reference kit. If things from practice are taken into account, accuracy and reliability of the tested commercial kits can vary compared to those obtained in this study where results were based on ideal functioning of the kits. When choosing the convenient commercial SARS-CoV-2 detection kit using RT-PCR method, many parameters need to be considered.

Key words: SARS-CoV-2, SARS-CoV-2 detection kits, real-time PCR

Presentation number: MG 34

PRENATAL RHD GENOTYPING BY NIPT METHOD: CROATIAN EXPERIENCE

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Croatian Institute of Transfusion Medicine (CITM) implemented non-invasive prenatal fetal RHD genotyping as a request for targeted antenatal anti-D prophylaxis. The preanalytical factors, diagnostic performance, and results of validated in house RT-PCR method are investigated. Materials and methods RHD genotyping of 205 RhD negative pregnant women in 12-36th week of gestation was performed in period between 2015 and 2019. QIA symphony SP DSP Virus Midi Kit was used with modification to obtain optimized cffDNA yield on QIA symphony SP device (Qiagen, Germany). Fragments of RHD exons 7, 10 and later exon 5 were RT-PCR amplified. As internal controls, fragments SRY or RASSF1A gene with β-actin genes digested with BsTUI were used. Results 70.72% (145/205) positive and 28.78% (59/205) negative fetal RHD genotypes were detected. One inconclusive result (0.5%) was due to the interference of maternal DNA with variant genotype RHD*09.02.00/01/*01N.01. Our method enables detection of fetal D variant inherited from the father in RHD*04.04/*01N.01 genotype. When compared to newborn's RhD phenotypes, no false negative and three false positive results (3/199, 1.50%) were observed. The test yielded 100% sensitivity, 95.08% specificity and 98.48% diagnostic accuracy. The negative and positive predictive test values were 100% and 97.86%, respectively. Conclusion Careful sample handling, automated cffDNA extraction and RT-PCR amplification of fetal RHD exons 5,7,10 and internal controls of SRY, RASSF1A fragments represents highly reliable system for determining fetal RHD status which enables targeted antenatal anti-D prophylaxis. To obtain high specificity of cffDNA extraction, strict and thoroughly decontamination protocol is required. Introduction the mandatory NIPT RHD screening for whole country requires a fully automated process on platform used only for this method. This would shorten the time to results, allow better standardization, and reduce cross-contamination risk.

Key words: non-invasive prenatal RHD genotyping, cell-free fetal DNA, anti-D immunoprophylaxis.

Presentation number: MG 35

PLACENTAL PATHOLOGY CHANGES OF THE THIRD TRIMESTER PREGNANT WOMEN FROM COVID-19: HISTOLOGICAL, BIOMOLECULAR AND IMMUNOHISTOCHEMICAL STUDY

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The present study aimed to report the analysis of histopathological, immunohistochemical, and molecular of a large series of placentas from SARS-CoV-2-positive mothers observed at a “San Giovanni di Dio” Hospital in Agrigento during the pandemic and to compare them with a control group to highlight any histopathological alterations attributable to SARS-CoV-2. Regarding placental disease in SARS COV-2 virus-positive pregnant women, only case reports or small, limited case series were reported during the pandemic. Twenty-one placentas from the third-trimester pregnancy women were studied. Twenty-one selected received singleton third-trimester placentas consecutively from SARS-CoV-2-negative women from the same time period were reviewed for comparison. All patients were cured, and no clinical or serological evidence pointed to vertical transmission of SARS-CoV-2. In SARS CoV-2 virus-positive pregnant women were only observed aspecific lesions: Maternal VascularMalperfusion (MVM) were present in 19 (90.4%) cases; Fetal Vascular Malperfusion (FVM) lesions occurred in 20 (95.2%) cases; Maternal/Fetal Inflammatory Response (MFIR) was observed in 3 (14.2) cases. In 17 cases (80%), MVP and FVM were associated; in 2 cases(10%), FVM and MFIR were associated; in 1 (5%) case, the MVM, FVM, and MFIR occurred together; in 1 (5%) case MVM occurred alone. A comparison of placental lesions between SARS COV-2 virus-positive pregnant women and the control group showed a statistically significant difference (p-value: 0.0089). In no cases does biomolecular and immunohistochemical analysis (RNASCOPE with probe SARS CoV-2; anti-spike protein) demonstrate viral mRNA or spike protein. The preterm newborns were significantly present (p-value: 0.0048) in pregnant women virus-positive during the third trimester of pregnancy “remote” from delivery. We found no evidence of vertical transmission and adverse maternal-fetal outcomes in the placentas of third trimester COVID-19 pregnancy women, which provided further information for the clinical management of those women in the third trimester. However, further studies are still needed for patients with infections in different stages of gestation, especially in the first and second trimesters.

Key words: Placental pathology, SARS-CoV-2 infection, vertical transmission, immunohistochemistry

Prenatal diagnostics

Presentation number: MG 36

INCOMPLETE PENETRANCE OF PATHOGENIC GREBL1 VARIANT CAUSED A DIFFERENT CLINICAL PRESENTATION IN 3 GENERATIONS OF ONE FAMILY

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Renal agenesis is a rare congenital malformation characterized by the complete absence of development of one or both kidneys. The prevalence of such malformation is estimated at around 1/2000 for unilateral and 1/8,500 for bilateral renal agenesis. Even though unilateral renal agenesis can often be detected as an incidental finding later in life, bilateral renal agenesis is unfortunately incompatible with life. Our patient previously had 2 failed pregnancies, both associated with urogenital malformations of the fetus. The patient had functional kidneys and no urogenital malformations even though her mother was diagnosed with unilateral renal agenesis and subsequent contralateral kidney hypertrophia and systemic hypertension. Her mother also had a miscarriage before our patient's birth. Her brother is a healthy individual and her husband reports no inherited diseases on his side of the family. Considering the patient's medical history, genetic testing was initiated in our hospital and included the "Invitae Congenital Anomalies of Kidney and Urinary Tract (CAKUT) Panel" which covers genes associated with congenital renal malformations. Even though these conditions can present with a seemingly similar phenotype, they are often associated with a high degree of genetic heterogeneity. Therefore, this broad panel testing allows for an efficient evaluation of several potential genes based on a single clinical indication. Results of the genetic testing revealed that the patient is a heterozygote for the pathogenic variant of the GREB1L gene (c.4115_4118dup (p.Trp1373Cysfs*4), associated with autosomal dominant renal hypodysplasia/aplasia. Preliminary evidence also correlates the GREBL1 gene with autosomal dominant inner ear malformations and deafness. It is important to note that the GREBL1 gene shows reduced penetration which was clearly evident by the absence of the symptoms in our patient despite the presence of the genetic mutation. In the case of our patient, there is a 50% chance that the pathogenic gene variant will be passed on to descendants. These findings further emphasize the importance of genetic testing which can be combined with prenatal ultrasonography to provide an optimal diagnostic evaluation.

Key words: renal agenesis, CAKUT, GREB1L, miscarriage, genetic testing

Protein glycosylation in diagnostics and therapy

Presentation number: MG 37

N-GLYCANS OF COMPLEMENT COMPONENT C3 ARE A MARKER OF EARLY ONSET TYPE 1 DIABETES MELLITUS

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Previously it was shown that children at the onset of type 1 diabetes have a higher proportion of oligomannose glycans in plasma N-glycome compared to their healthy siblings. The most abundant complement component, glycoprotein C3, contains two N-glycosylation sites occupied by this type of glycans. Also, C3 gene was recently associated with plasma N-glycosylation in type 1 diabetes population. Using our high-throughput workflow for human C3 N-glycosylation analysis, we wanted to see whether C3 is the carrier of aforementioned changes in plasma N-glycome. C3 enrichment from human plasma was done in a 96-well format using Concanavalin A lectin affinity matrix. We studied plasma samples from 61 children/adolescents (1-16 years) newly diagnosed with type 1 diabetes and 84 of their unaffected siblings (4-22 years). A glycan-based discriminative model was built using logistic mixed model elastic net regression. C3 N-glycan profiles were significantly changed in type 1 diabetes children compared to healthy siblings. Type 1 diabetes was associated with an increase in the proportion of unprocessed glycan structures with more mannose units. A model including C3 N-glycans showed notable discriminative power between children with type 1 diabetes and healthy siblings with AUC of 0.879. There are significant changes of C3 N-glycosylation accompanying the onset of type 1 diabetes, indicating that C3 is the carrier of the previously reported high-mannose glycan changes in the total plasma N-glycome. Our C3 glycan-based descriminative model could be valuable in assessment of type 1 diabetes risk in children.

Key words: C3 glycoprotein, glycopeptides, LC-MS, N-glycosylation, Type 1 diabetes onset

Presentation number: MG 38

CELL AGING AFFECTS GLYCOSYLATION OF IMMUNOGLOBULIN G SECRETED FROM MODEL CELL LINE FREESTYLE™ 293-F

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Glycosylation of the Fc fragment of immunoglobulin G (IgG) affects the role of this antibody in the adaptive immune system. Aging is associated with changes in IgG glycosylation, primarily galactosylation, which leads to an increased proportion of proinflammatory IgG antibodies in human plasma. FreeStyle ™ 293-F is a model cell line used for production of recombinant IgG and is thus appropriate for studies of IgG glycosylation. In addition, glycome of IgG secreted from FreeStyle ™ 293-F cells is similar to IgG glycome from human plasma. The aim of this study was to investigate if the aging of the model cell line affects IgG glycome and, if so, are these changes similar to the changes observed on IgG from human plasma in older people. Ultra-high performance liquid chromatography revealed that cell aging, monitored during 90 days, indeed led to changes of IgG glycome. The most significant changes were an increase in the proportion of agalactosylated and a decrease in the proportion of fucosylated glycan structures. Proportion of high-mannose glycans also increased significantly, while proportions of sialylated glycans and glycans with bisecting N-acetylglucosamine remained stable during the time course experiment. Next, we investigated if glycan changes resulted from differential expression of glycosyltransferases responsible for individual steps in the IgG glycosylation pathway. This analysis revealed that a decrease of core fucosylation was associated with changes in FUT8 expression, while changes in galactosylation were not a direct consequence of altered B4GALT1 expression. An increase in the proportion of high-mannose glycans was in correlation with reduced MGAT1 and MGAT2 transcriptional activity, and the downregulation of these genes could also explain the decrease of complex IgG glycan structures. Overall, changes of IgG glycome caused by FreeStyle ™ 293-F cell aging were similar to those observed during human aging, most notably changes of IgG galactosylation. Interestingly, not all of the detected changes could be explained by differential expression of the corresponding glycosyltransferases.

Key words: immunoglobulin G, N-glycosylation, IgG glycome, HEK293 FreeStyle, in vitro cell aging

Presentation number: MG 39

MAPPING THE ESTRADIOL SIGNALLING NETWORK THAT REGULATES IMMUNOGLOBULIN G GLYCOSYLATION USING CRISPR/dCas9 BASED FREESTYLE293-F TRANSIENT EXPRESSION SYSTEM

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Immunoglobulin G (IgG) is a glycoprotein with a central role in adaptive immunity. Glycosylation of Fc domain defines IgG function and different studies link the change in IgG glycosylation with disease and aging. In healthy women, the most prominent change coincides with perimenopause, and a recent study has revealed that estradiol (E2) is involved in regulation of IgG glycosylation. Analysis of the Signaling Pathway Projects (SPP) web knowledgebase revealed that E2 affects expression of four genes with yet unknown role in IgG glycosylation, three of them being associated in previous genome-wide asLCKsociation studies (GWAS) with galactosylation (RUNX1, RUNX3, SPINK4) and one with sialylation (ELL2). To map downstream pathways linking E2 signaling and IgG glycosylation we utilized our FreeStyleTM293-F transient system, expressing IgG antibodies, for targeted manipulation of candidate loci. This system exploits stably integrated CRISPR dCas9-VPR or dCas9-KRAB expression cassettes for targeted activation or silencing of genes via transient transfection of cells with plasmids carrying specific gRNAs and recombinant IgG. Using this cell system we upregulated and downregulated RUNX1, RUNX3, SPINK4 and ELL2 loci but only upregulation of RUNX3 (Runt-related factor 3) and SPINK4 (Serine Peptidase Inhibitor Kazal Type 4) resulted in alternative IgG glycosylation. Upregulation of RUNX3 resulted in a significant decrease of galactosylated glycans accompanied with an increase of agalactosylated glycans. Upregulation of SPINK4 was also accompanied with a decrease of galactosylated glycans, but the ratio of agalactosylated glycans was unchanged. We hypothesized that RUNX3 acts as B4GALT1 repressor considering its role as a transcription factor that can either activate or suppress transcription. However, following RUNX3 upregulation, expression of B4GALT1 gene remained stable. To further investigate RUNX3 signaling, possibly involved in alternative IgG glycosylation, the total cell transcriptome was analyzed following RUNX3 overexpression. In sum, the results suggest a novel mechanism through which E2 could regulate IgG glycosylation, specifically galactosylation. Moreover, this was the first in vitro functional validation of RUNX3 and SPINK4, the GWAS hits associated with IgG glycosylation.

Key words: IgG glycosylation, estrogen, gene regulation, RUNX3, CRISPR/dCas9

Presentation number: MG 40

EFFECTS OF LOW-CALORIE AND DIFFERENT WEIGHT-MAINTENANCE DIETS ON PLASMA N-GLYCOME COMPOSITION

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Over half of all proteins are altered by covalently bound glycans that are crucial for maintaining a normal physiological role of glycoproteins. Aberrant glycosylation is associated with a wide range of diseases, including diabetes, and cardiovascular and immunological disorders. Alterations in sialylation and fucosylation of circulating glycoproteins have recently been shown to be affected by the diet, however, this is the first study that considered plasma proteins' susceptibility to different dietary regimes for weight control after the initial weight loss. To investigate plasma protein glycosylation alterations due to weight loss and successive weight-maintenance diets, 1850 glycomes from participants of the Diogenes study were analyzed using Ultra-High-Performance Liquid Chromatography (UHPLC). Diogenes study is a large dietary intervention study in which participants were subjected to a low-calorie diet (LCD) followed by one of five weight-maintenance diets (low protein/low glycaemic index, low protein/high glycaemic index, high protein/low glycaemic index, high protein/high glycaemic index and control) in a period of 6 months when the participants were at risk of regaining the formerly lost weight. The most notable alteration of the plasma glycome was 8 weeks after the subjects engaged in the LCD; a significant increase of low-branched glycan structures, accompanied by a decrease of high-branched glycan structures. After the LCD period, there was also a significant rise in fucosylated N-glycan structures, both core and antennary. Moreover, we have observed a significant decrease in trigalactosylated and trisialylated glycans and a concomitant increase in tetragalactosylated and tetrasialylated glycan structures. Interestingly, we did not observe significant changes between different diets, and almost all effects we have observed immediately after the LCD period were annulled during the weight maintenance diets.

Key words: plasma N-glycans, weight loss, low-calorie diet, glycoproteins

Presentation number: MG 41

N-GLYCOSYLATION OF IgG IS NOT INFLUENCED BY THE LEVEL OF RENAL COMPLICATIONS OR RETINOPATHY IN TYPE 1 DIABETES MELLITUS

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N-glycosylation of immunoglobulin G (IgG) is known to influence the antibody function. Changes in IgG N-glycome associate with many inflammatory conditions and were reported in early stages of type 1 and type 2 diabetes mellitus (DM). IgG N-glycosylation was also studied in relation to disease progression in type 2 DM and was found to associate with diabetes complications. Although type 1 and type 2 DM share complications, these findings may not apply to type 1 DM due to different pathophysiology involved and a longer disease duration prior to complication manifestation. In this study we investigated IgG N-glycosylation of 190 patients (age 18-70, median 46, 81 M, 109 F) with type 1 complications. Complications included: hypertension, albuminuria and retinopathy. Patients were differentiated by the levels of complications but were all in later stages of disease progression. N-linked glycans from IgG were released, fluorescently labelled and analysed using HILIC-UPLC. Twenty-four glycan structures were identified and relatively quantified. In addition, nine derived traits, corresponding to different structural characteristics of glycans were calculated. Data was analysed using multiple linear regression with age and sex as covariates. Glycan traits were log transformed prior to analysis. We observed no statistically significant changes of IgG N-glycosylation with respect to type of complication or severity level. Previously reported changes with respect to age, sex and lifestyle aspects, e.g., smoking were replicated in this study, confirming their influence on the glycome. IgG N-glycosylation changes previously reported as connected to the severity of complications in type 2 DM were not replicated for type 1 DM patients in our study. This can be explained by the fact that pathophysiological changes leading to type 1 DM and influencing the N-glycome occur much earlier in life and are not further influenced by disease progression and development of complications in the adult age. Absence of differences with respect to type of complications may also be due to fact that they share some common pathophysiological processes which prevent their distinction based on IgG N-glycosylation profile.

Key words: glycosylation, N-glycosylation, IgG, diabetes, type 1

Presentation number: MG 42

N-GLYCOSYLATION OF TOTAL SERUM PROTEINS IN ADULTS WITH TYPE I DIABETES MELLITUS

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Previous studies have shown that aberrant plasma protein N-glycosylation exists in children newly diagnosed with T1D, but there is little information on the N-glycome in the adult life of patients. N-glycosylation has been associated with poor glycaemic control and nephropathy, but other common diabetic complications remain to be assessed. Therefore, we compared the serum protein N-glycosylation between patients and controls and analysed its changes in regard to complication status, duration of the disease, glycaemic control and biochemical parameters. Total serum protein N-glycans were analysed in 200 patients with T1D (aged 18-70) and 298 healthy controls (aged 18-79). N-glycome was divided in 39 glycan groups and 16 derived traits calculated based on structural similarities. Patients were further divided based on the status of hypertension, albuminuria and retinopathy. Smoking, a major risk factor for the development of diabetic complications, was noted. Patients with T1D exhibited significant differences in N-glycosylation compared to healthy controls (19/39 glycan groups and 4/16 derived traits), e.g., monogalactosylated glycans were lowered, while digalactosylated, monosialylated and antennary fucosylated were elevated. The duration of the disease or biochemical parameters, other than HbA1c, showed no correlation with N-glycome. HbA1c was positively associated with sialylated and highly branched glycans. The effect of smoking was observed through the increase of high-branched, trigalactosylated and trisialylated glycans and the decrease of low-branched, a- and monogalactosylated, asialylated, bisecting and core-fucosylated glycans. Presence of T1D in adults associates with major changes in total serum N-glycome, some of which were previously reported in children. The duration of T1D, as well as the development of diabetic complications, appear not to further affect these changes. Poor glycaemic control and smoking status are strongly reflected in the N-glycome.

Key words: Type 1 diabetes mellitus, N-glycosylation, Serum protein N-glycosylation

Presentation number: MG 43

AUTOMATED IgG N-GLYCAN SAMPLE PREPARATION METHOD FOR HIGH THROUGHPUT ANALYSIS

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Role of protein N-glycosylation and especially changes in IgG N-glycosylation pattern has repeatedly been confirmed as crucial in different physiological and pathological processes. Expanding knowledge requires more affordable, reliable, and higher throughput methods. New approaches to glycosylation analysis would allow its optimization and application in the biopharmaceutical industry, epidemiology as well as advance clinical diagnostics that would rely on glycan biomarkers. Though there were successful attempts to develop new automated strategies for glycan research, many laboratories still leverage manual sample preparation protocols that limit their throughput. In order to help address this issue, automated methods for glycosylation analysis should be developed further. This research proposes one possible approach to automation of IgG glycan sample preparation using the Tecan Freedom Evo liquid handling automated platform. To improve throughput and robustness, the manual method was substituted by the automated system that was equipped with a liquid handling arm (LiHa) and a robotic manipulator arm (RoMa), while the vacuum manifold operations were replaced with A200, a positive pressure unit which allows for higher pressure and flow-through. The adapted automated protocol features IgG isolation on a protein G plate, IgG deglycosylation, and glycan 2-AB labeling. This automated protocol allows the samples to be analyzed using ultra-high performance liquid chromatography (UPLC). UPLC analysis showed that peaks' number, retention time and peak area corresponded with the manual method. Peaks with the largest area showed small variation, while the smaller peaks exhibited larger variation. Method has yielded promising results and with development could encourage additional automation efforts in other protocols.

Key words: liquid handling, lab automation, glycomics, UPLC

Regenerative medicine

Presentation number: MG 44

ARE BIOLOGICAL TREATMENTS FOR KNEE OSTEOARTHRITIS EFFECTIVE? KOOS, WOMAC AND VAS SCORE ANALYSIS

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Osteoarthritis is the most common progressive musculoskeletal condition. It affects not only cartilage but all the joint tissues, causing irreversible morphological changes and decreased joint function. Changes are mediated by numerous cytokines, chemokines, adipokines, growth factors, and new, biological treatments are commonly used to slow down the natural course of disease but also to reduce patient's symptoms. In this study, we included 8 women and 8 men with grade 2 and 3 knee osteoarthritis. Patients were treated with the intraarticular application of 2 mL of autologous micro-fragmented adipose tissue (MFAT) containing stromal vascular fraction (SVF) (Arthrex ACP, Double-Syringe System, Arthrex, Munich, Germany) in combination with 5 mL of leukocyte-poor platelet-rich plasma (PRP) (The Angel System, Arthrex, Munich, Germany).. After the initial assessment, patients were followed up on their Knee Injury and Osteoarthritis Outcome Score (KOOS) score, Visual analog scale score and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score 3 and 6 months after intraarticular application. Statistical analysis revealed a difference in the total KOOS score, total WOMAC score and the VAS score, both for resting and movement, over time, and the Wilcoxon Signed Ranks Test showed a statistically significant improvement three months ($p = 0.001$) and six months ($p = 0.005$) after the application of SVF and PRP. However, we did not observe changes in the glycosaminoglycans level (GAG) by using delayed gadolinium (Gd)-enhanced magnetic resonance imaging of cartilage (dGEMRIC). In conclusion, biological treatments are effective in reducing signs and symptoms of knee osteoarthritis, measured three months after intraarticular application, with the effect still consistent six months after application.

Key words: knee osteoarthritis, stromal vascular fraction, KOOS, WOMAC, VAS

Presentation number: MG 45

THE POTENTIAL USE OF MESENCHYMAL STEM CELLS IN GYNECOLOGY

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Mesenchymal stem cells (MSCs) are our own body's mechanism for healing and regeneration, all due to the secretion of bioactive factors which are having an immunomodulatory and regenerative ability. However, in the past decade, there is a rising number of studies that indicated their exceptional role in regenerative medicine. Their potential is very promising and can be used as a treatment for diseases in rheumatology, orthopedics, neurosurgery, endocrinology and many other fields. Many clinical trials, as well as published scientific articles, provided evidence showing the beneficial effect of their application, due to their analgesic, anti-inflammatory, antiapoptotic, angiogenic and immunomodulatory effects. In gynecology, they are being used both in animal and human studies for treating various innate and acquired diseases and especially in conditions that until now, haven't been treated effectively. There are published case series evaluating the long-term (3 years) safety and effectiveness of MSC for urogenital atrophy which is according to literature and clinical practice, possibly a very effective new treatment for clinical problem that affects several millions of patients. Lichen sclerosus treatment is also very demanding and there have been published articles about successful results with MSC. As one of the world's most common disorders, infertility and its possible causes are very challenging and often with poor therapeutic results. Studies have shown that endometrial, menstrual, umbilical, bone marrow and adipose-derived MSCs are efficient as a treatment and can restore fertility in treated individuals. In many cases, the use of MSCs in gynecology has proved efficient, safe and with the possibility of a wide application. In Asherman syndrome, this therapy enabled restoring the endometrium thickness - the patients had improvement of menstrual cycles and had successful pregnancies. So far, in St. Catherine's specialty hospital, we have experience and great results with MSC in orthopedics - they have been successfully used in our hospital for more than six years. We are now preparing a clinical trial with MSC application in gynecology, hopefully with the same accomplishment. Yet more clinical and scientific studies are needed on the use of MSC in gynecology, but so far, they are very encouraging.

Key words: mesenchymal stem cell, gynecology, regenerative medicine, infertility, Asherman syndrome

Presentation number: MG 46

CLINICAL APPLICATION OF CULTURED KERATINOCYTES AS ADVANCED THERAPY MEDICINAL PRODUCTS: A TWENTY-YEAR EXPERIENCE IN CROATIA

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The aim of this study is to present development of tissue engineering and clinical application of cultured keratinocytes. Advanced therapy medicinal products (ATMPs) are medicines for humans based on gene therapy, somatic cell therapy and tissue-engineered products. Cultured keratinocytes regenerate the epithelium and belong to the category of ATMP as tissue-engineered products. The development of ATMPs in Croatia began during 2002 in collaboration with the Ruđer Bošković Institute, the Clinic of Traumatology and the Children's Hospital Zagreb on the project "Production of skin grafts in vitro". Tissue Engineering Laboratory was built in May 2005 in accordance with Good Manufacturing Practice and clean room technology. Tissue and Cell Bank (TCB) was established during 2007. The procedure includes isolation of keratinocytes from the skin biopsy (about 4-6 cm2/ 0.3 mm thick) after which they are seeded onto a feeder layer of 3T3 cells and incubated at 37 °C, 5% CO2. Preparation of the optimal number of grafts is accomplished within 3-4 weeks depending on the area of the injury. Quality control involves potency (yield, viability, CFE), purity (p63, CK14, CK19), impurities (rest of 3T3 cells) and safety (sterility, mycoplasma, bacterial endotoxins). The first successful production of epidermal grafts began in the Clinic of Traumatology in September 2002 with 10 epidermal grafts of 700 cm2. This retrospective analysis covers period from February 2002 to October 2003. The project included donors (n=15), from 2 to 66 years old with 92 cultured grafts. Microbiological control has proven the sterility of all keratinocytes, cell media as well as epidermal transplants. From July 2007 to March 2022 in TCB, donors (n=62) were from 2 to 74 years old with 2175 cultured grafts from which 88,9% were transplanted and 11,1% discarded. The most common reasons for discardment were patient's death, initial microbiological contamination, and technical reasons. Keratinocytes prepared as epidermal grafts or suspension with fibrin glue contributed to the survival of severely burned patients. Twenty years of successful cooperation between TCB employees and clinicians have resulted in successful application of cultured autografts in severely burnt patients.

Key words: tissue engineering, keratinocytes, ATMP

Presentation number: MG 47

INJECTION OF AUTOLOGOUS PLATELET-RICH PLASMA FOR TREATING ANDROGENIC ALOPECIA: PILOT STUDY OF A NOVEL TREATMENT PROTOCOL

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Autologous platelet-rich plasma (PRP) treatment has emerged as a valuable, effective, and affordable treatment for androgenetic alopecia in recent years. Androgenetic alopecia is the most common type of alopecia, affecting both men and women, characterized by diminished hair follicles mainly pronounced in the frontal region and vertex. A huge variety of PRP treatment regimens were described but so far there is no consensus for standardization of PRP preparation or administration protocol. Our study enrolled two patients (ages 56 and 33 years old) with the aim to test the efficacy of a new PRP application protocol of only 2 treatments by using a combination of a PRP collecting device and a conventional kit. Efficacy of treatment was assessed after a 6-month follow-up by AI-driven software on microscopic images of treated regions. An average number of hairs, cumulative hair thickness, and the number of follicular units increased in the vertex region of both patients by 30/59%, 35/53%, and 14/48% respectively ($P<0.05$). A novel PRP treatment regimen with a decreased number of treatments was shown significantly effective in only 6 months of follow-up.

Key words: hair loss, vertex, autologous platelet-rich plasma

Presentation number: MG 48

NOVEL CELL-BASED THERAPIES IN CROHN'S DISEASE

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Stem cells (SCs) are undifferentiated or partially differentiated cells with the potential to specialize in more mature cells and, at the same time, self-replicate in daughter stem cells. In addition to the high proliferative potential for regeneration, SCs have many other effects on tissues, including growth support, immunomodulatory effect, and effect on paracrine signaling. Mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate into a limited number of cell types of the same lineage. MSCs are considered new therapeutic agents for immune-mediated diseases, including Crohn's disease (CD) thanks to their differentiation potential into gut cells and their pro-angiogenic and immunomodulatory characteristics. There are currently two methods for MSC use in patients with CD: systemic (intravenous) use for systemic control of intestinal inflammation in luminal CD and local administration as a therapeutic approach for patients with perianal fistulizing CD. Current therapy for CD involves immunosuppressive drugs that promote remission of intestinal inflammation and related symptoms. Recent research shows that depending on the intercellular environment in which stem cells are located, they have an increased secretion of anti-inflammatory cytokines (IL-2, IL-4, IL-10, and TGF-β) and a decrease in pro-inflammatory cytokines (IL-1, IL-6, IL-17, and TNF-α). MSCs in a pro-inflammatory environment with high concentrations of TNF-α and IFN-γ secrete anti-inflammatory mediators and become so-called MSC-2, which can inhibit the activation of dendritic cells, T and B lymphocytes, and NK cells. The results obtained in a large number of clinical trials suggest that topical application of autologous as well as allogeneic adipose-derived stem cells is a safe and useful therapeutic approach for the healing of perianal fistulas in patients with CD. The safety of MSC-based therapies, after systemic administration of MSCs, remains to be investigated to be safely used as new therapeutic agents for the treatment of CDs due to their differentiating potential as well as their proangiogenic and immunomodulatory properties.

Key words: Stem cell, Crohn's disease, mesenchymal stem cell, TNF-α, adipose-derived stem cell

Stem cells

Presentation number: MG 49

N-GLYCOSYLATION OF INDUCED PLURIPOTENT STEM CELLS (IPSCS) AND NEURAL STEM CELLS (NSCS) DERIVED FROM PERSON WITH DOWN SYNDROME (DS) CAUSED BY TRISOMY 21 (T21)

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The goal was to explore the difference in N-glycosylation between euploid disomic (D21) and trisomic (T21) isogenic human induced pluripotent stem cells (iPSCs) and neural stem cells (NSCs) derived from person with mosaic Down syndrome (DS) trisomy of 21st chromosome. Cell pellets of isogenic disomic and trisomic iPSCs are derived from a person with mosaic DS. Cells were lysed and (glyco)proteins were extracted. N-glycans were released from cell lysates and then fluorescently labeled. After a clean-up by hydrophilic interaction liquid chromatography (HILIC), labeled glycans were obtained and subsequently separated by HILIC-UHPLC (ultra-high performance liquid chromatography). Data was processed into chromatograms which were separated into 45 glycan peaks. Comparison of iPSCs and NSCs using student's t-test revealed a clear difference in N-glycosylation. There was an increase in the relative abundance of five glycan peaks and a decrease in the abundance of nine glycan peaks in iPSCs when compared to NSCs. Comparison of disomic and trisomic iPSCs revealed no significant difference in the relative abundance of N-glycans. The same was true for disomic and trisomic NSCs. A significant difference was observed in N-glycosylation between isogenic iPSC and NSCs, which falls in line with recently published research that showed NSCs tend to have more N-Glycosylation of NrCAM and different Plexins compared to iPSCs. The lack of differences in N-glycosylation between disomic and trisomic cells might be explained by the small effect of increased gene expression of chromosome 21 during the pluripotent/stem cell stage, but also due to low levels of N-glycosylation in iPSCs (and NSCs).

Key words: N-glycosylation, Down syndrome, isogenic, stem cells

Cell therapy

Presentation number: MG 50

SYSTEMS APPROACH TO HEMOANALYTIC CHARACTERIZATION OF PLATELET RICH PLASMA AND BONE MARROW CONCENTRATE AND AI ALGORITHMIC DATA ANALYSIS OF DOSE-RESPONSE RELATIONSHIPS

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Autologous cell therapies, including Platelet Rich Plasma (PRP) and Bone Marrow Concentrate (BMC), continue to be limited by a lack of quality control. Existing commercial systems lack the ability to standardize or quantify cellular concentrations within these preparations. As a result, most research to date lacks any specific analysis of cell or platelet dose-response relationships defining what constitutes an optimized product. Presented is a systems approach to address quality deficiencies in autologous point of care cellular products (Greyledge Technologies USA). Acquired samples from peripheral and medullary blood respectively, along with manually processed preparations of PRP and BMC are quantified using a validated hemoanalyzer (Sysmex XN-350). Analyses include WBC (TNCC) with 6-part differential (Lymphocyte, Monocyte, Neutrophil, Basophil, Eosinophil and Immature Granulocyte), RBC and Platelet count. Sample analytics are imported into a cloud-based data platform (Greyledge Cloud) along with patient demographic information and body-part specific patient reported outcomes questionnaires. Outcomes are collected pre-treatment and at 1,3,6,12 and 18month intervals. Greyledge uses a secure HIPAA certified database along with the most advanced programming languages to digitally combine data sets which are transmitted into AI/ML algorithms. A CSV Document is exported that dynamically combines tailored cellular data sets which are examined using Python as the backend framework in combination with Rcode, Node and Angular JS libraries to perform dynamic artificial intelligence studies. At present the file contains 7,000 discreet variables attributable to 200 patients (4000 sample analytic data points and 3000 demographic data points with an average of 35 variables/patient). Cellular dose-response trends and relationships are under investigation to effectively develop predictive models of success and failure, along with optimal product parameters specific to patient demographics and treatment indication.

Key words: bone marrow-derived stem cells, platelet rich plasma, regenerative medicine, PRP, mesenchymal stem cell

Translation medicine

Presentation number: MG 51

MOLECULAR MECHANISMS OF THE RENOPROTECTIVE EFFECT OF EMPAGLIFLOZIN ON LLC-PK1 CELLULAR MODEL OF PROXIMAL TUBULAR CELLS

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Diabetic nephropathy (DN) is a chronic complication of diabetes mellitus, both type I and type II, which can lead to end-stage renal kidney failure. This research aimed to assess the effects of empagliflozin (SGLT2i) on cell viability and F-actin distribution in the LLC-PK1 model of DN. To mimic DN, cells were exposed to high glucose (HG30 mM) followed by 0,5 mM H2O2 and a combination of glucose and H2O2 for 24 hours. The cells were treated with different combinations of glucose and empagliflozin (100 and 500 nM) and combinations of glucose, H2O2, and empagliflozin. MTT colorimetric assay and Erythrosin B color exclusion test were used to determine cell viability. F-actin cytoskeleton was visualized with Phalloidin stain and subsequently quantified. MTT results revealed a significant reduction in cell viability when treated with the HG30/H2O2 combination ($p<0.001$). Cell viability was considerably increased with the addition of empagliflozin 100 and 500 nM to cells treated with HG30 and HG30/H2O2 compared to cells treated with HG30/H2O2 only ($p<0.001$). Furthermore, empagliflozin in the concentration of 100 nM decreased the total amount of F-actin in HG30 treated cells ($p<0.001$), while a higher dose of 500 nM had no effect. Cellular viability shows that empagliflozin has protective effects on renal injury, whereas the effect on F-actin structure was dose-dependent.

Key words: diabetic nephropathy, LLC-PK1 cell culture, empagliflozin

Presentation number: MG 52

MOLECULAR MECHANISMS OF THE RENOPROTECTIVE EFFECT OF LIRAGLUTIDE ON LLC-PK1 CELLULAR MODEL OF PROXIMAL TUBULAR CELLS

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Transforming growth factor-beta (TGF-β) has recently been associated with diabetic nephropathy (DN) development. It causes cell apoptosis induced by oxidative stress and cell proliferation and migration triggered by hyperglycemia and inflammation. Liraglutide is an antihyperglycemic agent that has a direct renoprotective effect. This study aimed to evaluate the effects of liraglutide on cell viability and TGF-β expression in the LLC-PK1 model of DN. Cell viability was determined by colorimetric MTT assay and erythrosine B color exclusion assay. The expression of mRNA TGF- β1 was measured by RT-PCR and β-actin was used as an internal control. LLC-PK1 cell culture was treated with different concentrations of glucose (1.5, 30 mM) and the combination of glucose and H2O2 (0.5 mM) for 24 hours. To study the renal effect of liraglutide, cells were treated for 24 hours with different combinations of glucose and liraglutide (10, 20 nM) and combinations of glucose, H2O2, and liraglutide. A significant decrease in MTT levels compared to control ($p < 0.01$; $p < 0.001$) was observed after treatment with a combination of HG30/ H2O2 and HG30 alone. Cell viability was improved by the addition of liraglutide (10 nM) to cells treated with HG30, while 20 nM had no effect. There was no significant difference in cell survival with the addition of HG30 and HG30/ H2O2 compared to control. The addition of liraglutide at both concentrations to cells treated with HG30 and HG30/H2O2 improved cell survival, although significance was only numerical, not reaching statistical significance. TGF-β1 expression levels were significantly increased in cells treated with HG30 ($p < 0.001$). Liraglutide inhibited TGF-β1 expression except in HG30/H2O2 treated cells. Our results support a protective role of liraglutide in LLC-PK1 cells, mediated by inhibition of TGF-β1, thus reducing oxidative stress damage.

Key words: diabetic nephropathy, LLC-PK1 cell culture, liraglutide

Presentation number: MG 53

THE ACTIVITY OF GARLIC EXTRACTS INHIBITS EPITHELIAL DAMAGE CAUSED BY BILE SALT IN A CELLULAR MODEL OF PEPTIC ULCER DISEASE

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Peptic ulcer disease (PUD) is a common digestive disorder and global problem with a lifetime risk of development ranging from 5% to 10%. Proton pump inhibitors such as lansoprazole (LPZ) are used as a first-line therapy to treat gastric ulcers worldwide. On the other hand, garlic extracts (GE) have been shown in several studies to be beneficial in the treatment of ulcers. The goal of this research was to establish a cell culture model of ulcer disease using bile salts; sodium taurocholate (NaT), to investigate the effects of pretreatment with GE and addition of LPZ on oxidative stress and F-actin distribution in a cell model of PUD. The establishment of the NaT model was determined by the MTT test. The ability of GE to protect the gastric cells against the damage induced by NaT was performed by determining glutathione (GSH) and prostaglandin E2 (PGE2) levels by ELISA, proliferation of the human gastric cell line by cell counting, expression of nuclear factor kappa B subunit 2 (NFKB2), thioredoxine 1 (TRX 1) by RT PCR, and visualization of the F-actin cytoskeleton by semi-quantification of rhodamine-phalloidin staining. Our results showed that gastric cells pretreated with LPZ ($p<0.001$) and increasing concentrations of GE ($p<0.001$), exhibited a significant reduction in cell damage after incubation with NaT. In a cell culture model of PUD, pretreatment with LPZ and various concentrations of GE increased PGE2 and GSH levels ($p<0.001$). Positive correlation of NFKB2 ($p<0.01$), and TRX 1 ($p<0.001$) with LPZ and GE pretreatment was confirmed. Treatment with NaT as oxidative stress on F actin structure was less pronounced, while the highest concentration of GE led to a statistically significant increase of total amount of F-actin ($p<0.001$). In a cell model of PUD, pretreatment with GE showed a gastroprotective effect. However, further experiments are needed to confirm protective role of GE in PUD.

Key words: peptic ulcer disease, garlic extracts, lansoprazole, sodium taurocholate

Presentation number: MG 54

ETA POLYCAPROLACTONE (ε-PCL) IMPLANTS APPEAR TO CAUSE A PARTIAL DIFFERENTIATION OF BREAST CANCER LUNG METASTASIS IN A MURINE MODEL

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Cells in every epithelium can be roughly divided in three compartments: stem cell (SC) compartment, transient amplifying cell (TA) compartment and mature or functional cell (FC) compartment. Maturation of stem cells is characterized epithelial stromal interaction and sequential maturational movement of stem cell's progeny through those compartments. In this work we hypothesize that providing an artificial stroma, which murine breast cancer metastatic cells can infiltrate, will induce their differentiation. BALB/c female mice were injected with 106 isogenic 4T1 breast cancer cells labeled with GFP. After 20 days primary tumors were removed, and artificial ε-PCL implants were implanted on the contralateral side. After 10 more days mice were sacrificed and implants along with lung tissue were harvested. Mice were divided in four groups: tumor removal with sham implantation surgery (n=5), tumor removal with ε-PCL implant (n=5), tumor removal with VEGF enriched ε-PCL implant (n=7) and mice without tumor with VEGF enriched ε-PCL implant (n=3). Differentiational status of GFP+ cells was assessed by Ki67 and activated caspase 3 expression, thus dividing the population in SC like cells (Ki67+ aCasp3-), TA like cells (Ki67+ aCasp3+) and FC like cells (Ki67- aCasp3+/-) on flow cytometry. Lung metastatic load was reduced by 20% in mice with simple ε-PCL implant when compared to tumor bearing group with no implant ($p<0.0001$). Mice with VEGF enriched implants had 90% increase in lung metastatic load in comparison to tumor bearing mice with no implants ($p<0.0001$). Likewise, amount of GFP+ cells doubled in simple ε-PCL implant in comparison to VEGF enriched implants ($p<0.0001$). Differentiation wise, simple implants in comparison to sham group reduced the SC like cells by 25% and VEGF enriched implants reduced it further by another 20 % (i.e., 45% reduction in total) ($p<0.0001$). On the other hand, TA like cells were increased by amounts identical to SC-like cells decrease. Effects of both type of implants on FC like cells were minute. Both types of implants cause lung metastasis differentiation by shifting cancer cells from SC to TA compartment, leaving the FC compartment unaffected. VEGF enriched ε-PCL implants appear to decrease further migration of lung metastasis.

Key words: metastasis, breast cancer, differentiation, ε-PCL implant