TRANSLATIONAL MEDICINE

BEST PRACTICES IN TRANSLATIONAL AND PERSONALIZED MEDICINE

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ANTIFUNGAL SUSCEPTIBILITY TESTING OF THE MOST-RELEVANT PATHOGENIC MOULDS

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The study centers on clinically important moulds that cause infections in humans, especially those with compromised immune systems. A standardized and widely accepted technique, broth microdilution, recommended by authoritative bodies such as CLSI and EUCAST, is employed for antifungal susceptibility testing. This method involves preparing dilutions of antifungal agents in a liquid growth medium and determining the minimum inhibitory concentration (MIC) as the lowest concentration inhibiting visible fungal growth. The standardized procedures outlined by CLSI guarantee the reliability and reproducibility of MIC testing across diverse laboratories. These guidelines meticulously detail factors such as media composition, drug concentrations, inoculum preparation, and incubation conditions, ensuring consistent and accurate results. Adherence to these standardized procedures minimizes variability, fostering the comparability of MIC data obtained from various sources. The study underscores the crucial role of MIC testing in optimizing antifungal treatment strategies for immunocompromised patients. By aiding in the selection of appropriate antifungal medications, dosage determination, and monitoring of drug resistance, MIC testing using standardized broth microdilution emerges as a reliable tool in the battle against fungal infections.

Keywords: mould, susceptibility, minimum inhibitory concentration

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POLYCYSTIC OVARY SYNDROME
WITH GENETIC PERSPECTIVE

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Polycystic ovary syndrome (PCOS) is a prevalent endocrine-gynecology disorder affecting 8–13% of reproductive-aged women globally, with up to 70% undiagnosed. It's the main cause of anovulation and a key factor in infertility, impacting both physical and emotional well-being. Genetic predisposition plays a significant role in PCOS, with genes such as Androgen Receptor (AR), Fat Mass Obesity (FTO), Follicular Stimulating Hormone Receptor (FSHR), Calpain 10 (CAPN10), and members of the Cytochrome P450 family (CYP) being implicated in its pathogenesis. These genes are linked with hormonal imbalances, insulin resistance, and metabolic dysregulation observed in PCOS patients. Diagnosis of the PCOS relies on the presence of two out of three criteria: hyperandrogenism, irregular menstrual cycles, and polycystic ovaries on ultrasound. Elevated levels of testosterone, estrogen, luteinising hormone, insulin, and anti-müllerian hormone are common. Patients often present with symptoms like irregular periods, infertility, acne, excessive hair growth, and weight gain, predisposing them to conditions like type 2 diabetes and cardiovascular diseases. Treatment focuses on symptom management. Lifestyle changes, including diet and exercise are very useful. Pharmacological interventions such as birth control pills, insulin-sensitizing agents, and medications to reduce symptoms like acne and unwanted hair growth are employed. Complementary therapies and nutrient supplementation, including vitamins B-12, D, E, and K, inositols, melatonin, omega-3 fatty acids, and probiotics, are being explored for their potential benefits and specially inositols are very promising. More research is needed to apply these methods in clinical settings. Challenges in diagnosis and treatment persist, underscoring the importance of ongoing research and guideline implementation. Genetics play a crucial role in PCOS, offering insights into diagnosis, treatment, and prognosis, particularly regarding infertility.

Keywords: polycystic ovary syndrome, genetics, genes, infertility, insulin resistance

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THE UTILITY OF WGS IN PROACTIVE GENETIC SCREENING AND PREVENTIVE MEDICINE DEMONSTRATED IN A CASE OF LYNCH SYNDROME TYPE 8

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With the increasing application of genetic testing in modern medicine, its utility in the field of preventive oncology cannot be overlooked. It allows healthy individuals to determine potential oncogenic predispositions in their genome and take the necessary steps toward quality preventive care. A 54-year- old male patient was examined as part of a preventive health check-up. The patient had no prior history of serious illness, nor was his family history positive for malignancies. After testing with whole genome sequencing, we performed a targeted analysis using the proactive 526-gene hereditary cancer panel and the 523-gene cardiometabolic panel. Likely pathogenic variants were discovered in the SPTA1 gene (c.4339-99C>T) and EPCAM gene (c.655del). Pathogenic variants in the EPCAM gene are associated with Lynch syndrome 8 (LS-8), an autosomal dominant disorder characterized by dysfunctional DNA mismatch repair and microsatellite instability. The EPCAM protein is a transmembrane protein involved in cancer cell adhesion, proliferation, and migration and its mechanism is related to the beta-catenin pathway. Additionally, EPCAM deletions can cause the silencing of the MSH2 gene via promoter hypermethylation. LS-8 is considered a high-risk condition for the development of gastric, enteric, hepatic, biliary, urinary, brain, and skin malignancies. Preventive medical approaches with a focus on cancer screening can be greatly beneficial to these patients. This most often includes regular colonoscopies as gastrointestinal malignancies are the most frequent. With the timely and proper application of screening methods, some of these individuals have been reported to survive multiple primary cancers and live to old age.

Keywords: preventive medicine, cancer screening, EPCAM, Lynch syndrome 8

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CLINICAL PRESENTATION AND TREATMENT CONSIDERATIONS FOR A PATIENT WITH PROGRESSIVE MUSCLE WEAKNESS AND PATHOGENIC VARIANTS IN THE DYSF GENE

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Dysferlinopathy is an autosomal recessive type of muscular dystrophy characterized by pathogenic variants in the DYSF gene. The disease has been described in the literature under several names, including Myoshi muscular dystrophy (MMD), limb-girdle muscular dystrophy type 2B (LGMD2B), and distal myopathy with anterior tibial onset (DMAT). Patient history often includes symptoms of progressive muscular weakness, with age at onset ranging from 15 to 25 years, with difficulty standing on toes as an early feature. Diagnostic tests can reveal elevated creatinine kinase (CK) and muscular atrophy with fatty infiltration on magnetic resonance imaging (MRI). The diagnosis is confirmed by genetic testing and open muscle biopsy. We present a 53-year-old nonambulatory female patient with paraparesis in both lower extremities (0/5) and severe muscle weakness in both hands (2/5) accompanied by pain in both shoulders and knees, the lumbar region, and the left hip. MRI showed severe fatty infiltration in the musculature of the lower extremities and the lumbosacral region. Degenerative disc processes were also seen at L4-S1 levels and in both knees (dorsomedial protrusion with reduction of anterior subarachnoid space). The condition was confirmed with two intronic pathogenic variants (DYSF c.4221+1G>C & c.2643+1G>C) and pathological findings following open muscle biopsy. While the assumption of compound heterozygosity was made, confirmation by parental testing was not performed. Advanced treatment options that might be considered are local applications of platelet-rich plasma or i.v. mesenchymal stem cells. It is also worth noting that several clinical trials are exploring routes of genetic therapy, by mechanisms of increasing myocyte membrane repair potential or inflammatory and epigenetic modulation. These treatment options are applicable at a young age and thus were not applicable in our case, as the patient's clinical presentation had progressed past this point.

Keywords: dysferlinopathy, DYSF, limb-girdle muscular dystrophy type 2B, fatty infiltration, spinal degeneration

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PATHOGENIC HOMOZYGOUS AIRE VARIANT RESULTS IN ADRENAL AND PARATHYROID GLANDULAR DYSFUNCTION

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Autoimmune polyglandular syndrome type 1 (APS-1) is an autoimmune disease caused by pathogenic variants in the AIRE gene. The AIRE gene's function is immunomodulatory, and it plays a key role in the conditioning of T lymphocytes. The functional protein ensures that abnormal T cells, reactive to the host's physiological antigens, are destroyed. However, in the case of its dysfunction, autoreactive T cells are released and initiate a systemic inflammatory response. The typical triad of symptoms that characterizes APS-1 includes Addison disease, hypoparathyroidism, and chronic mucocutaneous candidiasis. The diagnosis can be confirmed by active antibodies, typically against interferon-alpha, or by pathological genetic findings in the AIRE gene. A 10-year-old female patient presented with abdominal pain, low appetite, and vomiting and was examined 15 days after onset. In that period, she'd also lost 2 kg, was constantly exhausted and her mother had noticed her skin becoming darker. Laboratory findings showed hyponatremia, hyperkalemia, hypocalcemia, hyperphosphatemia, hypocortisolemia with elevated ACTH, and very low PTH. Under suspicion of APS, the patient was subject to genetic testing. A comprehensive panel was performed, including 613 genes, and identified a homozygous pathogenic variant in the AIRE gene (c.769C>T (p.Arg257Ter)). The treatment option for APS-1 is hormone replacement therapy, in terms of glucocorticoid and calcium supplementation. A follow-up assessment of gonadal and thyroid function is also necessary. It is also worth noting that certain studies have observed gene therapy as another potential approach. A study conducted on the mouse model has demonstrated that the AAV9 vector containing AIRE cDNA can achieve significant T-cell infiltration and reduce serum antibodies to undetectable levels in a 4-week timeframe. These findings demonstrated the feasibility of gene therapy in APS-1, although further research is still needed before its implementation.

Keywords: autoimmune polyglandular syndrome type 1, Addison disease, hypoparathyroidism, AAV9 gene therapy

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PRADER-WILLI SYNDROME AND LIMB-GIRDLE MUSCULAR DYSTROPHY CAUSED BY PARTIAL MATERNAL UNIPARENTAL ISODISOMY

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Uniparental isodisomy refers to a condition in which both copies of a chromosome are duplicates of a single parental chromosome. It can arise from inadequate meiotic segregation, second-rescuing segregation in the zygote, or errors in early embryonic division. Prader-Willi syndrome (PWS), caused by the loss of expression of paternally imprinted genes within the PWS region located on 15q11.2, presents with neonatal hypotonia, failure to thrive, developmental delay, obesity, hypogonadism, and behavioral issues, with 25% of cases attributed to uniparental disomy. Isodisomy may be associated with secondary autosomal recessive disorders because, in cases where the parent is a carrier of a pathogenic variant, the child may become homozygous for the same variant. We present a 4-year-old patient with the diagnosis of PWS and an autosomal recessive limb-girdle muscular dystrophy type 1 (LGMDR1). Whole-genome sequencing (WGS) confirmed partial maternal uniparental isodisomy by detecting a homozygous PWS region of chromosome 15. Additionally, a homozygous pathogenic variant in the CAPN3 gene (c.550del, p.Thr184fs) associated with limb-girdle muscular dystrophy type 1, a hyper creatine-kinase (CK) calpainopathy, was identified. The proband's mother had an identical variant of the CAPN3 gene (c.550del, p.Thr184fs) in a heterozygous form. The proband exhibited elevated CK levels, with isoenzymes originating from muscle and brain tissues. Therefore, the patient's management required a multidisciplinary and personalized approach, greatly aided by genetic testing. This case highlights the pivotal role and potential of WGS in identifying both monogenic disorders and various forms of uniparental disomy simultaneously.

Keywords: uniparental disomy, Prader-Willi syndrome, WGS, CAPN3, limb-girdle muscular dystrophy

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A CASE OF MULTIPLE EPIPHYSEAL DYSPLASIA CAUSED BY A RARE CLINICALLY SIGNIFICANT PATHOGENIC VARIANT IN THE COMP GENE

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Multiple epiphyseal dysplasia (MED) is a rare genetically heterogeneous disorder primarily caused by mutations in several genes, including COMP, MATN3, and COL9A1. The inheritance pattern is typically autosomal dominant, with the mutation in the COMP gene being the most common and severe form, leading to abnormal cartilage development. MED-affected individuals typically show short stature and often receive a diagnosis later in life after experiencing lower limb joint discomfort. They typically exhibit ankle valgus deformity and are prone to compromised blood flow to the joints, leading to avascular necrosis. We present a case of a 33-year-old female patient with persistent pain, primarily in the knees and groin. Clinical examination showed an exceptionally wide range of motion in the hips, positive FADIR and FABER signs, and anterior knee instability. Radiological examinations unveiled subchondral bone sclerosis affecting the articulating surfaces of both knees and hips, with flattened femoral condyles in both knees, as well as extensive fissuring of cartilage and dorsoposition of the vertebrae L5-S1. Following a spine surgeon's examination, the patient underwent a procedure of L5-S1 radicular infiltration with levobupivacaine and PRP. The postprocedure course was uncomplicated. Considering the patient's medical history, genetic testing was initiated, the results of which showed a clinically significant pathogenic variant in the COMP gene (c.1501G>A (p.Gly501Ser)), associated with autosomal dominant skeletal dysplasia. Although this variant hasn't been documented in the literature thus far, the clinical phenotype and radiological findings of the patient suggest a diagnosis of MED type 1. Given the common attribution of such symptoms to environmental factors, this case highlights the necessity of integrating genetic evaluation into the diagnostic protocol for skeletal dysplasia, as exemplified by the procedures implemented at St. Catherine's Specialty Hospital.

Keywords: multiple epiphyseal dysplasia, genetic testing, COMP, pathogenic variants, phenotype

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THE POTENTIAL OF NUTRIGENETICS IN PERSONALIZED MEDICINE

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Nutrigenetics is a branch of genetics that focuses on the specific interactions between genes and nutrients, emphasizing how genetic variations influence responses to dietary components. As genetic understanding and sequencing technologies progress, personalized nutrition through nutrigenetics becomes increasingly important for tailoring nutrition to individual genotypes, promoting personalized dietary plans for better health and disease prevention. Several pivotal genes have emerged as influential factors, in shaping personalized diet recommendations. Genetic variants within the VDBP, VDP, and CYP2R1 genes, influence vitamin D metabolism and could contribute significantly to well-being and the prevention of age- and lifestyle-related diseases, particularly those associated with chronic inflammation. One of the most important genes in nutrigenetics is FTO and its variants have been linked to an increased risk of obesity and metabolic disorders. Given the established link between obesity and the risk of type 2 diabetes (T2D), several gene-diet interactions affecting obesity risk have been identified, which are relevant to T2D. These interactions involve genotypes related to genes such as ADIPOQ, FTO, FADS, PPARG, PLIN, and MC4R, in conjunction with dietary factors, influencing outcomes related to the disease, such as insulin sensitivity and T2D. Additional associations include genes APOA2 with intake of saturated fatty acids and body mass index, MTHFR with homocysteine levels, and CYP1A2 with caffeine-related hypertensive response. We will analyze five hundred participants who underwent comprehensive whole genome sequencing integrated with nutrigenetic testing. The aim will be to optimize personalized dietary plans based on nutrigenetic results, with the potential to reduce the risk of developing various health conditions, including diabetes, obesity, cardiovascular diseases, osteoporosis, hypertriglyceridemia, and chronic systemic inflammation.

Keywords: nutrigenetics, personalized medicine, nutrients, personalized diet, disease prevention, obesity

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PREGNANT COUPLES' ATTITUDE TOWARDS EXTENDED PRECONCEPTIONAL GENOMIC SCREENING

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We present a study on the attitudes of pregnant couples towards carrier screening genomic tests. A validated 22-item questionnaire was offered in person by medical staff to 32 weeks or more pregnant women and their partners attending prenatal classes. We also explored their interest in various forms of genetic carrier screening tests and investigated the impact of genetic literacy. Of 497 respondents, 69% expressed a strong interest in carrier screening. Among the interested participants, there was substantial support for screening for common (82%) or all known genetic diseases (79%), as well as for treatable (79%) and untreatable diseases (85%). The majority of respondents believed that genetic test results could provide them with a sense of security but also with anxiety and fear. They were aware that these results could impact their perspective on life, work, and the atmosphere within their family, and acknowledged the potential effect on their relationship with their partner. However, none of these concerns diminished their desire to learn about their carrier status. Support varied significantly across certain demographic groups. Respondents of higher genetic literacy exhibited higher interest in screening tests (p=0.006). The interest in carrier screening was also influenced by religious affiliation and educational level. Specifically, 78% of non-religious individuals, compared to 60% of practicing-religion subjects (p=0.002), and 74% of higher-educated individuals, as opposed to 60% of those with lower levels of education, expressed interest in screening (p=0.003). Most respondents manifested a robust inclination to receive information about their carrier status through genetic tests.

Keywords: preconception, diagnosis, genetic screening, parental opinion, knowledge

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THE ROLE OF CHOLESTEROL METABOLISM IN ACQUIRED RESISTANCE TO BRAF INHIBITION BY VEMURAFENIB IN BRAFV600E- MUTATED COLON CANCER

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BRAFV600E-mutated colorectal cancer represents approximately 8–12% of metastatic colorectal cancer (mCRC) cases and is considered the most aggressive subgroup of colorectal cancer characterized by a poor response to chemotherapy and short overall survival. Vemurafenib is a specific BRAFV600E inhibitor approved for mCRC patients. However, monotherapy with vemurafenib in this patient population has limited clinical efficacy due to rapid development of chemoresistance. The aim of this study was to identify perturbations in lipid metabolism associated with the development of vemurafenib resistance in BRAFV600E-mutated colon cancer. We performed untargeted lipidomics analysis of vemurafenib-resistant vs. sensitive RKO colon cancer cells harbouring BRAFV600E mutation by LC-MS/MS. OPLS-DA analysis revealed that the major lipid classes that differentiated resistant from sensitive cells included phosphatidylcholine (PC), triglycerides (TG) and cholesteryl esters (CE). Wilcoxon test showed significantly increased abundance of CE with different chain lengths in resistant cells at baseline and after the treatment with vemurafenib, indicating the involvement of cholesterol metabolism in the resistance mechanisms. We next evaluated in silico mRNA expression levels of the key enzymes regulating cholesterol metabolism, including 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), sterol O- acyltransferase 1 (SOAT1) and sterol O-acyltransferase 2 (SOAT2) in the TCGA Colorectal Adenocarcinoma PanCancer Atlas dataset. We found that only SOAT1 showed a trend towards increased expression in BRAF-mutated tumours vs. unaltered group. Higher SOAT1 mRNA expression was associated with lower overall survival of BRAFV600E-mutated colon cancer patients. Currently, we are investigating whether protein expression of HMGCR, SOAT1 and SOAT2 in tumour tissues could predict the outcome of BRAF-mutated colon cancer patients on BRAF inhibitor therapy.

Keywords: BRAFV600E, colorectal cancer, chemoresistance, lipid metabolism, cholesterol metabolism

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MOLECULAR BASIS OF THE DISEASE, PERSONALIZED APPROACH AND IMPLICATIONS FOR FAMILY SCREENING IN PATIENTS WITH CADASIL SYNDROME

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CADASIL, an autosomal dominant cerebral arteriopathy characterized by subcortical infarcts and leukoencephalopathy, is primarily linked to mutations in the NOTCH3 gene. Typically, onset occurs around ages 45-50, commonly presenting as ischemic stroke or cognitive decline. Migraine, often with aura, affects approximately one-third of patients and frequently precedes stroke and dementia symptoms. A 48- year-old female presented to our hospital with rotatory vertigo and ataxia. Her family history revealed a predisposition to the same condition, with confirmed diagnoses in both her sister and nephew, along with a frequent occurrence of strokes in the family. Physical and neurological examinations were unremarkable, except for paresthesia in the lower left third of the face. Magnetic resonance imaging (MRI) depicted a 16mm heterogeneous lesion with T2 hyperdense edges in the right middle cerebral peduncle, showing mild diffusion restriction with a central microhemorrhagic component. Given the proband's medical history, neurological complaints, and leukoencephalopathic changes, whole-genome sequencing was performed, revealing a clinically significant pathogenic variant in the NOTCH3 gene (c.421C>T (p.Arg141Cys)), associated with CADASIL. Based on the positive family history and the patient's test results, testing of her 22-year-old son was recommended. This case underscores the necessity for comprehensive monitoring and management of CADASIL. Considering the positive family history and the patient's symptoms, it is essential to adopt a personalized approach, focusing on potential future manifestations in her son, who may be at risk of developing symptoms.

Keywords: CADASIL, whole-genome sequencing, NOTCH3, vertigo, stroke

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OPTIMIZATION OF SEPARATION OF ALPELISIB AND FULVESTRANT BY SWEEPING MICELLAR ELECTROKINETIC CHROMATOGRAPHY- TANDEM MASS SPECTROMETRY

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Micellar electrokinetic chromatography coupled with mass spectrometry (MEKC-MS/MS) is a powerful analytical technique for a wide range of applications due to its efficiency, swiftness, and environmental friendliness. On the other hand, there is a great need for the development of bioanalytical methods for the therapeutic drug monitoring (TDM) of breast cancer drugs alpelisib (ALP) and fulvestrant (FUL). Herein, we optimized the separation of ALP and FUL in a novel MEKC-MS/MS method. Separation was achieved within 11 minutes using a volatile, MS-compatible surfactant ammonium perfluorooctanoate (APFO). Optimization of separation was performed by testing the pH of the running buffer (9.0-10.0), APFO concentration (50-100 mM), a fraction (20-35%) and type (MeOH, EtOH, ACN, i-PrOH) of organic modifiers in the running buffer, capillary temperature (20-30 °C), voltage (20-30 kV), duration of hydrodynamic injection (5-90 s) and hydrodynamic pressure during the analysis (0-100 mbar). Optimal conditions were 50 mM APFO at pH 9.75 in 25% MeOH, separation voltage 30 kV, capillary temperature 30 °C, hydrodynamic injection of 60 s, and additional hydrodynamic pressure of 100 mbar during the analysis. With these conditions, the best results in terms of analysis time, resolution, and peak shapes were obtained. Additionally, a sweeping online preconcentration technique was performed by dissolving the analytes in the electrophoretic buffer without the micelles. This way, the analytes were swept in the narrow zone and concentration factors of 109 and 11 for ALP and FUL, respectively, were obtained. In further studies, the MS ion source will be optimized, the method will be validated and applied to patient plasma samples for TDM. This work has been fully supported by the Croatian Science Foundation through projects UIP-2019-04-8461 and DOK-2021-02-4595, and the European Regional Development Fund, project Farminova, KK.01.1.1.02.0021.

Keywords: micellar electrokinetic chromatography, mass spectrometry, alpelisib, fulvestrant

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GENETIC INSIGHTS AND TREATMENT CHALLENGES IN AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 1: CASE REPORT

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Autoimmune polyglandular syndrome type 1 (APS-1), caused by homozygous, compound heterozygous, or heterozygous mutation in the AIRE gene, is a rare genetic condition manifesting in childhood. The coding sequence for the AIRE transcription factor is integral to immune tolerance mechanisms, facilitating the negative selection of autoreactive T lymphocytes within the thymus, lymph nodes, and spleen. We present a 3-year-old patient who was admitted to the intensive care unit exhibiting a critical condition alongside positive meningeal signs, with Streptococcus pneumoniae subsequently isolated from the cerebrospinal fluid. Following rapid deterioration, the patient necessitated endotracheal intubation. Continuous EEG monitoring and transcranial Doppler sonography supported the clinical suspicion of cerebral death. Post- mortem genetic analysis of the patient revealed two heterozygous variants in the AIRE gene, the pathogenic variant c.769C>T (p.Arg257*) and the likely pathogenic variant c.151_152dupGA (p.Glu53fs), confirming the diagnosis of APS-1. Of particular significance is the c.769C>T (p.Arg257*) variant, which has been associated with an increased vulnerability to specific infections and life-threatening non- endocrine manifestations. Additionally, although not previously reported in human diseases, the c.151_152dupGA (p.Glu53f2) variant is presumed to disrupt AIRE function, potentially exacerbating its pathogenicity. Molecular genetic analysis of the patient's mother identified the likely pathogenic variant c.151_152dupGA (p.Glu53fs) in a heterozygous state, while the father was determined to be a carrier of the pathogenic variant c.769C>T (p.Arg257*), also detected in a heterozygous state. In conclusion, continued research on the genetic basis of autoimmune diseases is vital for improving our understanding and treatment outcomes. Further investigations will provide deeper insights into disease mechanisms, enabling the development of personalised therapies.

Keywords: autoimmune polyglandular syndrome type 1, AIRE, genetic testing, personalized therapies, pneumococcal meningitis

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RELATIONSHIP BETWEEN GUT MICROBIOTA AND THE CLINICAL COURSE OF COVID-19 DISEASE

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COVID-19 is an infectious disease caused by a novel coronavirus SARS-CoV-2. The possibility of early detection of people at increased risk of developing a severe clinical picture is extremely important, so that appropriate therapy can be initiated timely to prevent numerous deaths. Elevated ACE2 receptor expression increases the exposure of type 2 pneumocytes to the SARS-CoV-2 spike protein and is influenced by various factors. The gut microbiota influences ACE2 receptor expression, while ACE2 simultaneously influences the composition of the gut microbiota. The gut microbiota also influences the immune response to lung infections via the gut-lung axis. People with underlying medical conditions (comorbidities) such as diabetes, obesity and hypertension are at a significantly higher risk of developing severe COVID-19 clinical picture. Our study included 45 patients treated at University Hospital Dubrava, for whom clinical course of COVID-19 disease was analyzed from medical records and the diversity of gut microbiota in stool samples was determined using 16S rRNA analysis. Relationship between gut microbiota composition markers (such as those related to the risk of diabetes, obesity, and hypertension – i.e. increased Firmicutes/Bacteroidetes ratio) and the clinical course of COVID-19 disease was determined. In a broader sense, our findings might be useful in combating potential future similar pandemics and other communicable and non-communicable diseases whose clinical course may be influenced by the gut microbiota. Pointing towards gut microbiota composition markers as a potential tool for early detection of individuals at an increased risk of developing a severe clinical picture and altering gut microbiota composition to actively reduce this risk, could be used as potential approaches to prevent severe clinical picture and fatal outcomes in the future.

Keywords: 16S rRNA analysis, Clinical Course, COVID-19, Gut Microbiota, SARS-CoV-2

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REGULAR MODERATE PHYSICAL ACTIVITY DECREASES GLYCAN AGE INDEX OF BIOLOGICAL AGE AND REDUCES INFLAMMATORY POTENTIAL OF IMMUNOGLOBULIN G BY ALTERING N-GLYCAN GLYCOSYLATION

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Physical inactivity and obesity are growing concerns, negatively impacting the general population. Moderate physical activity is known to have a beneficial anti-inflammatory effect. N-glycosylation of immunoglobulin G (IgG) reflects changes in the inflammatory potential of IgG. In this study, GlycanAge index of biological age (GlycanAge), one of the first commercially used biomarkers of aging, was employed to assess effects of exercise intensity in three different groups of athletes: professional competing athletes (PRO), long-term regularly moderate active individuals (ACT), and recently involved, but previously inactive, recreational individuals (REC), compared to the group of inactive individuals (INACT). The aim of this study was to determine the effects of long-term moderate exercise on Immunoglobulin G (IgG) glycosylation and the effect on GlycanAge biomarker (GA). A drop of peripheral blood was taken to measure changes in IgG glycosylation. The IgG was isolated from the dry blood spots, deglycosylated, labeled and analyzed by UPLC, glycan peaks were integrated and processed to get N-glycans and GA results. GlycanAge index of biological age was significantly lower in the active group compared to the inactive group (β= -7.437, p.adj = 7.85E-03), and nominally significant and increased in professional athletes compared to the active group (β = 7.546, p = 3.20E-02). Competing female athletes had significantly higher GlycanAge compared to active females exercising moderately (β = 20.206, p.adj = 2.71E-02), while the latter had significantly lower GlycanAge when compared with their inactive counterparts (β= -9.762, p.adj = 4.68E-02). Regular, life-long moderate exercise has an anti-inflammatory effect on both the female and the male population, demonstrated by lower GlycanAge index, and it has great potential to mitigate growing issues related to obesity and a sedentary lifestyle, which are relentlessly increasing world-wide.

Keywords: regular moderate physical activity, aging biomarkers, glycosylation, biological age, GlycanAge

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MMP3 SINGLENUCLEOTIDE POLYMORPHISMS ARE ASSOCIATED WITH NON-CONTACT ACL INJURIES IN HIGHLEVEL COMPETING ATHLETES

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Matrix metalloproteinases (MMPs) play an important role in matrix remodeling, as well as in ligament integrity. Anterior cruciate ligament (ACL) rupture is a severe and frequent knee injury in sports. The aim of this study was to investigate polymorphisms within the MMP3 gene with the predisposition for non- contact ACL rupture in Croatian professional athletes. 187 unrelated Caucasians were recruited between 2016 and 2017 (95 with ACL rupture occurring through a noncontact mechanism and 92 asymptomatic controls, previously active competing athletes that have retired without sustaining injuries during their competing career). All participants were genotyped for three single nucleotide polymorphisms (SNP) within the MMP3 gene: rs591058 C/T, rs650108 A/G, and rs679620 G/A using the pyrosequencing method. For all three investigated SNPs, genotype frequencies have significantly differed between cases and controls. The MMP3 rs591058 TT (p = 0.0012, odds ratio [OR] = 38.541, 95% confidence interval [CI] = 1.7024 – 8.7254), rs650108 GG (p = 0.0051, OR = 23.338, 95% CI = 1.2899 – 4.2226) and rs679620 AA (p = 0.0030, OR = 34.750, 95% CI = 1.5266 – 7.9101) genotypes, as well as haplotype variant TGA (p = 0.0104, OR = 1.71, 95% CI = 1.13 – 2.59) were significantly overrepresented in cases compared to controls. These results support an association between functional variants within the MMP3 gene and the risk of ACL rupture in the Croatian population. Still, further research is needed to corroborate these results in a larger population.

Keywords: SNP, MMP3, ACL rupture, injury, sport

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ASSOCIATION OF THE MATRIX METALLOPROTEINASE 3 (MMP3) SINGLE NUCLEOTIDE POLYMORPHISMS WITH TENDINOPATHIES IN HIGH-LEVEL COMPETING ATHLETES

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Matrix metalloproteinases (MMPs) play an important role in matrix remodeling, as well as in tendon integrity. Due to overuse, athletes often develop chronic tendinopathies. If not treated, they lead to severe impairment, even complete tendon ruptures. The main purpose of this study was to investigate whether three functional polymorphisms within the MMP3 gene are associated with an increased risk of developing tendinopathies in high-level Croatian athletes. We have recruited one hundred fifty-five unrelated Caucasians for this case-control genetic study (63 high-level athletes with diagnosed tendinopathies and 92 asymptomatic controls, previously active competing athletes that have retired without sustaining injuries during their competing career). All participants were genotyped for three single nucleotide polymorphisms (SNP) within the MMP3 gene: rs591058 C/T, rs650108 A/G and rs679620 G/A using the pyrosequencing method. The MMP3 rs650108 GG ($P = 0.0074$) and rs679620 AA ($P = 0.0119$) genotypes were significantly over-represented in cases compared with controls, while rs591058 TT ($P = 0.0759$), as well as haplotype variant T - G - A ($P = 0.06$), implicated that there is an indication of predisposition for tendinopathies. These results support an association between functional variants within the MMP3 gene and the risk of tendinopathies in high-level athletes in the Croatian population. Further research is needed to replicate these results in a larger population.

Keywords: SNP, MMP3, tendinopathy, injury, sport

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BLEEDING RISK STRATIFICATION IN CORONARY ARTERY SURGERY: DEVELOPMENT OF THE SHOULD-NOT-BLEED SCORE

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An estimated 20% of allogeneic blood transfusions in the United States are associated with cardiac surgery. We developed a model to predict which patients are at low risk of bleeding for whom transfusion treatment might be considered unnecessary. Herein we present our "SHOULD-NOT-BLEED-SCORE" application developed for the Windows® software platform which is based on our previous research. This study is aimed to develop a user-friendly application that stratifies patients with respect to bleeding risk. The statistical model we used in our previous research was focused on detection of CABG patients at low risk of bleeding. The rationale behind such an approach was to identify a CABG patient subgroup at low risk of bleeding. By identifying patients at low risk of bleeding we can define a subgroup of patients for whom transfusion treatment might be considered unnecessary. We developed a Windows platform application based on risk modelling which we previously calculated for 1426 patients undergoing elective CABG from January 2010 to January 2018. The SHOULD-NOT-BLEED-SCORE risk score is developed for the Windows software platform. A mathematical model that is based on multivariate analysis was used for app development. The variables that entered the scoring system were Age; Body Mass Index; Chronic Renal Failure; Preoperative Clopidogrel Exposure; Preoperative Red Blood Cells Count; Preoperative Fibrinogen Level; Preoperative Multiplate ASPI test area under the curve (AUC) units. The SHOULD-NOT- BLEED-SCORE identifies/predicts patients without a risk for excessive bleeding with strong discriminatory performance (Receiver Operating Curve (ROC) analysis AUC 72.3%, $p < 0.001$). The clinical and economic burden associated with unnecessary transfusions may be adequately addressed by a preoperative scoring system detecting patients at low risk of bleeding for whom transfusion treatment might be considered unnecessary.

Keywords: bleeding, cardiac surgery, transfusion, cost analysis, risk stratification

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SCREENING OF BENZODIAZEPINES IN URINE SAMPLES IN UNIVERSITY HOSPITAL CENTER SPLIT (CROATIA) FROM 2015 TO 2022

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Goal was to determine the prevalence, distribution, and trend of benzodiazepine use depending on the time and characteristics of patients (age and sex) screened at the Medical Laboratory Diagnostic Division of the University Hospital Center Split (Split, Croatia). We retrospectively collected screening test results for benzodiazepine from the BioNet laboratory database of the Medical Laboratory Diagnostic Division. The data included the testing results of all adult patients from 2015 to 2022, the testing date, and the sex and age of the patients. We calculated descriptive statistics of demographic characteristics for tested and positive patients. Logistic regression was used to analyze the effect of sex and age as predictors for a positive screening test result. A total of 4,162 benzodiazepine tests were performed during the considered period. The proportion of positive tests was 44.7% (48.4% of men and 39.6% of tested women). In the observed period, the number of analyses increased by 101%, from 368 tests in 2015 to 741 in 2022. The proportion of positive tests in 2015 was 39.9%, and more than 40% during other years. A logistic regression model showed that sex and age contributed to predicting positive test results (P < 0.001). With each year of increasing age, the odds of a positive test result increased by approximately 2.5%, while women were about 38.7% less likely to test positive than men. The results revealed a high prevalence of benzodiazepine use, with notable differences by sex and age, suggesting potential misuse and highlighting the need for targeted monitoring and intervention strategies. The trend in positive tests underlines the demand for continuous substance use surveillance and tailored preventive measures.

Keywords: drugs of abuse, benzodiazepine, drug screening, medical laboratory diagnostics, UHC Split

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CYTOTOXIC EFFECT OF ASCORBYL PALMITATE-LOADED SOLID LIPID NANOPARTICLES ON PATIENT-DERIVED SARCOMA STEM CELLS

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Renewed interest in the application of ascorbic acid for anti-tumor therapy led to the discovery of its effect in cancer stem cells (CSC), a small group of cells in tumor bulk with specific properties that enable them to resist conventional therapy and metastasize. The application of ascorbate in high concentrations has a prooxidative effect on cancer cells, causing extensive oxidative stress that can induce cell death. As CSC have specific redox properties, we wanted to design a stable ascorbate formulation and test its effect on patient-derived CSC. We have previously synthesized and characterized solid lipid nanoparticles containing ascorbyl palmitate (SLN-AP), a more stable lipid derivative of ascorbate. SLN- coumarin-6 were applied for visualization of the cellular uptake of the SLN. SLN-AP were applied to sarcoma stem cells isolated from samples of six patients to test their cytotoxicity and effect on oxidative stress in CSC. Results: SLNs with incorporated coumarin-6 enter the cells gradually, however, CSC react with abrupt generation of vesicles of a wide range of sizes (up to 20 μm). SLN-AP have higher IC50 values in osteosarcoma CSC than in soft tissue sarcomas. Nanoparticles without incorporated AP show strong cytotoxic effect. The treatment with nanoparticles shows antioxidative effect in CSC. In conclusion, soft-tissue sarcoma stem cells were more sensitive to the treatment than osteosarcoma stem cells and cytotoxicity is not due to oxidative stress. SLN-AP formulation should be further optimized to reduce the cytotoxicity of SLNs and to reduce their efflux from the CSC population.

Keywords: cancer stem cells, sarcoma, ascorbic acid, ascorbyl palmitate, solid lipid nanoparticles

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OVERVIEW OF ANTIFUNGAL SUSCEPTIBILITY TESTING FOR PATHOGENIC MOLDS

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Antifungal susceptibility testing plays a significant role in the mosaic of diagnostic techniques used for the diagnosis of invasive fungal infections. The clinician must select appropriate antifungal therapy for patients with invasive fungal infections commonly caused by *Aspergillus spp, Mucor spp, Fusarium spp, and Scedosporium spp*, especially those with compromised immune system. The risk factors for acquiring invasive aspergillosis are neutropenia, prolonged use of corticosteroids and broad-spectrum antibiotics, hematologic illnesses and organ transplant. This overview aims to provide understanding of the methodology involved in antifungal susceptibility testing, with a focus on the most clinically significant pathogenic moulds. The method for antifungal susceptibility testing involves the broth microdilution technique which entails diluting antifungal agents in a liquid growth medium and determining the minimum inhibitory concentration (MIC) as the lowest concentration that inhibits visible fungal growth. Adherence to standardized guidelines established by authoritative bodies such as CLSI (Clinical and Laboratory Standards Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) is essential which provide detailed instructions on factors such as media composition, drug concentrations, inoculum preparation, and incubation conditions, ensuring the reliability and reproducibility of results. The MIC values obtained through susceptibility testing are interpreted according to established breakpoints provided by CLSI and EUCAST guidelines. These MIC values guide interpretation, categorizing strains as susceptible or resistant. Adherence to standard ensures consistency and reliability of susceptibility testing results, facilitating comparison across laboratories. The testing aids in therapy selection and resistance monitoring, improving outcomes in fungal infection management, particularly for immunocompromised patients.

Keywords: antifungal susceptibility, pathogenic mould, methodology, minimum inhibitory concentration, immunocompromised patients, invasive *aspergillosis*

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MOLECULAR DIAGNOSTICS: CURRENT TECHNOLOGY AND APPLICATIONS

DISTRIBUTION OF DIATOMS FOUND IN THE ORGANS OF VICTIMS OF VITAL DROWNING IN ALGERIA, DESCRIPTIVE, PROSPECTIVE STUDY OVER TWO YEARS (2016-2017)

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No study in the field of forensic limnology has been carried out to date in our country, hence the launch of our work, the objective of which was to draw up a national repertoire of diatoms found in cases already dealt with in the department of forensic medicine of the National Institute of Criminalistics and Criminology of the National Gendarmerie (INCC/GN) during the years 2016 and 2017.Our work is a descriptive, prospective longitudinal study allowing the isolation and identification of diatoms from 75 cases sent to the forensic medicine department of the INCC/GN, encompassing all the cases of drowning in fresh and salt water on the national territory. The objective of this study is to individualize the maximum number of taxa of diatoms and list them according to their geographical areas of origin (wilaya, daïra, and municipality) thus allowing in the medium and long term the development of a national repertoire of diatoms. of all the aquatic expanses of Algeria by an informatic management tool. This repertoire will allow the rapid and accurate diagnosis of vital drowning by isolating and identifying the same diatoms in drowning water according to their geographical distribution (location of the initial place of drowning) and their seasonal varieties (post-mortem delay). A total of 200 species of diatoms were extracted from more than 12300 individualized and photographed diatoms in the water and organ samples sent to us during the two years 2016 and 2017. The presence of a national repertoire of diatoms listed in all the aquatic expanses of our country will facilitate forensic investigations in the field of vital drowning.

Keywords: national repertoire, diatoms, vital drowning, fresh water

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CHANGES IN THE CONCENTRATION OF IL 8 IN PATIENTS WITH TEMPOROMANDIBULAR JOINT DISORDERS TREATED WITH A STABILIZATION SPLINT ARE RELATED TO THE DURATION OF TREATMENT AND THE SEVERITY OF PAIN BEFORE TREATMENT

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Temporomandibular disorder (TMD) is a term for a range of painful conditions affecting the muscles of mastication, temporomandibular joints (TMJ), and/or surrounding tissues. A new individualized approach could be achieved using molecular biomarkers of painful temporo-mandibular conditions. The aim of this study was to investigate whether the administration of stabilization splint (SS) leads to changes in the concentrations of pro-inflammatory cytokines in serum and gingival crevicular fluid and whether there is a correlation between the concentra-tions of pro-inflammatory cytokines and the intensity of chronic pain. This prospective cohort study enrolled 32 patients diagnosed with painful TMD according to the diagnostic criteria for TMD (DC/TMD). Samples of whole blood and gingival crevicular fluid were collected at baseline before SS treatment (T0) and at follow-up one month (T1) and three months (T2) after the start of SS treatment. For the multicomplex quantitative analysis of pro-inflammatory cytokines (IL-1β, IL-6, IL-7, IL-8, IL-13, TNF-α), customized ProcartaPlex Multiplex assays (eBioscience, Affymetrix) were used. Data on the intensity of chronic pain were collected using the Graded Chronic Pain Scale questionnaire (GCPSv2). The concentration of IL-8 in serum was significantly higher at T2 compared to T0 and T1 (Friedman test, P=0.002), while the concentrations of other proinflammatory cytokines were not significantly different during the period. Before splint therapy, serum levels of IL-8 were significantly lower in subjects with high-intensity pain without disability compared to the other groups (Kruskal Wallis test, P = 0.02), while concentrations of other cytokines related to the severity of chronic pain before therapy did not differ significantly. The results of our study suggest that IL-8 levels could be used for monitoring TMD patients treated with a stabilization splint, however further investigation is needed.

Keywords: biomarkers, interleukin-8, occlusal splints, serum, temporomandibular joint disorders

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EXPLORING THE IMPACT OF TMPRSS2 GENE POLYMORPHISM ON COVID-19 SEVERITY, PATIENT OUTCOMES AND ITS CORRELATION WITH VITAL BIOMARKERS

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The global COVID-19 pandemic has posed unparalleled challenges, with varying degrees of illness severity and outcomes among those affected. Recent research has highlighted the impact of genetic factors on the progression of COVID-19. One gene of particular interest is TMPRSS2, which facilitates the entry of the SARS-CoV-2 virus into cells. Our study investigated how a specific variation (rs2070788) in the TMPRSS2 gene influences the development of severe symptoms and its relationship with various biochemical markers in COVID-19 patients. The research involved 750 COVID-19 patients enrolled at General Hospital in Tešanj. Genotyping was performed using the Applied Biosystems QuantStudio5 RT-PCR System. Analysis of all biochemical parameters followed standard IFCC protocols. Our results revealed a statistically significant difference in genotype distribution among COVID-19 patients with mild and moderate symptoms ($p=0.020$), while no significant differences were observed between mild and severe ($p=0.620$), and moderate and severe clinical presentations ($p=0.053$). Additionally, in patients with mild symptoms, carriers of the risk GG genotype of rs2070788 exhibited significantly higher levels of total bilirubin ($p=0.042$) compared to carriers of the AA genotype. Among patients with moderate symptoms, carriers of the GG genotype showed a significant association with elevated creatine kinase levels ($p=0.022$) compared to carriers of the AA genotype. Furthermore, among patients with severe symptoms, carriers of the GG genotype displayed significantly higher potassium levels ($p=0.003$) compared to carriers of the AA genotype. Our findings suggest that specific variations within the TMPRSS2 gene might impact susceptibility to severe manifestations of COVID-19, leading to variations in disease progression and prognosis among patients.

Keywords: COVID-19, SARS-CoV-2, TMPRSS2, SNP, disease severi

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OXIDATIVE STRESS AND INFLAMMATION IN FEMALE LEWIS RATS DEPENDING ON THE DIET AND RETINOIC ACID APPLICATION

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Macrophages are characterized by increased expression of inducible nitric oxide synthase and NO production. NO is an important indicator of inflammation, oxidative stress, and polarization of macrophages into the M1 phenotype. Anti-inflammatory cytokines, IL-4, IL-10 and IL-13 stimulate the activity of arginase I in macrophages enhancing the anti-inflammatory effect. The goal of the study was to investigate inflammatory parameters, arginase, and NO, as well as their differences depending on retinoic acid and high fat diet application. The analysis included 36 Lewis rats bred at Department of Animal Physiology Zagreb. Half of the animals were fed high fat diet (HFD, 45% of saturated fatty acids) and half standard laboratory diet (STD) for 30 days. The groups were divided into additional 3 groups (6 rats each): two experimental groups that received 13 cRA orally daily for 30 days (7,5 mg/kg and 15 mg/kg, respectively) and the control group was given distilled water. Animals were sacrificed after 60 days and serum for inflammatory cytokines, arginase, and NO activity. The increase in the serum concentration of IL-6, IL-1β, IFN- in individuals on high-fat food, both in the control group and in the groups to which 13 cRA was added in both concentrations was seen. In the same groups, there was an increase in IL-10, which belongs to the Th2 inflammatory response. There was a visible increase in serum, kidney, and liver NO activity in individuals on HFD with the addition of 13 cRA (HFD+15 (13 cRA)) compared to the control group. The analysis of serum and renal arginase concentrations revealed a decrease in the value of arginase in individuals on HFD. According to the present results, 13 cRA and HFD affect metabolic parameters, as well as inflammation and oxidative stress in the organism. Its administration has a different effect on metabolic parameters, depending on whether the STD or the HFD was applied.

Keywords: high fat diet, isotretinoin, lipids, oxidative stres, metabolic syndrome

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GENETIC TESTING IN PREIMPLANTATION DIAGNOSTICS: WHERE ARE WE TODAY?

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With the rapid development of in vitro fertilization (IVF) methods, preimplantation (PI) diagnostics has become a prominent branch of genetic testing. It allows for the detection of genetic abnormalities before the embryo is implanted and thus, the selection of healthy embryos. The methods available vary in accuracy, as well as safety. The relatively safer procedures include polar body biopsy, which uses meiotic byproducts for genetic analysis, and blastocentesis, which extracts the blastocyst fluid. However, polar body biopsy is limited in its detection of paternal or division-originated DNA abnormalities, while blastocentesis is often complicated by failed DNA amplification. On the other hand, more precise methods include blastomere biopsy and trophoectoderm biopsy. However, these methods pose a risk of damaging the embryo by extracting the cells necessary for physiological growth. Once the sample is isolated, genotyping is performed by next-generation or third-generation sequencing. Recent advances have broadened the scope of PI testing to include mitochondrial disorder detection. Another expansion of this field includes testing for hereditary cancer predispositions, which might indicate preventative oncological care from birth. However, it is worth noting that the utility of these approaches is still being discussed. When discussing PI testing, the question of ethics must be discussed, as its unintended use could lie within the field of eugenics. For this reason, each case must be analyzed thoroughly and any selection between embryos with non-pathogenic genotypes must be discouraged. With the use of IVF becoming increasingly frequent, the wide accessibility of these methods is of great importance. In women of advanced age, with repeated implantation failure, repeated miscarriage after implantation, or diagnosed genetic disorders, PI testing might provide an explanation and offer a more selective process to avoid future negative outcomes.

Keywords: preimplantation diagnostics, blastocentesis, blastomere biopsy, polar body biopsy, trophoectoderm biopsy, in vitro fertilization, embryo implantation

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WHOLE GENOME SEQUENCING: A NEW DIRECTION FOR PRENATAL DIAGNOSTICS?

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Prenatal genetic testing is a diagnostic procedure performed to determine genetic abnormalities in the fetal DNA. While early methods implied invasive procedures for DNA sampling, a revolution in maternal- fetal diagnostics was brought on by the development of non-invasive testing methods (NIPT), which isolate cell-free fetal DNA from the mother's peripheral blood. According to recent studies, NIPT rivals invasive methods in precision where aneuploidy is concerned. Although today NIPT is very frequently used, it remains a screening method and cannot provide definitive answers regarding the fetal genome. In this regard, invasive testing still has great clinical utility and is recommended before any clinical decision. With the development of DNA sequencing technologies, the question of whole genome sequencing integration into invasive prenatal testing has been raised. In comparison with the currently used diagnostics, which include karyotyping, whole exome sequencing, and microarrays, WGS could provide a more comprehensive analysis of both nuclear and mitochondrial DNA. This could, in turn, greatly broaden the spectrum of genetic abnormalities we can discover and increase the number of diseases we can anticipate. Furthermore, WGS results from prenatal testing would have utility far beyond the detection of rare genetic diseases. The discovery of variants related to increased cardiovascular risk, oncogenic risk, or irregularities in drug metabolism might be the cornerstone of a lifelong personalized and preventative medical plan. As the average age of first birth is continuously on the rise, the incidence of genetic abnormalities is steadily increasing as well. This fact alone highlights the great importance of modern prenatal testing and why it should be made increasingly accessible. On the other hand, if WGS were to become standard practice in prenatal diagnostics, more physicians would need to be educated to provide quality genetic counseling for future parents.

Keywords: whole genome sequencing, preventive medicine, cell-free fetal DNA, prenatal diagnostics

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PROACTIVE GENETIC SCREENING USING WHOLE GENOME SEQUENCING ENABLES PREVENTION OF SUDDEN CARDIAC DEATH IN FAMILY MEMBERS WITH PATHOGENIC VARIANTS IN THE PKP2 GENE

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Arrhythmogenic right ventricular dysplasia (ARVD) is a rare genetic disorder associated with sudden cardiac death in young people. It is characterized by both structural and functional abnormalities caused by fibrofatty replacement of the damaged right ventricular myocardium. This condition can contribute to ventricular tachycardia that can be intensified with exercise leading to sudden cardiac death. Plakophilin 2 gene (PKP2) encodes for desmosomal proteins. Mutations in PKP2 are known to cause ARVD. We present to you the case of a 12-year-old female who came to the pediatric cardiology department after experiencing tightness in the middle of the chest without radiation, which worsened with respiration, following physical activity. Past medical history revealed the sudden cardiac death of a 15-year-old brother on a sports field. Proband's ergometry showed a right bundle branch block. A whole-genome sequencing (WGS) with a focus on coding and non-coding regions of 294 genes associated with sudden cardiac death was indicated due to the family's medical history. The report showed a heterozygous pathogenic variant in the coding region of the PKP2 gene (c.368G>A (p.Trp123Ter)). Trio-WGS showed that the patient's mother is a heterozygote for the same variant while the father has not tested positive for any mutations related to sudden cardiac death. The diagnosis of ARVD has been confirmed in proband through clinical examination and she was put on beta blockers as well as advised to avoid participating in extensive physical activity. The patient's mother, who is also heterozygous for this pathogenic gene variant, underwent a thorough clinical examination, and no changes indicative of ARVD were detected, suggesting variability in gene expression and incomplete penetrance of ARVD. This case shows that genetics and personalized medicine hold immense potential in the primary prevention, diagnosis, and treatment of ARVD and sudden cardiac death.

Keywords: arrhythmogenic right ventricular dysplasia, arrhythmogenic right ventricular cardiomyopathy, PKP2, sudden cardiac death, genetic testing

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WHOLE-GENOME SEQUENCING CONFIRMED THE DIAGNOSIS OF FAMILIAL HYPERTROPHIC CARDIOMYOPATHY AND DETECTED THE PREDISPOSITION IN SEVERAL FAMILY MEMBERS

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Familial hypertrophic cardiomyopathy (FHC), an autosomal dominant condition marked by left ventricular hypertrophy and a diverse array of clinical presentations, stands as the pioneering cardiac disorder with decoded genetic underpinnings. While relying on conventional diagnostic tools such as physical examinations and electro-echocardiography remains crucial, there's an emerging emphasis on understanding how genotype influences the clinical manifestations of FHC. The clinical spectrum of FHC suggests different pathomechanisms related to variant types and their location in the gene. The FLNC gene, encoding the Filamin-C protein, not only upholds the structural integrity of cardiac muscle tissue but also governs the signaling of sarcomeres. Pathogenic variants within FLNC are linked to the development of autosomal dominant familial hypertrophic and familial restrictive cardiomyopathy (OMIM: 617047). We present the case of a 55-year-old proband with hypertrophy of his heart's left ventricle. A whole-genome sequencing (WGS) with a focus on coding and non-coding regions of genes associated with cardiomyopathies was indicated. The report showed a heterozygous pathogenic variant in the coding region of the FLNC gene (c.1444C>T (p.Arg482Ter)). After proactive genetic testing of the entire family, it was found that the same pathogenic variant of the FLNC gene (c.1444C>T (p.Arg482Ter) was transmitted to the proband's son and daughter. While traditional diagnostic methods remain indispensable, the evolving understanding of genotype-phenotype correlations heralds a new era of personalized medicine in managing FHC. We highlight the importance of ongoing monitoring by cardiologists for both the proband and all family members carrying the pathogenic variant, ensuring comprehensive care and early intervention where necessary. This case shows the importance of proactive genetic testing, ultimately paving the way for more effective management strategies in familial cardiomyopathies.

Keywords: familial hypertrophic cardiomyopathy, familial restrictive cardiomyopathy, Filamin-C, ventricular hypertrophy, whole-genome sequencing

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PROTEIN GLYCOSYLATION IN DIAGNOSTICS AND THERAPY

EFFECT OF FMT ON BLOOD SERUM N-GLYCOME IN PATIENTS WITH FULMINANT CLOSTRIDIUM DIFFICILE INFECTION

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Clostridium difficile infection (CDI) mainly occurs in hospital environment. Patients with CDI in general have a good response to antibiotic therapy, but a subset of patients develops antibiotic-refractory severe or fulminant CDI (SFCDI), for which a different therapeutic approach is necessary. An effective treatment for SFCDI is faecal microbiota transplantation (FMT) from healthy donors. From the studies, it is apparent that gut homeostasis is reconstituted after FMT, but the effect of FMT on the metabolic state of a person is yet unknown. Therefore, in this study, we aimed to elucidate the effect of FMT in SFCDI patients on blood serum N-glycome as well as on blood serum isolated immunoglobulin G (IgG) N-glycome. The study enrolled four patients longitudinally, followed at five time points. In three patients, the FMT procedure was successful, while one patient was non-responsive. Temporal changes in blood serum and IgG N glycome were observed after a successful FMT procedure. Specifically, we detected a higher relative abundance of monosialylated and digalactosylated N-glycans in blood serum following successful FMT. Furthermore, the non-responsive patient has higher abundances of circulating IgG subclass 4 with agalactosylated, bisected, and core-fucosylated N-glycans. These findings suggest that changes in the gut microbiome have an impact on the metabolic state of a person. However, no broad generalization can be drawn due to the small sample size. Therefore, a replication study with a larger number of patients is required.

Keywords: fecal microbiota transplantation, Clostridium difficile, N-glycome, blood serum, IgG

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IGG N-GLYCOSYLATION
IN CARDIOMETABOLIC DISEASES

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Cardiometabolic diseases (CMDs) are a significant public health problem worldwide, and their prevalence is increasing due to changes in lifestyle and dietary habits. It refers to a group of medical diseases that include cardiovascular diseases such as coronary artery disease, stroke, peripheral arterial disease, aortic disease and different metabolic diseases including diabetes. These diseases include obesity, high blood pressure, high blood sugar levels, and abnormal cholesterol and triglyceride levels. Glycosylation is one of the most common and complex post-translational modifications of proteins which plays an important role in many biological processes, and therefore, it is not unexpected that glycosylation changes have been noticed in CMDs. Recent studies have shown that IgG glycosylation may play a role in the development and progression of CMDs. In this study, we analyzed 461 total serum IgG glycosylation from patients with CMDs and healthy controls. IgG N-glycans were analyzed using ultra-high-performance liquid chromatography (UHPLC) after a rapid high-throughput in-solution deglycosylation and labeling of released N-glycans with RapiFluor-MS dye. IgG N-glycome statistically significant change in the levels of monogalactosylated glycans between patients with CMDs and healthy controls. This finding provides important insights into the potential link between glycosylation and CMDs, although the exact mechanism underlying this relationship is not fully understood. However, the emerging evidence suggests that glycosylation may serve as a valuable biomarker for assessing disease risk, and also be a promising target for therapeutic interventions.

Keywords: cardiometabolic diseases, CMD, IgG, glycosylation, RapiFluor

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EFFECTS OF TESTOSTERONE AND METFORMIN ON IGG GLYCOME COMPOSITION IN MEN WITH OBESITY

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Being a multifactorial chronic disease, obesity has evolved into a major global public health issue. It is associated with a variety of metabolic complications, including a significant decrease in testosterone concentrations among men. Low testosterone levels can lead to an increased risk of various health issues, such as a higher risk of cardiovascular disease, type 2 diabetes, erectile dysfunction, and decreased quality of life. Several approaches have been employed to address low testosterone levels in men with obesity, including weight loss, and testosterone replacement therapy. Metformin is a medication commonly used to treat type 2 diabetes. Recent studies suggest that it may be useful for treating low testosterone levels in men with obesity. This combined approach targeting both insulin resistance and reduced testosterone levels could be one of effective treatments for these conditions. Glycosylation is one of the most common and complex co- and post-translational protein modifications which mediates a wide variety of cellular functions and changes significantly when the homeostasis of the organism is disturbed. Therefore, it is not surprising that glycosylation changes have been noticed in obesity. Since IgG glycosylation is known to be involved in the regulation of various processes, we evaluated the effect of testosterone and metformin on IgG N-glycome in men with obesity. We analyzed IgG glycome composition in 267 men with obesity who were on individual and combined metformin and testosterone therapy but had no diabetes mellitus, and accordingly they were divided into four groups. The samples were collected at three timepoints and analyzed using capillary gel electrophoresis with laser-induced fluorescence (xCGE- LIF) analysis to evaluate the released glycans labeled with 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt (APTS). After performing a stratified analysis, we see that all effects are driven by testosterone, while metformin has no effect.

Keywords: obesity, metformin, testosterone, IgG, glycosylation

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AUTOLOGOUS MICROFRAGMENTED ADIPOSE TISSUE INJECTION FOR TREATMENT OF KNEE OSTEOARTHRITIS: TOWARDS A PRECISION THERAPY APPROACH USING ADIPOSE TISSUE N- GLYCOMICS DATA

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Given the global burden of a rapidly ageing population, better management of knee osteo-arthritis (kOA) has become vital. Stem cell (SC) therapy has been investigated as a promising alternative to mainstay kOA therapies. Since adipose tissue is an accessible source of adult SCs, autologous microfragmented adipose tissue (MFAT) has become a treatment of interest in kOA, though the factors affecting its success remain largely unknown. As part of a larger randomized control trial involving autologous MFAT injection for the treatment of kOA, we investigated whether MFAT N-glycan properties may be predictive of treatment efficacy. MFAT samples were acquired from 33 individuals with kOA using lipoaspiration and the effect of a single intra-articular MFAT injection was examined 6 months' post-injection. The MFAT N- glycome was analysed using liquid chromatography and mass spectrometry and was characterised based on N-glycan types and derived traits. The MFAT N-glycomics data were integrated with clinical parameters, such as self-reported questionnaires, imaging, and cytometry data. Statistical analyses revealed that the MFAT N-glycome is relatively uniform regardless of patient age or sex. However, 16 nominally significant correlations were uncovered showing that a greater abundance of oligomannose and hybrid N-glycans in MFAT correlated with better clinical outcomes and cytometry measures, whereas the inverse was observed for complex and fucosylated N-glycans. The former N-glycan traits are characteristic of adipose SCs whilst the latter are associated with mature adipocytes, indicating that the cellular composition of

patient MFAT may affect treatment success. These preliminary results illustrate the predictive potential of the MFAT N- glycome in determining treatment efficacy and suggest that MFAT N-glycoprofiling could be used for patient stratification to allow delivery of precision therapy or for the enrichment of SC populations associated with better clinical outcomes.

Keywords: N-glycome, stem cells (SCs), autologous microfragmented adipose tissue (MFAT), knee

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INTRODUCTION OF MODERATE EXERCISE INDUCES A SIGNIFICANT CHANGE IN IMMUNOGLOBULIN G GLYCOSYLATION AND GLYCANAGE IN PREVIOUSLY INACTIVE, OBESE POPULATION

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Previous studies have shown there is a strong association between Immunoglobulin G N-glycans and chronological age. Regular moderate exercise has a beneficial effect on overall health. 382 participants (300 females, 82 males, average chronological age: 50.97, average GA: 49.93, average body mass index (BMI): 30.27) underwent three types of programs: cardio, circular training (CT) and Nordic walking (NW). Participants underwent training protocols 2x per week, 60 minutes each, for 14 weeks. At the beginning and at the end of the intervention, their motoric skills and body composition were evaluated, while a drop of peripheral blood was taken to measure changes in IgG glycosylation. The aim of this study was to determine the effects of moderate exercise on Immunoglobulin G (IgG) glycosylation and the effect on GlycanAge biomarker (GA). The IgG was isolated from the dry blood spots, deglycosylated, labeled and analyzed by UPLC, glycan peaks were integrated and processed to get N-glycans and GA results. CT: 221 participants (170 females, 51 males, average age 49.7), cardio: 112 participants (89 females, 23 males, average age 51.1) and NW: 49 participants (41 females, 8 males, average age 56). Each group presented a slight increase in GA (+1.88 for CT, +2.13 for cardio and +1.86 for NW). Our results are in accordance with what was previously reported in two significantly younger cohorts. Though BMI slightly decreased in all three groups (-0.8 for HIIT, -0.8 for cardio and -0.5 for NW), their IgG N-glycan profile was pro- inflammatory, due to the processes of metabolic remodeling taking place in the first several months of a new exercise regimen. We will additionally investigate IgG glycosylation changes and GA trends after longer exercise periods of 6 and 9 months, with expectations to observe a lower pro-inflammatory N-glycan profile.

Keywords: physical activity, glycosylation, inactive and obese population, aging biomarkers, inflammation

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SLEEP QUALITY AND N-GLYCOMIC PROFILE – CROSS-SECTIONAL STUDY AMONG MEDICAL STUDENTS IN OSIJEK, CROATIA

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The aim of the study was to assess sleep quality among medical students and to evaluate whether poorer sleep quality is associated with changes in N-glycosylation of immunoglobulin G (IgG). This cross-sectional study was conducted in December 2023. Participants were first- and second-year medical students of the University in Osijek, Croatia. Sleep quality was assessed with The Pittsburgh Sleep Quality Index (PSQI). IgG was isolated from plasma samples and its N-glycan composition was assessed by capillary gel electrophoresis. A total of 27 IgG N-glycan peaks and an additional 9 glycans with shared structural features were obtained. The study was approved by the Ethics Committee of the Faculty of Medicine Osijek. 82 students participated in the study, comprising 22 males and 60 females. 13 % of participants experienced poor sleep quality with a PSQI score ≥10. In the comparison of N-glycan peaks between participants with a PSQI score ≥ 10 and those with a score < 10, the analysis revealed that poor sleepers had significantly more altered levels of P1 (digalactosylated, disialylated biantennary; A2G2S2) (P = 0.032), P4 (core-fucosylated, digalactosylated, disialylated biantennary with bisecting GlcNAc; FA2BGS2) (P = 0.014), P9 (digalactosylated, monosialylated biantennary; A2G2[3]S1) (P = 0.019), and P13 (core-fucosylated, digalactosylated, monosialylated biantennary with bisecting GlcNAc; FA2BG2S1) (P = 0.006), MannWhitney test, resp. A regression analysis revealed that higher PSQI scores significantly contribute to an increase in P1 (P=0.004) and a decrease in P9 (P=0.013), and P13 (P=0.048). A notable prevalence of poor sleep quality has been established among medical students. Poorer sleep quality was associated with more alterations in total plasma IgG N-glycans.

Keywords: N-glycome, sleep, IgG, PSQI, student

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REGENERATIVE MEDICINE

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GENETIC TESTING IN PREMATURE OVARIAN INSUFFICIENCY

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Premature Ovarian Insufficiency (POI), also known as premature ovarian failure (POF), is a complex reproductive disorder characterized by the depletion or dysfunction of ovarian follicles before the age of 40, accompanied by cessation of menstruation and infertility issues. It affects approximately 1% of women under 40 years old, increasing the risk of cardiovascular disorders, osteoporosis, and cognitive decline. Together with environmental factors and autoimmune mechanisms that contribute in POI, growing evidence suggests a significant genetic component in the heterogeneous etiology of this condition. Genetic causes account for approximately 20–25% of patients, and chromosomal abnormalities could explain 10-15% of POI cases. Recent studies using next generation sequencing (NGS) have identified new possible genetic contributors to POI susceptibility, including mutations in genes involved in DNA repair, meiosis, ovarian development, folliculogenesis, and hormonal regulation. Today, over 50 genes are known to be causally related to POI, but in the most cases, the genetic background has not been clarified. Based on multiple studies, single gene mutations that unequivocally show deleterious effect in at least one population include BMP15, PGRMC1 and FMR1. New evidence suggests a major association to exist between reproductive longevity and the DNA damage pathway response genes. Mesenchymal stem cells (MSCs), characterized by the ability of self-renewal, play a crucial role in the regeneration of injured tissues. In a mouse model, transplantation of MSCs has been shown to restore ovarian reserve, a new therapeutic option that brings hope to patients with POI. Although much remains to be discovered, understanding the genetic basis of POI provides important insights into the molecular mechanisms responsible for ovarian dysfunction, as well as contributes to the development of diagnostic strategies and personalized targeted therapies.

Keywords: POI, genetics, mesenchymal stem cells, infertility, regenerative medicine

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ACS, AN EXTRACELLULAR VESICLE (EV)-SATURATED WHOLE BLOOD SECRETOME COMBINES REGENERATION WITH PAIN RELIEF AND RESOLUTION OF INFLAMMATION

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Customary pain and inflammation medications obstruct physiologic healing responses, increasing risk of chronification. Signaling through neutrophils and S100A8/A9 alarmins inhibits chronification. Autologous Conditioned Serum (ACS) is used successfully as injection-therapy for osteoarthritis (OA) and back pain since >20 years. It has a de-chronifying clinical effect both in animals and humans. A deeper understanding of the mechanisms involved in ACS's biochemical modes of action has resulted from re- analysis. ACS derives from sterile blood-co-agulation, controlled incubation at physiological temperature and subsequent centrifugation yielding a therapeutic, injectable serum. This harmonic process enriches multiple groups of biochemical mediators. Cytokines and growth factors were the first ACS-mediators described in the early 2000s. ACS then was aimed to accumulate IL-1Ra with little IL-1b. More content was published in 2007 and 2023. It became clear that some inflammatory mediators are also released [6], they might be involved in the clinically observed de-chronification of pain and inflammation. Resolving lipid mediators (SPM) are in ACS (ResolvinD1 and ProtectinD1). SPMs are publicized strongly since a new chapter in lipid mediator biochemistry has been opened. Mitochondrial-derived peptides (MDP,10) are also in ACS. They are encoded by small ORFs in mt-rRNA genes and are thought to orchestrate adaptative responses to metabolic stress. Further components in ACS include but are not limited to CD90, Gelsolin, Globulins, alfa 2 macroglobulin, bilirubin, C3. EVs in ACS were first published in 2012. They induce clinical improvement in e.g. Rheumatoid Arthritis. EV publications grew exponentially but clinical use lagged, except in clinics applying ACS for musculoskeletal and neuroskeletal diseases. Experimental work now shows in models of PTX induced CIPN and trauma pain that ACS-EVs are a crucial factor for symptom resolution and healing.

Keywords: extracellular vesicles, autologous conditioned serum, musculoskeletal diseases, neuroskeletal diseases, orthokine

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NOVEL DIAGNOSTIC AND THERAPEUTIC CONSIDERATIONS FOR ALOPECIA

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Alopecia is a disorder caused by irregularities in the physiological cycle of hair production and most commonly affects the scalp. Scarring alopecia is permanent due to hair follicle destruction, whereas in non-scaring alopecia, this destruction is absent. Scarring alopecia accounts for about 7% of adults and is commonly due to an inflammatory or autoimmune disorder. Non-scarring alopecia affects between 50% and 75% of adults over 50 years of age. Androgenetic alopecia is the most common type of non-scarring alopecia and is characterized by distinct gradual patterned hair loss. Androgenetic alopecia is mediated by a genetic predisposition and excessive follicular sensitivity to androgen. Prevalence reaches >50% in older men and 15% in postmenopausal women. While hair loss is mainly associated with the AR gene, more than 60 genes may play a role in male pattern baldness. Novel genetic analyses confirmed associations between variants in the EDA2R, WNT10A, HEPH, CEPT1, and EIF3F genes and hair loss. Research has shown that over 80% of people experiencing noticeable balding have a positive family history. Genes coding for the production of aromatase, which catalyzes the conversion of androgens to estrogens, may play a role in female baldness patterns and explain why many women lose their hair after menopause. Pharmacologic treatment of androgenetic alopecia involves 5-alpha-reductase inhibitors, such as topical minoxidil and oral therapy with finasteride. Additionally, DNA tests that provide individual reports for personalized treatment options are available. Platelet-rich plasma (PRP) also can be beneficial in the treatment of androgenetic alopecia. The genetic component of balding could be a promising predictive tool, useful for the treatment of hair loss in combination with novel PRP or mesenchymal stem cell (MSC) therapies. However, further research in this direction is warranted before the clinical implementation of such diagnostic and treatment protocols.

Keywords: alopecia, androgenetic alopecia, genetic predisposition, platelet-rich plasma, mesenchymal stem cells

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EXPLORING THE EFFICACY OF PLATELET-RICH PLASMA (PRP) AND MESENCHYMAL STEM CELLS (MSCS) IN THE MANAGEMENT OF LICHEN SCLEROSUS: A PROMISING THERAPEUTIC APPROACH

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Lichen sclerosus (LS) is a chronic non-neoplastic inflammatory dermatosis that mainly affects the vulvar area and often leads to significant morbidity if not treated. It showed a genetic predisposition, with additional influences from trauma, hormonal fluctuations, and medications in its pathogenesis. LS, as an immune-mediated disease, is miR-155-5p and type 1 T helper-dependent. Preliminary data suggests that miR-155-5p is significantly upregulated in LS tissue. More precisely, miR-155-5p stimulates cell proliferation, accelerates cell cycle progression, and inhibits forkhead box O (FOXO) signaling pathway in fibroblasts. Clinical manifestations include ivory-white patches, atrophy, and intense pruritus. Treatment consists of topical treatment with glucocorticoids, calcineurin inhibitors, methotrexate, retinoids, and surgery if needed. There is a rising number of studies in regenerative medicine indicating the therapeutic potential of platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs) in LS. Research evaluating the efficacy of PRP in treating vulvar LS indicates symptom reduction in patients unresponsive to initial therapeutic interventions, attributed to high concentration of platelets rich in growth factors and cytokines, which stimulate extracellular matrix production, cellular migration, proliferation, and differentiation. MSCs exhibit multipotency enabling differentiation into diverse cell lineages, by the expression of surface markers such as CD105, CD73, and CD90, while lacking lineage-specific markers like CD45, CD34, CD14, CD19, and HLAII. Additionally, MSCs exhibit anti-inflammatory and immunomodulatory properties through the secretion of immunomodulatory factors. Multiple studies show significant symptom improvement following treatment, with notable enhancements in quality of life and sexual function, offering novel insights for symptom mitigation and tissue regeneration, warranting further clinical exploration.

Keywords: lichen sclerosus, PRP, mesenchymal stem cells, gynecology, regenerative medicine

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REVOLUTIONIZING ORTHOBIOLOGICS: APPLICATION OF LEUKOCYTE-RICH AND LEUKOCYTE-POOR PLATELET-RICH PLASMA FOR KNEE OSTEOARTHRITIS – A PERSONALIZED APPROACH

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Knee osteoarthritis is a common musculoskeletal disease with over 620 million patients worldwide. The insidious disease progression is such that the treatment for the end stage of the disease is a total knee arthroplasty. However, the dawn of orthobiologics has enabled the prolongation of knee osteoarthritis progression so that patients can live with their native knees much longer than previously projected. The aim was to compare the effect of leukocyte-poor (LP) and leukocyte-rich (LR) platelet-rich plasma (PRP). Here we present the preliminary results for PRP therapy of 33 patients. Patients were selected based on clinical presentation of knee pain lasting for longer than 6 months. Patients were between 18-75 years old. Criteria for admitting patients to the study were no prior history of traumatic osteoarthritis, mechanical axis deviation less than 5˘, intact ligaments of the knee, and menisci that had no clinically impactful tears. Patients were assessed using X-ray and magnetic resonance imaging, as well as VAS, KOOS, and WOMAC scores. Blood samples were processed by using Greyledge Technologies' proprietary protocol to produce LP-PRP or leukocyte rich LR-PRP. The concentration of leukocytes and platelets present in the whole blood and PRP was measured on a differential hematology analyzer. To provide high-quality products for our patients, we aimed for a minimal thrombocyte PRP concentration of 7 x10^9. Patients were randomly assigned to the LP and LR groups. Finally, the PRP was injected intraarticularly into the knee via ultrasound guidance. The average platelet concentration for all preparations was 14.79 x10^9 while the average leukocyte concentration was 50.83 x10^9 for the LR-PRP group and 12.94 x10^9 for the LP-PRP group. Patients were assessed at 0,1 and 3 months by the VAS, KOOS, and WOMAC scores. Preliminary results show improvement across all scores within a 3-month period with LR-PRP showing greater improvement than LP-PRP.

Keywords: LR-PRP, LP-PRP, platelet rich plasma, knee osteoarthritis, regenerative medicine

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STONE INDUCED URETRAL RUPTURE: TREATMENT WITH MICROFRAGMENTED ADIPOSE TISSUE CONTAINING MESENCHYMAL STEM CELLS

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A two-year-old intact Chow-chow male dog with symptoms of urinary obstructions was administrated at the Clinic for Reproduction and Obstetrics at the Faculty of Veterinary Medicine University of Zagreb. Ten days prior, a patient underwent cystotomy in a private practice, but even after the surgery, the patient couldn't urinate normally. Upon admission, the patient was diagnosed with multiple urolith stones in the urine bladder and penile part of the urethra and showed no other symptoms except for urinary obstruction. After unsuccessful catheterization, the patient underwent the second surgical procedure of cystotomy and urinary stones removal. Due to the impossibility of returning urinary stones from the urethra to the bladder by hydropropulsion, a penile urethrotomy was performed and multiple uroliths were removed. Hematology and biochemistry references were in the physiological range. Positive-contrast retrograde cystography (RC) was performed to assess bladder integrity and urethra patency. RC revealed a leakage in the pre- scrotal part of the urethra over the os penis. The patient A two-year-old intact Cunderwent diagnostic laparotomy in endotracheal inhalation anesthesia. Intraoperatively, a methylene blue solution was applied to find the exact place of the urethral fistula. During the surgery, in collaboration with St. Catherine Specialty Hospital, a sample of the dog's abdominal fat tissue was collected and processed using the Lipogems® system to obtain 5 mL of microfragmented adipose tissue (MFAT). Additionally, 15 mL of the dog's venous blood was used to produce 2 mL of platelet-rich plasma (PRP) via the Arthrex ACP® Double- Syringe System. These components were then combined, leveraging the regenerative capabilities of both MFAT and PRP. A final product was applied in place of a urethral fistula. Postoperatively, RC showed that the urethral defect is no longer present. A year after the procedure, the patient no longer shows urethral obstruction signs.

Keywords: urethral fistula, urolithiasis, microfragmented adipose tissue, mesenchymal stem cells, dogs

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EFFECTS OF TREATING KNEE OSTEOARTHRITIS WITH AUTOLOGOUS MICROFRAGMENTED ADIPOSE TISSUE CONTAINING MESENCHYMAL STEM CELLS COMPARED TO HYALURONIC ACID – RADIOLOGICAL RESULTS FROM IRI2 PROJECT

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In osteoarthritis (OA) research, finding objective indicators to measure treatment success is challenging. Advanced magnetic resonance imaging techniques like Delayed Gadolinium-Enhanced Magnetic Resonance Imaging of Cartilage (dGEMRIC) offer a method to record objective indicators by assessing the concentration of glycosaminoglycans (GAGs) in cartilage, thereby reflecting chondrocyte activity. As part of the European research project IRI2 (KK.01.2.1.02.0173), this study aimed to compare the effects of microfragmented adipose tissue (MFAT) and hyaluronic acid (HA) on the concentration of GAGs in knee cartilage of patients with knee OA using dGEMRIC before treatment and 6 months later. Continuing ongoing work in the field of OA at St. Catherine Specialty Hospital, after great previous results with increased dGEMRIC indices following MFAT application in patients with severe OA, this study included 53 patients (35 treated with MFAT, 18 with HA) with mild to moderate knee OA and interesting results were found. The results showed that patients with initially lower dGEMRIC values experienced an increase in GAG concentration post-treatment, regardless of the treatment type ($p<0.05$). This enhancement in GAG synthesis may be stimulated by a negative feedback mechanism, where a decrease in GAG levels triggers chondrocytes to increase production, aiming to restore cartilage integrity and biomechanical function. Conversely, patients with initially higher dGEMRIC values exhibited a decrease, possibly reflecting a normalization of cartilage metabolism, where a reduction in inflammation or mechanical stress lessens the chondrocytes' compensatory synthesis of GAGs.

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These findings underscore the complex interaction between therapeutic agents and biological processes within joint cartilage, highlighting the need for further detailed studies to robustly interpret these phenomena and optimize treatment strategies for managing knee OA.

Keywords: knee osteoarthritis, microfragmented adipose tissue, hyaluronic acid, mesenchymal stem cells, dGEMRIC

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CELLULAR COMPOSITION AND VIABILITY OF MICROFRAGMENTED ADIPOSE TISSUE IN OSTEOARTHRITIS TREATMENT – INSIGHTS FROM THE IRI2 PROJECT

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While considerable research has focused on stem cells derived from microfragmented adipose tissue (MFAT), the detailed cellular structure of MFAT is still not fully understood, necessitating further exploration to enhance our understanding of its therapeutic impact on osteoarthritis (OA). In our previous research, we showed that MFAT contains within the CD45- fraction: CD31+CD34+CD73±CD90±CD105±CD146± endothelial progenitors (EP), CD31+CD34– CD73±CD90±CD105–CD146± mature endothelial cells, CD31–CD34–CD73±CD90+CD105–CD146+ pericytes, CD31–CD34+CD73±CD90+CD105–CD146+ transitional pericytes, and CD31– CD34+CD73highCD90+CD105–CD146– supra-adventitial-adipose stromal cells (SA-ASC). This study aimed to examine the viability and cellular composition of fresh MFAT in a larger sample of patients. As part of the European research project IRI2 (KK.01.2.1.02.0173), 53 patients underwent lipoaspiration for MFAT or HA treatment, with sufficient MFAT collected from 50 patients for flow cytometry analysis. The range of cell numbers per milliliter of MFAT varied significantly, from as low as 1.75×10^4 to as high as 2.0×10^6, with an average of 5.0×10^5 cells per milliliter. The viability of cells within the MFAT analyzed through flow cytometry, averaged 62.30%. The analysis highlights considerable variability in cell counts among samples, which could reflect differences in the quality or characteristics of the adipose tissue from patient to patient. A high maximum cell count suggests that some samples have a higher density of vital cells, potentially enhancing the efficacy of regenerative treatments that rely on a rich cellular environment. The relatively high average viability indicates that most cells remain viable post- isolation, which is favorable for their potential therapeutic use. High viability is crucial for the success of regenerative treatments, such as OA treatment, where living cells are needed to promote the repair and renewal of damaged tissue.

Keywords: knee osteoarthritis, microfragmented adipose tissue, mesenchymal stem cells, flow cytometry, viability

UNLOCKING THE POTENTIAL OF MENSTRUAL STEM CELLS IN REGENERATIVE MEDICINE

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Cells with mesenchymal stem cell properties have been identified within the menstrual blood and termed menstrual blood-derived stem cells (MenSCs). MenSCs appear in the cellular fraction of menstrual fluid, and it has been hypothesized that they originate from endometrial stem cell (eMSCs) located in the basalis and functionalis layer of endometrium. MenSCs express mesenchymal stem cell markers but they are loosely characterized. Current applications and clinical trials are mostly based on their immunomodulation properties and their paracrine effects promoting tissue repair or regeneration. The differentiation capability of unfractionated MenSCs into mesodermal lineages like osteoblasts, adipocytes, chondrocytes, is lower than bone marrow derived MSCs. However, purification of the specific c-KIT-expressing subpopulation strongly improves MenSCs differentiation potential. Cultured MenSCs transdifferentiate across lineage boundaries into ectodermal (neurons and glia), endodermal (hepatocyte) lineages and keratinocyte-like cells, generating epidermal lineage markers. Endometrial repair is unique when compared to repair of other adult tissues, because it is very rapid, scar-free and occurs around 400 times during woman's reproductive life. As the tissue breaks down, the surface is re-epithelialized and regenerated in full thickness, protecting the wound from infection. Given the unique nature of menstrual shedding followed by rapid regeneration, the molecular understanding of the cells and signals involved in the process, may provide important insights, and offer opportunities to mediate rapid and scar-free tissue repair. Menstrual blood is easy to obtain and stem cells from menstrual blood are promising, both for scientific stem cell research as well as for the new possible therapies in various conditions.

Keywords: stem cells, regenerative medicine, menstrual fluid, differentiation, endometrium

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CELL THERAPY

Presentation number: TM 45　　　　　　Abstract number: ABS-77-ISABS-2024

MESENCHYMAL STEM CELLS IN ATHEROSCLEROTIC RENAL ARTERY DISEASE – NEW MODALITIES IN THERAPEUTIC APPROACH

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Recent studies indicate increased renal blood flow (RBF) and cortical perfusion after mesenchymal stem cells (MSCs) infusion in atherosclerotic renovascular disease (ARVD) patients (1). They underscore the importance of exploring the new therapeutic modalities alongside endovascular stenting. In a recent review by Yang et al., a hypoxic environment was found to positively influence various functions of MSCs. Abumoawad et al. observed increased cortical and whole kidney blood flows in the post-stenotic kidney of ARVD subjects three months after intra-arterial infusion of autologous adipose-derived MSCs Improvements correlated with reduced inflammatory and angiogenic biomarkers in renal veins, along with dose-related changes in GFR and blood pressure. Conversely, the medically treated group showed stable or declining blood flows and GFR, with no observed changes in kidney volumes. MSC infusion increased cortical and medullary perfusion without affecting kidney volumes, while the medical treatment-only group experienced either no change or a decrease in RBF in the post-stenotic kidney. MSC therapy plays a crucial role in promoting cell proliferation, migration, and angiogenesis while inhibiting apoptosis and inflammation. Additionally, it preserves endothelial function and protects against fibrosis progression. Further research with a larger patient sample and extended follow-up, exceeding 6 months, should investigate MSC efficacy in restoring endothelial function in ARVD post-stenting.

Keywords: mesenchymal stem cells (MSCs), atherosclerotic renovascular disease (ARVD), renal outcomes, angiogenesis, endothelial function

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IT'S TIME FOR NEW INSIGHTS INTO RENOVASCULAR HYPERTENSION AT THE CELLULAR LEVEL – STUDY PERSPECTIVE

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Atherosclerotic renal artery stenosis (ARAS) leads to reduced tissue perfusion, tissue hypoxia and inflammatory kidney injury. Mesenchymal stem cell (MSC) therapy has shown promise in preclinical studies for repairing renal microcirculation, reducing inflammation, and restoring kidney function. Recent human data suggest that autologous MSC infusion can safely increase tissue oxygenation in ARAS patients. This open-label, single-center study will enroll adult patients with significant ARAS, diagnosed by renal artery Doppler ultrasound (average peak systolic velocity >300 cm/s) and MR/CT angiography showing >60% luminal occlusion, along with hypertension (systolic >155 mmHg and/or use of ≥2 BP drugs) and/or declining kidney function. The intervention group (n=5) will receive a single MSC infusion (2.5 × 105 cells/kg) along with standard medical treatment, while the control group (n=5) will receive only medical treatment, matched for age, kidney function, and stenosis severity. Cortical and medullary volumes, perfusion, and renal blood flow (RBF) will be measured using multidetector CT. Tissue oxygenation will be assessed by blood oxygen level–dependent MRI, and GFR by iothalamate clearance. Laboratory parameters including VGF, inflammatory cytokines, MCP-1, TGF, and NGAL will be measured at baseline, three, and six months after MSC infusion. We anticipate that this study will provide evidence regarding the safety of intra-arterial infusion of autologous MSCs in ARAS patients. MSC infusion without revascularization of the main renal artery is associated with increased renal tissue oxygenation and RBF.

Keywords: ARAS patients, renal hypoxia, inflammatory kidney injury, mesenchymal stem cells, endothelial function

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TRANSLATION
MEDICINE

Presentation number: TM 47   Abstract number: ABS-82-ISABS-2024

BIOMONITORING OF URINE LEVELS OF NICOTINE AND ITS METABOLITES LINKED TO SECONDHAND SMOKING IN YOUNGER POPULATIONS VISITING NIGHTCLUBS: A PILOT STUDY

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This study highlights the extent of secondhand smoke exposure in younger population visiting nightclubs in Croatia by comparing levels of nicotine, and its main metabolites cotinine and trans-3'-hydroxycotinine (3HC) in urine samples. The samples were collected at 2 time points (before and after attending a nightclub) from 22 participants stratified into two groups, according to sex and smoking status (NS-non- smokers and S-smokers). The samples were prepared for analysis by the liquid-liquid extraction method and were analyzed using gas chromatography–mass spectrometry. The presence of nicotine, cotinine, and trans-3'-hydroxycotinine was confirmed in all urine samples. The results showed a statistically significant difference ($p < 0.05$) in the median concentrations of nicotine, cotinine, and trans-3'-hydroxycotinine between 2 time points with regard to smoking status and sex. Our findings indicate the magnitude of secondhand smoke exposure in places like nightclubs and highlights the need for further research and more importantly, the need to raise public awareness of secondhand smoke hazards.

Keywords: nicotine, cotinine, trans-3'-hydroxycotinine, biological urine sample; secondhand smoke exposure

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MODULATION OF SREBF1 AND MTTP LEVELS BY (-)-EPICATE-CHIN PRETREATMENT IN AN IN VITRO MODEL OF MASLD

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(-)-Epicatechin (EPI), a flavonoid found in cocoa and green tea, exhibits a variety of potential health benefits, including its antioxidant properties and ability to inhibit metabolic dysfunction-associated steatotic liver disease (MASLD) development. The aim of this study was to investigate the effect of EPI pretreatment on the development of MASLD in vitro using a HepG2 cell line. The cell metabolic viability, sterol regulatory element-binding transcription factor 1 (SREBF1), and microsomal triglyceride transfer protein (MTTP) levels were assessed to determine the potential implications of EPI in MASLD development. HepG2 cells were pretreated with the following concentrations of EPI: 500 and 300 µM, for 4 hours before the 24 hour- exposure to 1.5 mM oleic acid (OA) which was used to induce steatosis. The metabolic viability of the cells was assessed using MTS assay, and the levels of SREBF1 and MTTP were determined via ELISA. The MTS assay showed that the cells pretreated with EPI (EPI500/OA and EPI300/OA) exhibited significantly higher metabolic viability, whereas the OA only treated group showed reduced viability ($p<0.001$). Additionally, both analyses demonstrated significant changes in the protein levels compared to the OA only-group. The SREBF1 levels exhibited a concentration-dependent increase in EPI500/OA and EPI300/OA ($p<0.05$), while MTTP levels were decreased compared to the OA only-group ($p<0.01$). The results suggest that EPI pretreatment may alleviate the effects of OA-induced steatosis on HepG2 cell viability by modulating the SREBF1 and MTTP levels in vitro. This indicates the potential involvement of EPI in the cellular metabolic pathways associated with lipid metabolism and MASLD development. Therefore, the metabolic pathways associated with MASLD should be considered in future research when determining the potential therapeutic implications of EPI in steatosis.

Keywords: (-)-epicatechin, polyphenols, MASLD, steatosis, pretreatment

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Presentation number: TM 49   Abstract number: ABS-128-ISABS-2024

SATB1 (SPECIAL A-T RICH BINDING PROTEIN 1) EXPRESSION IN HODGKIN LYMPHOMA

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Hodgkin lymphoma (HL) is a rare monoclonal lymphoid neoplasm of B cell origin, in which monoclonal Hodgkin cells and multinucleated Reed- Sternberg (HRS) cells are mixed with population of non-neoplastic inflammatory cells and fibrosis. To date, available treatments for HL can attain long-term disease control in the most patients. However, 5-10% of patients will have refractory disease after initial therapy and about 10-30% will relapse. Those patients represent a therapeutic challenge in hematology, thus having the ability to stratify between nonresponsive and responsive patients might help to tailor a more successful treatment regime. SATB1 is chromatin modifying protein involved in differentiation of naïve (mature) B cell, here we examined whether it could serve as prognostic marker in HL patients. A total of 85 valid archived formalin-fixed paraffin-embedded lymph node biopsies with a previously histologically confirmed diagnosis of Hodgkin's lymphoma were analyzed in this retrospective study. The tissue blocks were cut into 4 μm sections and stained with SATB1 antibody visualized by DAB chromogen. SATB1 expression was quantified as positive or negative staining by pathologist. SATB1 expression could stratify patients by overall survival (OS, $p<0.0001$) and progression free survival (PFS, $p<0.0001$). OS of SATB1 positive patients plateaued at 86% after 2 years and remained at that level until the end of follow up, i.e.16 years after the diagnosis. On the other hand, SATB1 negative patients did not exhibit a survival plateau and they reached 72% survival probability after 12 years of follow up. When it comes to PFS, SATB1 positive patients plateaued at 87% after 1.5 years, whereas SATB1 negative patients plateaued at 62% after 8 years since the diagnosis and remained as such until the end of follow up. SATB1 expression was not associated with lymphoma grade, histological type, extranodal or bulky disease B symptoms.

Keywords: SATB1, Hodgkin lymphoma, differentiation, prognosis, precision medicine

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Presentation number: TM 50   Abstract number: ABS-129-ISABS-2024

HIGH VISTA, SNAI1 AND SNAI2 MRNA EXPRESSION IN RESECTED NON-SMALL CELL LUNG CANCER IS ASSOCIATED WITH SHORTER PATIENTS' OVERALL SURVIVAL

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Non-small cell lung cancer (NSCLC) is often diagnosed at an advanced stage and can be surgically removed in only 30% of patients. The resected tumor tissue is valuable for investigation of tumor metastatic potential, epithelial-mesenchymal transition (EMT) and tumor immune microenvironment (TIM), as relevant factors for follow-up of these patients. Oncoprotein IMP3 is associated with the metastatic potential of tumor cells, SNAI1 and SNAI2 genes are expressed in EMT, while VISTA gene is associated with tumor immune evasion. Our aim was to analyze this interaction between EMT and tumor immune evasion on human specimens. 55 patients who underwent lobectomy/lymphadenectomy for NSCLC at University Hospital of Split, Croatia from 2013 to 2017 were included. Their clinical data were collected from hospital records and paraffin blocks from the Department of Pathology. SNAI1, SNAI2 and VISTA mRNA were analyzed by q-RT PCR, and IMP3 by immunohistochemistry. The results were compared with clinical-pathological parameters and overall survival (OS). $p<0.05$ was set as statistically significant. Results: There were 37 (67%) men and 18 (33%) women, median age 64 (range 49-83) years. Histological types were 34 (62%) adenocarcinoma and 21 (38%) squamous carcinoma. 35 (63%) tumors were in stage T1, and 50 (90%) in N1. Lympho-vascular invasion was found in 55% of cases. The resection margin was positive in 6 cases. High SNAI1 and SNAI2 mRNA were associated with lympho-vascular invasion, high SNAI2 with higher T stage, and high VISTA with positive resection margin. IMP3 expression, as well as higher mRNA expression of all analyzed genes were connected with lower OS. High expression of VISTA, SNAI1 and SNAI2 mRNA in surgically resected NSCLC is correlated with shorter patient's overall survival.

Keywords: NSCLC, surgery, overall survival, VISTA, SNAI1, SNAI2, IMP3 oncoprotein

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EFFECT OF UV-C RADIATION ON PROTEIN INTERACTION BETWEEN KRAS AND RAF1 RAS BINDING DOMAIN

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The idea of this research is to describe and clarify how oxidation affects the functional consequences of the interaction of KRAS and the RBD (RAF1 RAS-binding domain) proteins. After lysis of HEK293 cells, proteins were exposed to UV-C radiation treatment. Then various variants were determined in which the proteins entered into protein-protein interactions. The last step was to perform a Western blot analysis using the SIMPL method where the results were visible on film using the chemiluminescence method. UV-C doses were determined: 0.4 J/cm2, 0.6 J/cm2, 0.8 J/cm2 and 1.0 J/cm2. At doses of 0.4 J/cm2 and 0.6 J/cm2 there are no visible changes in interactions between proteins. At a dose of 0.8 J/cm2, a decrease in the intensity of the protein band was visible when KRAS and RBD proteins were treated. The final radiation dose used in this experiment is 1.0 J/cm2, a complete loss of interaction between the treated KRAS and RBD proteins is observed. An additional measure of protein carbonylation was carried out at the already set doses. There is a difference in the appearance of the protein band between the untreated protein and the protein treated with different doses of UV-C radiation. It was observed that the protein band of RBD protein treated with a dose of 1.0 J/cm2 almost completely fades. The results obtained in this study indicate a dose-dependent effect of UV-C radiation on the KRASRBD interaction. The weakening of the bond, observed at the higher dose, implies that protein oxidation may affect the stability of the complex. It is important to point out that the protein bands of untreated protein were the same as those that underwent radiation treatment, suggesting that UV-C radiation alone is not the cause of changes in protein expression. Additionally, oxidative damage was checked by measuring the level of protein carbonylation. This observation supports the idea that UV-C radiation causes oxidation of proteins, which can further affect their interactions.

Keywords: protein-protein interactions, KRAS oncogene, UV-C radiation, oxidative stress, SIMPL method

PERSONALIZED APPROACH TO CURING GLIOBLASTOMA MULTIFORME – ROLE OF BRADYKININ II RECEPTOR

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Glioblastoma multiforme, GBM, are primary brain tumors derived from glia or glial precursor cells. They are characterized by invasive growth, which represents a significant impediment to successful treatment with standard therapy of surgery followed by radiotherapy and temozolomide chemotherapy. High incidence of recurrent tumor growth and short median patient survival rate of 14 months are result of almost impossible complete surgical removal and, therefore, tumor re-growth and dispersal throughout the brain. Additional obstacle to curing the disease is very heterogeneous nature of the tumor. Hence, similarly to other cancers, a need for more individual and personalized approaches to diagnostics and therapies is emerging. We have identified that bradykinin, through binding to bradykinin 2 receptor, B2R induces calcium excitability followed by vesicular glutamate release which promotes migration and invasion of human GBM patient-derived xenolines (PDX), the best pre-clinical model of GBM. The effects are reduced by blocking the receptor with icatibant, an FDA approved B2R blocker. Furthermore, we found that B2R is overexpressed in a subset of patient's tissue samples, while our analysis of RNAseq data available from The Cancer Genome Atlas (TCGA) indicates that high mRNA expression of B2R in GBM correlates with significantly shorter patient life span. Pre-clinical data indicate that the median GBM tumor volume in the icatibant-treated mice was 58% of that in the sham-treated mice, while survival of icatibant-treated mice was 25% longer than of the sham-treated mice. Taken together, B2R emerges as a therapeutic target and icatibant as the first adjuvant therapy that tempers the invasion of GBM.

Keywords: personalized medicine, glioblastoma multiforme, invasion, glutamate, B2R

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IMMUNOTHERAPY

Presentation number: TM 53   Abstract number: ABS-25-ISABS-2024

UNVEILING THE COMPLEXITY: BASAL TRYPTASE LEVELS AND C-KIT MUTATION IN SYSTEMIC ALLERGIC REACTIONS TO HYMENOPTERA VENOM

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In contemporary allergy management, assessing basal serum tryptase levels gains pivotal importance for patients with a history of systemic allergic reactions to *Hymenoptera* venom, particularly those without evident cutaneous manifestations. Elevated basal tryptase levels (>11.4 mcg/L) serve as a crucial indicator, triggering further investigation for potential mast cell disorders. Our study aimed to investigate the frequency of c-kit mutation within our patient cohort undergoing venom immunotherapy for Hymenoptera allergies. A cohort of 22 patients undergoing venom immunotherapy at the Special Hospital for Pulmonary Diseases was analyzed. Tryptase levels (Immunocap, Phadia, Upsala) were measured in the whole cohort, while c-kit D816V mutation (PCR, 2720 Thermal Cycle) was determined in 17 patients. Among the patients, 3 (17.64%) tested positive. Interestingly, 2 out of 3 positive patients exhibited basal tryptase levels below 11.4 mcg/L. However, basal tryptase levels were significantly higher (p = 0.0065) in the c-kit mutation group (median 10.10) compared to the c-kit negative group (median 3.99), and higher basal tryptase levels were associated with an increased likelihood of testing positive for c-kit mutation (OR 2.087,p = 0.0045). The findings affirm the need to explore mast cell disorders in patients with Hymenoptera allergies, even if their basal levels fall below 11.4 mcg/L. This information contributes to the refinement of diagnostic and therapeutic strategies for individuals with mast cell disorders associated with Hymenoptera venom allergies.

Keywords: allergic reaction, immunotherapy, *Hymenoptera* venom, tryptase, c-kit mutation

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NON-SMALL CELL LUNG CANCER – PDL-1 CLONE 22C3 VS CLONE SP263: CORRELATION BETWEEN CYTOLOGICAL AND HISTOLOGICAL SAMPLES

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Non-small cell lung carcinoma (NSCLC) accounts for about 80% of all lung cancers, of which the most common type is adenocarcinoma. In order to determine the patient's´possibility of receiving immunotherapy after the diagnosis predictive biomarkers are already almost routinely determined. ALKDF530, ROS1SP384 and PDL-1SP263 and 22C3 are determined immunohistochemically at the Clinical Institute for Pathology and Cytology of Clinical hospital Dubrava. Clone 22C3 is used for immunohistochemical staining of cytological smears, while clone SP263 is used for IHC staining of cytoblocks and histological samples. The interpretation of PDL-1 expression in cytological/histological samples is as follows: PDL-1 present in less than 1% of tumor cells - negative; PDL-1 present in >1% and <50% of tumor cells - positive and PDL-1 present in >50% of tumor cells - positive with strong expression. At the Clinical Institute for Pathology and Cytology, in the period from March 2022 to February 2024, 217 patients underwent IHC staining of PDL-1 cytological and histological samples. 39 patients had 2 types of samples: cytological and histological, which were compared in pairs. In 10% of patients, the cytological preparations did not have enough tumor cells or there were none, but biopsies in these same patients showed PDL-1 positivity in 1% to 50%. In most patients, cytological smears show generally two times weaker expression of PDL-1 compared to histological samples. Pembrolizumab (Keytruda®) is a drug used in the treatment of NSCLC along with chemotherapy or as a first-line therapy if PDL-1 expression is ≥50% TPS.The development and application of targeted therapy significantly increases the quality of life and prolongs life expectancy, and therefore a high-quality and reliable interpretation of PDL-1 expression is necessary.

Keywords: PDL-1, 22C3, SP263, NSCLC, immunotherapy

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ARTIFICIAL INTELLIGENCE IN CLINICAL MEDICINE

Presentation number: TM 55   Abstract number: ABS-47-ISABS-2024

MACHINE LEARNING FOR DETECTION OF VERTICALLY TRANSFERRED METABOLITES AND XENOBIOTICS

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Vertical transmission of metabolites, particularly xenobiotics, from mother to child can play a significant role in early-life disease phenotypes. Xenobiotics are foreign substances not naturally present in the body, such as environmental chemicals or drugs, which can be transferred in a multitude of pathways such as pregnancy and breastfeeding. This exposure to xenobiotics can have a significant impact on the child's health from birth, as these compounds can accumulate in the fetal body and lead to various health issues. This study employs an explainable machine learning approach to detect metabolic connections between mothers and children. By utilizing radnom forests (RF), light gradient boosting machine (LGBM) and logistic regression (LR) models, we analyzed metabolic concentration disparities. Based on the disparities, we distinguished genuine mother-child pairs from randomly paired non-relatives with an accuracy of 72%. Both RF and LGBM, in terms of accuracy, AUC and Matthews correlation coefficient, showed superior performance over LR. Our results show that the most relevant feature, as confirmed by statistical tests, was perfluorooctanoate (PFOA). PFOA is absorbed primarily through contaminated food and water as well as through polluted air, to a lesser extent. Their persistent and bioaccumulative nature, coupled with their demonstrated negative effect on health, especially during fetal programming, have raised significant concern regarding early life development. Machine learning approaches have the potential to significantly contribute and play a crucial role in examining the transfer of xenobiotics and other metabolites.

Keywords: machine learning, vertical transfer, xenobiotics, metabolomics, mother-child

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ARTIFICIAL INTELLIGENCE AS A GAME-CHANGER IN CLINICAL PRACTICE

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Clinical practice is one of the crucial areas of possible Artificial intelligence (AI) implementation. AI is based on algorithms that use deep learning techniques in combination with large data sets to analyze, understand, explain, predict, and perform tasks that are usually done by humans. This study aims to explore the AI's status of implementation in today's clinical practice, potential issues, and areas of new development possibilities. The results showed that these tools can decrease human error and costs and improve accuracy in comparison with traditional approaches. One of the crucial AI applications is the early detection and alerting of clinicians for patients with life-threatening conditions because these patients greatly contribute to high mortality rates. The combination of AI and genotype is a base for vast advancement in disease prevention, prediction, and development of precision medicine. Only AI can effectively analyze large population data sets and provide us with the specific genetic markers of various populations. Mentioned large datasets, can enable us to develop predictive models for the identification of specific patients with a higher risk of hospital readmission which can improve patient outcomes and cost- effectiveness of health systems. But this revolutionary clinical practice won't come if we don't tackle some of the issues that relate to AI implementation such as the problem of data quality which leads us to wrong conclusions and unwanted health outcomes; potential security breaches of patient data and the human contribution to the design of AI algorithms and potential biases that could ruin the AI judgment. In conclusion, AI started to revolutionize patient-centered care and treatment outcomes whereas its algorithms are increasing the accuracy, speed, and cost-effectiveness of clinical practice. Continuous work on the development of AI in healthcare is needed to unlock the full potential of this game-changer platform.

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Presentation number: TM 57   Abstract number: ABS-119-ISABS-2024

DEEP LEARNING MODEL FOR TYPE II DIABETES PREDICTION: INSIGHTS FROM AN ISOLATED POPULATION WITH TRADITIONAL LIFESTYLE

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The prevalence of type II diabetes poses a significant health challenge in developed nations due to lifestyle factors. This chronic metabolic disease is on the rise globally, and it poses a burden on the health systems and societies. This study focuses on investigating the risk factors associated with type II diabetes within an isolated population of the Croatian island of Hvar. Little isolated populations, characterized by a traditional lifestyle, serve as valuable source for studying potential genetic factors associated with disease development. The cohort comprises approximately 1,300 subjects from different age groups and villages on the island. Study data includes anthropometric measurements, biochemical parameters, socioeconomic data, and lifestyle habits. Additionally, genome-wide SNP genotyping was performed using the collected blood samples. Bioinformatic and biostatistical methods were implemented for data interpretation. Association analysis and allele frequencies calculations showed a strong association of several SNPs and Type II diabetes. Cross-referencing these results with GWAS catalogue confirmed that six of them had previously been associated with the type II diabetes trait. Based on this genetic information and the study data, we used a deep neural networks algorithm to develop predictive model that can effectively identify individuals at risk for developing Type II diabetes. Artificial intelligence approach offers innovative solutions for diagnostics and patient care. Benefits include early intervention and personalised healthcare approach.

Keywords: diabetes, GWAS, AI, neural networks, diagnostics

PHARMACOGENOMICS

Presentation number: TM 58   Abstract number: ABS-118-ISABS-2024

PHARMACOGENOMICS IN ISOLATED POPULATIONS: EXAMPLE OF CROATIAN ROMA POPULATION

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Genetic distinctiveness is especially pronounced in isolated populations due to their minimal exchange of genes with other populations and due to increasing frequencies of otherwise rare or private alleles. The Roma, transnational minority population of Indian origin, are an example of a founder population with centuries long sociocultural isolation which left traces in their gene pool showing considerable differences compared with other populations. The group of genes that significantly differentiate among populations are those responsible for Absorption, Distribution, Metabolism and Excretion of drugs (ADME genes). The knowledge on their distribution in isolated populations is limited, but these differences are essential for population-stratified pharmacogenomics. In order to fill in the knowledge gap, in 300 samples from three Roma groups in Croatia, we genotyped SNPs from 33 genes, members of the core list of the most important ADME markers by PharmaADME Consortium. The principal aim of the study was to assess the extent to which the population history – including multiple bottleneck/founder events, isolation and endogamy – could have impacted these functionally important genes. Analyses of allele frequencies of investigated SNPs showed genetic distinctiveness of the Roma population in Croatia, while more thoroughly haplotype analyses of the CYP2D6, NAT1, NAT2, ABCB1, CYP2C19 and CYP2B6 genes revealed distinctive metabolizing activity even at the level of different Roma groups in Croatia. Our data confirm that isolated populations have a specific position within the global ADME genetic landscape. This also highlights the fact that pharmacogenetics guidelines of the well-defined majority populations cannot be used in pharmaco-therapeutic practice in population isolates and confirms the necessity for defining their specific genetic profile.

Keywords: Roma population, ADME genes, pharmacogenetics, isolation

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SIGNIFICANCE OF CYP2C9 IN PRESCRIBING SIPONIMOD THERAPY IN PATIENTS WITH MULTIPLE SCLEROSIS

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Siponimod is a medication used in the treatment of secondary progressive (SPMS) and relapsing forms of multiple sclerosis (MS). The drug selectively acts on sphingosine-1-phosphate (S1P1), specifically type 1 and 5 receptors, which are involved in immunomodulation and anti-inflammatory processes, and have promyelinating and neuroprotective effects. CYP2C9 is responsible for the metabolism of approximately 20% of clinically important drugs, including siponimod. The CYP2C9 gene is highly polymorphic, which contributes to significant inter-individual variability of therapeutic response. An individual's ability to metabolize CYP2C9 substrates can be classified into the following phenotypes: normal metabolizer (*1/*1 genotype), intermediate metabolizer (*1/*1; *1/*3; and *2/*2 genotype), and poor metabolizer (*2/*3 and *3/*3 genotype). It is important to consider the genetic polymorphisms of CYP2C9 in patients receiving siponimod therapy, due to contraindications in patients carrying CYP2C9 *3/*3 genotype. Patients with other CYP2C9 genotypes may require dose adjustments. The aim of this study was to determine allelic distribution in the population of patients from Bosnia and Herzegovina, as well as to determine the precise dosage of siponimod according to the CYP2C9*2 and CYP2C9*3 genotypes. Material and methods: The study included 57 patients with MS in B&H. The CYP2C9 genotypes were determined by real-time PCR using Taqman Drug Metabolism Genotyping assays. Results: The prevalence of CYP2C9*2 and *3 alleles was 7.02 and 6.14%, respectively. Based on these frequencies, of the 57 participants 15 (26.32%) were predicted to be intermediate metabolizers (IM), and the remaining 42 (73.68%) were normal metabolizers (NM). The IM required a dose adjustment by 50%. Conclusion: All patients requiring siponimod therapy should be genotyped for these polymorphic variants, due to the high possibility of toxicity and side effects if an inappropriate dosage of a drug is administered.

Keywords: siponimod, CYP2C9, multiple sclerosis, polymorphism, pharmacogenetics

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PHARMACOGENOMIC TESTING IN CROATIA: ALLELE AND GENOTYPE FREQUENCIES OF DRUG-METABOLIZING ENZYMES, RECEPTORS, AND OTHER ASSOCIATED PROTEINS

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This retrospective cross-sectional study investigated the allele frequencies of genes encoding enzymes, receptors, transporters, and other drug metabolism constituents of 28 different genes in a Croatian population. Secondarily, the obtained data was compared to publicly available databases on the European population. A retrospective analysis of patient health records was performed, which included 522 patients who underwent pharmacogenetic testing by the RightMed PGx panel to determine the SNPs in the 28 targeted genes. Allele frequency for each gene was determined by dividing the total count of that allele by the total number of alleles in the patient pool for the respective gene. Genotype frequency was determined for each gene by dividing the total count of each unique allele combination by the total number of genotypes in the patient pool for the respective gene. The analysis demonstrated wild-type alleles to be the most frequent for most of the analyzed genes, consequently demonstrating the normal metabolizer phenotype to be the most frequent for utmost of genes. No statistically significant differences were found between the Croatian and European populations for the majority of analyzed genes. Conclusion: The allele frequencies observed in our study can serve as a valuable reference for future studies investigating drug efficacy and safety in the Croatian population. This study contributes to the growing data on genetic diversity that influences drug response in different populations.

Keywords: : pharmacogenomics, genetics, ADR, population genetics, allele frequency

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TISSUE ENGINEERING

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ADVANCEMENTS IN BIO-INK TECHNOLOGY FOR PRECISION DERMATOLOGICAL RECONSTRUCTION IN ACID ATTACK VICTIMS

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This paper introduces a pioneering method for dermatological reconstruction tailored to acid attack victims, merging bio-ink technology with in-vitro tissue engineering and advanced 3D modeling. The process initiates with cultivating healthy skin tissue in vitro to establish a robust cell reservoir, succeeded by digitally replicating burnt area dimensions for customized skin graft fabrication. The bio-ink, comprising hyaluronic acid (HA, $C_{14}H_{21}NO_{11}$) at 2%, collagen ($C_4H_6N_2O_3$) at 3%, fibrinogen ($C_{72}H_{104}N_{18}O_{19}S_2$) at 1%, silver nanoparticles (Ag) at 0.1%, and Transforming Growth Factor-Beta (TGF-β) at 0.05%, is meticulously applied onto the wound bed to foster hydration, structural reinforcement, hemostasis, antimicrobial shielding, and immunomodulation. HA ensures optimal hydration and moisture retention, while collagen provides structural support for tissue remodeling. Fibrinogen promotes clot formation, creating a stable scaffold, and silver nanoparticles confer antimicrobial properties. TGF-β regulates inflammation and promotes tissue regeneration. This specialized bio-ink acts as a scaffold, facilitating cellular infiltration and tissue integration, thereby augmenting wound healing and skin regeneration. Preliminary trials indicate promising success rates surpassing 75% in moderate to severe cases, underscoring the potential of this innovative approach to enhance outcomes and quality of life for acid attack survivors. Through the amalgamation of bio-ink technology, this methodology represents a paradigm shift in dermatological reconstruction, offering a ray of hope and healing to individuals grappling with traumatic skin injuries.

Keywords: acid attack, bioengineering, 3D modelling, bio-ink, tissue

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BIOINFORMATICS AND COMPUTATIONAL APPROACHES IN MICROBIAL BIODEGRADATION

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Increased human activity on energy reserves, dangerous farming methods, and rapid industrialization have all contributed to an increase in environmental contamination over the last few decades. Bioremediation is a convenient solution for environmental issues. Bioremediation is a biological technique for recycling wastes into a form that other species may utilize and reuse. Microorganisms are used in bioremediation to decrease, eliminate, contain, or alter harmful pollutants in soils, sediments, water, and air. The type of bioremediation known as "microbial bioremediation" uses microorganisms and/or their derivatives (enzymes or used biomass) to remove pollutants from the environment. Pollutants are broken down or transformed by microbes using their natural metabolic processes, with or without slight pathway modifications to direct the pollutant into the usual microbial metabolic pathway for breakdown and biotransformation. With a focus on plastic degradation and polyethylane compounds, Ideonella sakaiensis, *Klebseialla pnuemoniae*, and *Pseudomonas* were thoroughly researched using computational and bioinformatic tools. Protein sequences of mentioned bacteria were retrieved from NCBI, and tertiary structures were constructed using the SWISS-MODEL tool. Sequence alignments and comparisons were conducted using BLASTP tool, and the final results showed that enzymes produced by *Ideonella sakainesis* were the most reliable and useful. This is because PETase and MHETase enzymes produced by *I. sakainesis* were thoroughly researched, and identical sequences demonstrated in BLASTP results could be used as possible alternative enzymes for plastic degradation.

Keywords: bioremediation, biodegrading bacteria, *Ideonella sakaiensis, Klebsiella pneumoniae, Pseudomonas*

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MATURATION AND ELECTROPHYSIOLOGICAL PROPERTIES OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES (HIPSC-CMS) ON ELECTROMECHANOACTIVE SCAFFOLD

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Heart-on-a-chip for drug testing seems attractive approach to evaluate the possible drug effects on cardiomyocyte viability and electrophysiology. For the advancement of human induced pluripotent stem cell derived cardiomyocyte (hiPSC-CM) maturation, the need in recapitulation of the native cardiac microenvironment becomes increasingly evident. Our aim was to create a 3D hiPSC-CM culture system with a biocompatible electromechanoactive scaffold, coated with electroconductive layer, suitable for long term culturing and maturation of hiPSC-CMs and compatible with electrophysiology measurement techniques. Elastic scaffold was fabricated by electrospinning of gelatin-glucose (Gel/Glu) microfibers followed by the vapor-phase and electrochemical deposition of conductive polymer polypyrrole (PPy) to create electroconductive layer. Electrophysiological properties of hiPSC-CMs were analysed using intracellular calcium imaging (Cal520) and sharp electrodes. Synchronous calcium oscillations were induced using electrical stimuli. Calcein AM and DAPI staining showed high level attachment of hiPSC-CMs to Gel/Glu /PPy scaffold demonstrating its biocompatibility. The cells remain abundant even during long term cultures (5-7 weeks) and increasingly express maturation markers NKX2- 5, Troponin T, etc. Intracellular calcium imaging and analysis with sharp electrodes showed generation of synchronous spontaneous calcium oscillations by hiPSC-CMs on Gel/Glu/PPy scaffolds, while external electrical stimulation synchronised calcium oscillations. Gel/Glu /PPy scaffold is biocompatible for hiPSC-CMs maturation, and suitable for evaluation of cardiomyocyte electrophysiology properties. Such electromechanoactive scaffold can be further employed for development of heart-on-a- chip model and tissue engineering applications.

Keywords: human iPSC derived cardiomiocytes, electrophysiological properties, electrome-chanoactive scaffold, maturation, electric stimulation

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WHOLE GENOME SEQUENCING FOR IMPLEMENTATION OF PRECISION MEDICINE

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COMPREHENSIVE WHOLE-GENOME, BIOCHEMICAL, AND GLYCAN ANALYSIS IN PATIENTS WITH MORBID OBESITY

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Obesity is a growing health problem worldwide, imposing a significant burden on the health-care system and society in general, particularly morbid obesity. It is a multifactorial disease influenced by genetic and environmental factors. Pathophysiologically, obesity represents a chronic inflammatory state that leads to various metabolic disorders such as diabetes mellitus and hyperlipidemia, as well as conditions like hypertension, cardiovascular diseases, and tumors. This study is structured into two phases. Firstly, a case-control study aims to elucidate the molecular basis of morbid obesity and its potential correlation with genes associated with cardiovascular and metabolic diseases, and hereditary cancer syndromes. Sixteen participants of both genders and aged between 20 and 55 years, with morbid obesity (BMI >40 kg/m2), were enrolled. The control group comprised subjects with a normal BMI range (18.5-24.9 kg/m2). Both groups underwent genomic testing for 1130 genes, including panels for cardiometabolic, nutrigenetic and hereditary cancer-related genes. The second phase involves a prospective interventional study focusing exclusively on the group with morbid obesity. The aim is to observe the impact of weight loss on inflammatory conditions and specific metabolic disorders. Initially, participants underwent clinical assessments, laboratory analyses and plasma N-glycom and N-glycom Ig assessments. Subsequently, participants received consultations regarding nutritional plans and exercise regimens for the next 6 months, during which their progress was closely monitored. N-glycom assessments were repeated after 3 months. Another comprehensive clinical assessment was conducted at the 6-month mark, including laboratory analyses and N-glycom assessments. The final clinical assessment is scheduled for 12 months after the commencement of the study. The collected data are currently undergoing analysis, and preliminary results will be presented at a congress.

Keywords: morbid obesity, whole genome sequencing, glycosylation, metabolic disease

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WHOLE GENOME SEQUENCING: ADVANTAGES AND CHALLENGES OF CLINICAL IMPLEMENTATION

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Whole genome sequencing (WGS) is a revolutionary diagnostic method in clinical genetics that allows for a complete and comprehensive analysis of the human genome. On one hand, WGS generates exceedingly large volumes of data, providing the necessary raw information for the detection of more complex genetic variants. On the other hand, the correct interpretation of this data relies on computational statistical algorithms and predictive machine learning models trained on large datasets of genotype- phenotype correlations. This powerful tool has already begun its integration into many areas of applied medicine, the most prominent being cancer genomics, neonatal screening, pharmacogenomics, and rare disease genomics. Another aspect of WGS utility is in biomedical research, as the method has led to the discovery of many new intronic, structural, and copy number variants. By determining everyone's entire genomic profile, WGS has become one of the main pillars of personalized medicine and multi-omics development. Through the integration of data with other -omics branches, such as epigenomics, transcriptomics, proteomics, metabolomics, and metagenomics, a complete biomolecular profile can be determined for each patient. It should be stated that certain limitations of WGS are presenting challenges in particular clinical cases. These include predictive model imperfections, genetic mosaicism, non- Mendelian genetic disorders, and variability in variant penetrance. Additional options, such as 'trio testing', in which the proband's parents' genetic profiles are also determined, can be explored in these cases to provide a more complete picture. However, DNA sequencing technologies are continuously evolving, currently through the development of third and fourth-generation sequencing. With their increasing implementation into clinical practice, these technologies show great potential in revolutionizing the field of medicine.

Keywords: whole-genome sequencing, genomics, multi-omics, pharmacogenomics, cancer-genomics

SLOVENIAN GENOME PROJECT – A NATIONAL RESOURCE OF POPULATION AND DISEASE-ASSOCIATED GENETIC VARIABILITY IN SLOVENIANS

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Understanding a nation's genetic makeup is crucial for efficient genetic testing and development of national disease screening programs. Recent population sequencing initiatives focused on larger, mostly Anglo-Saxon populations, leaving many populations, including the Slovenians, underrepresented. To address this, we aggregated sequencing data from 8,025 Slovenian individuals, establishing a comprehensive genetic variability database. We combined exome (ES) and genome sequencing (GS) data from 7,566 ES and 459 GS individuals. Variants were called against the hg38 reference genome, following GATK guidelines. Stringent variant quality filters retained accurately genotyped variants in over 90% of ES or GS cohorts, ensuring precise frequency estimations. Variant were annotated via the Variant Effect Predictor, stored in a local SQL database and collected in an on-line genome browser. In the dataset, 26,439,624 distinct variants were identified, mostly (96.3%) in the GS cohort. Notably, 3,070,774 variants (11.6%) were absent in gnomAD 2.1.1, indicating novel human genome variation. Significant variant frequency disparities were observed, with 8.3% variants (MAF>0.01%) showing over two-fold differences compared to global gnomAD populations. For ClinVar variants, 77.0% of pathogenic variants either differed significantly or were absent in gnomAD populations. In conclusion, our study presents a novel resource on Slovenian genetic variability, crucial for understanding this population and the wider European region. Given distinctiveness from commonly used gnomAD populations, our resource is valuable for genetic diagnosis and population screening efforts.

Keywords: genome project, genomics, rare diseases, next-generation sequencing, population genetics

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THE CROSEQ GENOME PROGRAM: THE FIRST LARGE-SCALE PEDIATRIC CROATIAN GENOME ANALYSIS AND AGGREGATE DATABASE

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The genetic diagnosis of Mendelian diseases is often challenging due to their rare nature and complex phenotype. Population-specific variant allele count (AC) and allele frequency (AF) are important parameters for the clinical assessment of variants. Here, we present the development of the CROseq Genome Program in collaboration with the University Hospital Centre Zagreb (UHCZ) and the Brigham and Woman's Hospital (BWH) in the US, funded by the Mila za Sve Foundation, Rijeka. The aim of the CROseq is twofold: 1) analyze complex diseases by the joint genome analysis of the affected children and their unaffected family members to discover disease etiology, and 2) develop the first Croatian Genome Aggregated Database (CGAD) and Croatian Genome-Phenotype Database (CGPD). A total of 754 participants enrolled in the CROseq Genome Program (female=374; male=380; affected=274; unaffected=490). Whole genome sequenced data were analyzed at the BWH using the latest analytical tools. An automated computational infrastructure was developed to transfer the data to Croatia. The aggregate databases were generated using annotated variant parameters including population frequency, in silico scores, zygosity, and inheritance pattern, and a minimum of 20 Human Phenotype Ontology per case. A total of 54.8 million genomic variants have been analyzed. Of those, 16.9×10^6 variants were absent from gnomAD database and exclusively found in the Croatian population. A total of 18.5×10^3 variants absent from gnomAD were frequently observed in the CROseq cohort (AF>0.5). Aggregate databases were generated to enable elastic search by using any unique phenotype or genotype features. CROseq Genome Program is the first collaborative program to address the rare disease and complex genome in the pediatric population in Croatia. The CGAD and CGPD datasets are the first aggregated genome knowledgebases in Croatia. They are an invaluable tool for genetics and population-based studies in Croatia and beyond.

Keywords: CROseq Genome Program, Trio Whole Genome Sequencing, joint analysis, Croatian Genome Aggregated Database (CGAD), Croatian Genome-Phenotype Database (CGPD)

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MIRNA AS A PERSONALIZED MEDICINE TOOL FOR TREATMENT OF KNEE OSTEOARTHRITIS

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Knee osteoarthritis (OA) is a disease characterized by loss of cartilage and inflammation of all tissues in the joint, causing changes in tissue architecture, its metabolism, and function. These changes are mediated by a complex interplay of proinflammatory and anti-inflammatory mediators regulated with miRNA. We explored the miRNA profiles of OA patients who were treated with two types of treatments: with the intra-joint administration of micro-fragmented fat tissue (MFAT) and with hyaluronic acid. We measured the miRNA profiles in plasma, synovial fluid, and MFAT (from the test group) in different time points. The aim was to find miRNAs, predictors of treatment outcomes for the two therapies. miRNA profile was measured with the next-generation sequencing (NGS). NGS QC and absolute quantification of miRNAs were done using spike-ins based on a linear regression model. For the MFAT we didn't find any miRNA that would serve as a good predictor for treatment outcome, even if we stratify the patients by gender. For plasma, we can observe several candidate miRNAs (7 downregulated, 4 upregulated) as predictors for MFAT treatment outcome while no miRNAs were found as a good predictor for HA treatment. For the synovial fluid, no miRNAs were defined as predictors for MFAT treatment, while for HA treatment 13 miRNAs were up regulated and 1 down regulated. These candidate miRNAs need to be validated with other analytical techniques, to check for the possible false positivity. Nevertheless, candidate biomarkers as ones described in our project are of huge interest to orthopedists while knee OA can be treated with different treatments. The personalized approach is needed for the best possible treatment outcome. Conducted as part of the European research project IRI2 (KK.01.2.1.02.0173)

Keywords: knee osteoarthritis, miRNA, marker, treatment outcome, personalized medicin

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CLINICAL AND MOLECULAR ANALYSIS OF BETA-THALASSEMIA MINOR: CASE REPORT OF A PATIENT WITH HETEROZYGOUS HBB VARIANT

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Beta-thalassemias are heterogeneous groups of hereditary anemias characterized by reduced or absent synthesis of the beta-globin chain of hemoglobin (Hb), which is encoded by the HBB gene located on chromosome 11. There are three main forms: beta-thalassemia major (TM), beta-thalassemia intermedia (TI), and thalassemia minor (TMI). The clinical presentation may vary depending on the type of mutation and zygosity. We present the case of a 29-year-old male patient who underwent further laboratory examination due to microcytic anemia with iron and unsaturated iron-binding capacity within the normal range and an enlarged occipital lymph node. Hemoglobin electrophoresis showed the patient's hemoglobin consisted of 71.6% HbA0, 3.5% HbA2, <0.10% HbF, and 25% HbE. HbS was not detected. Finally, whole- genome sequencing revealed a single heterozygous variant (c.79G>A (p.Glu27Lys)) in exon 1 of the HBB gene. This pathogenic variant leads to the synthesis of abnormal HbE, characterized by a change in the amino acid sequence from glutamic acid to lysine. The βE mutation also affects β-globin gene expression by creating an alternate splicing site in the mRNA at codons 25–27. This explains our electrophoresis assessment, confirming the diagnosis of TMI. Although HbE alone does not cause significant clinical problems, its interactions with other thalassemias produce syndromes of varying severity. Despite the lack of a severe clinical presentation, individuals with TMI are carriers of the pathogenic variant, and genetic counseling is recommended for family planning by the patient or biological relatives in whom genetic tests confirm the pathogenic variant.

Keywords: beta-thalassemia, thalassemia minor, HBB, hemoglobin electrophoresis, whole-genome sequencing

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UTILIZING ARTIFICIAL INTELLIGENCE FOR ANALYSIS OF WHOLE GENOME SEQUENCING DATA TO DESIGN PERSONALIZED NUTRITION INTERVENTIONS FOR LIFE QUALITY IMPROVEMENT

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Each individual possesses a distinct genetic composition that profoundly influences various facets of their health and lifestyle, notably impacting nutrient processing mechanisms. The comprehension of these genetic disparities stands as a pivotal element in tailoring nutrition strategies. Leveraging cutting-edge genomics technologies such as whole-genome sequencing (WGS), Nutrigenetics 365 project employs sophisticated algorithms and data analysis methodologies to dissect this genetic landscape. Through meticulous analysis, specific genetic markers dictating nutrient metabolism, dietary inclinations, and the impact of food on health are discerned. These genetic markers may elucidate the efficiency of processing certain vitamins, highlight sensitivities to particular foods (e.g., lactose intolerance), or unveil predispositions to diet-influenced ailments (e.g., heart disease). Armed with this knowledge, nutritionists harness AI-powered platforms to craft bespoke nutrition regimens that harmonize with an individual's genetic blueprint, optimizing nutrient absorption and mitigating the risk of nutrition-related disorders. A crucial component entails the ongoing monitoring of an individual's health and dietary responses, facilitating iterative refinement of the nutrition plan through constant feedback integration into the analytics framework. The overarching objective is to elevate the overall quality of life and health outcomes. Personalized nutrition interventions hold the promise of superior weight management, heightened energy levels, diminished susceptibility to chronic ailments, and holistic wellness. In conclusion, the integration of artificial intelligence and whole-genome sequencing heralds a new era of precision and personalized medicine. By decoding the intricate genetic makeup of individuals, we can tailor nutrition strategies with unprecedented accuracy, optimizing health outcomes and enhancing quality of life.

Keywords: whole-genome sequencing, nutrigenetics, personalized nutrition, artificial intelligence

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RARE LIKELY PATHOGENIC VARIANT ASSOCIATED WITH STARGARDT DISEASE IN THE CROATIAN POPULATION

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Stargardt Disease (STGD) stands as the most prevalent inherited retinal dystrophy, marked by subretinal lipofuscin-like deposition and bilateral vision loss. It is the most common genetic retinal disease, affecting 1 in 10,000 individuals. STGD's phenotypic spectrum spans diverse age-related manifestations, ranging from rapidly progressive cone-rod dystrophy with significant vision loss to late-onset maculopathies with preserved central vision. Autosomal recessive or dominant STGD links to pathogenic variants of ELOVL4, PROM1, BEST1, PRPH2, and ABCA4 genes. A 45-year-old male patient with late-onset maculopathy and preserved central vision came to our hospital for a routine eye check-up. All ophthalmologic diagnostic evaluation for Stargardt disease was made: a personal and family history, visual acuity, slit lamp examination, tonometry, fundus examination, visual field testing, fluorescein angiography, fundus autofluorescence (FAF), electroretinography (ERG) and optical coherence tomography (OCT). A whole- genome sequencing (WGS) was conducted with a focus on coding and non-coding regions of 438 genes related to the most prevalent inherited ocular disorders. The analysis revealed a rare likely pathogenic variant in the coding region of the ABCA4 gene (c.3272G>A (p.Gly1091Glu)). Experimental studies have demonstrated that this missense alteration impacts ABCA4 function. However, further data are requisite to confirm the variant's pathogenicity, hence its classification as likely pathogenic. We highlight the importance of ongoing monitoring by an ophthalmologist for both the proband and all family members carrying the likely pathogenic variant, ensuring comprehensive care and early intervention where necessary. This case underscores the significance of genetic testing in identifying at-risk individuals and guiding targeted interventions, ultimately paving the way for potential prevention of visual impairment among these patients.

Keywords: Stargardt disease, retinal dystrophy, ABCA4, whole genome sequencing, electroretinograph

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