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3rd Congress of Geneticists in Bosnia and Herzegovina with International Participation – CONGUB&H

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The Official Publication of the
Institute for Genetic Engineering and Biotechnology
University of Sarajevo

3RD CONGRESS OF GENETICISTS IN BOSNIA AND HERZEGOVINA WITH INTERNATIONAL PARTICIPATION – CONGUB&H

2ND - 4TH OCTOBER 2023, SARAJEVO, BOSNIA AND HERZEGOVINA

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The abstracts underwent a streamlined submission process, wherein authors retained full responsibility for both the content and the language therein.

Dear colleagues,

With great pleasure and enthusiasm that we extend our warmest welcome to all participants of the Third Congress of Geneticists in Bosnia and Herzegovina with International Participation. This Book of abstracts, as a special issue of the Genetics and Applications, stands as a testament to the remarkable collective effort and intellectual curiosity that brings us together.

In the pages that follow, you will find a diverse and inspiring array of abstracts that represent the cutting-edge research, innovative ideas, and profound insights into various fields and disciplines of genetics. These abstracts reflect the passion and dedication of scholars, researchers, and practitioners who are shaping the present and the future of genetics.

As you delve into the abstracts within these pages, we encourage you to engage, question, and explore. Let this book be your guide through the boundless world of ideas, and may it inspire you to embark on your own journey of inquiry and innovation.

We extend our heartfelt gratitude to all the contributors who have shared their work with us, as well as to the organizing committee, volunteers, and sponsors who have made this event possible. Your dedication and commitment are the driving force behind the success of this abstract book and the Congress in general.

We hope that the knowledge and inspiration you gain from the contents of this book will serve as a catalyst for further research, collaboration, and personal growth. Together, let us continue to push the boundaries of knowledge and make a positive impact on our world.

Thank you for joining us in this celebration of intellectual exploration and discovery. We wish you an enriching and thought-provoking journey through the abstracts.

Warm regards,

Prof. dr. Kasim Bajrović,
Editor-in-Chief

CONGRESS OF GENETICISTS IN BOSNIA AND HERZEGOVINA WITH INTERNATIONAL PARTICIPATION

2nd - 4th October, 2023
Sarajevo, Bosnia and Herzegovina

SCIENTIFIC PROGRAMME

3rd Congress of Geneticists in Bosnia and Herzegovina with International Participation
Sarajevo, Bosnia and Herzegovina, 02nd - 04th October, 2023.

MONDAY 2nd October

12:00 **Registration desk opening and poster mounting**

13:30 **Opening ceremony: Welcome note**
Chairs: **Marko Gerić, Cristiano Vernesi, Irem Deniz**

13:45 – 14:20 **I1 Marko Gerić** *(Institute for Medical Research and Occupational Health, Zagreb, Croatia):* ASSESSING THE GENOTOXICITY EFFECTS OF AIR POLLUTION IN THE HUMAN POPULATION FROM ZAGREB, CROATIA

14:20 – 14:55 **I2 Cristiano Vernesi** *(Research and Innovation Centre - Fondazione Edmund Mach via E. Mach, 1- San Michele all'Adige 38010 (TN), Italy):* THE FOUR-YEARS JOURNEY OF A VERY PECULIAR BIKE, THE G-BIKE

14:55 – 15:10 **O1 Alicia Mastretta-Yanes** *(Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Mexico):* THE STATE OF GENETIC DIVERSITY CONSERVATION ACROSS NINE COUNTRIES FROM ALL CONTINENTS

15:10 – 15:40 **Coffee Break/Poster Session**

15:40 – 16:15 **I3 Irem Deniz** (*Manisa Celal Bayar University, Department of Bioengineering, Faculty of Engineering, Manisa, Turkey):* APPLICATIONS OF RECOMBINANT MICROORGANISMS FOR THE PRODUCTION OF PHAS

16:15 – 16:35 **O2 Lada Lukić Bilela** (*University of Sarajevo, Faculty of Science, Department of Biology, Sarajevo, Bosnia and Herzegovina):* BIOTECHNOLOGICAL POTENTIAL AND CONSERVATION OF MICROBIAL COMMUNITIES IN DINARIC KARST CAVES

16:35 – 16:50 **O3 Kamil Matulka** *(QIAGEN):* PATHOGEN DETECTION USING THE DPCR MICROBIAL DNA DETECTION ASSAYS ON THE QIACUITY

19:30 **WELCOME RECEPTION** (Botanic Garden of National Museum of Bosnia and Herzegovina, Zmaja od Bosne 3)

TUESDAY 3rd October

08:30 **Registration desk opening**

Chairs: **Duan Chen, Amina Kurtović-Kozarić**

09:00 - 9:35 **I1 Duan Chen** *(Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine, Trondheim, Norway):* TRANSLATIONAL RESEARCH ON GASTRIC CANCER: BIOMARKERS AND DRUG REPURPOSING

09:35 – 10:10 **I2 Chun-Mei Zhao** *(Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine, Trondheim, Norway*): PRECLINICAL DEVELOPMENT OF TREATMENT FOR PANCREATIC CANCER: COMPARATIVE PROTEOMICS AND TARGETING EXOCYTOSIS/ENDOCYTOSIS COUPLING

10:10 – 10:45 **I3 Zoran Galić** *(University of California, Los Angeles, USA):* POTENTIAL OF HUMAN PLURIPOTENT STEM CELLS FOR THERAPEUTIC APPLICATIONS

10:45 -11:15 **Coffee Break/Poster Session**

Chairs: **Chun-Mei Zhao, Lejla Pojskić**

11:15 – 11:30 **O1 Jadranka Vraneković** *(University of Rijeka, Faculty of Medicine, Department of Medical Biology and Genetics, Rijeka, Croatia):* RARE RECIPROCAL TRANSLOCATION IN A PATIENT WITH ASTHENOZOOSPERMIA: CASE REPORT

11:30 – 11:45 **O2 Amina Kurtović-Kozarić** *(Genomenon, Inc. Ann Arbor, MI, USA):* THE ROLE OF GENETICS IN THE DISCOVERY OF CANCER TREATMENTS

11:45 – 12:00 **O3 Saša Likić** *(Biosistemi Grupa d.o.o., Zagreb, Croatia):* A VIEW FROM THE OUTSIDE: FLUORESCENT IMAGING AND FLOW CYTOMETRY IN VARIOUS RESEARCH AREAS

12:15 – 14:00 **Lunch Break**

Chairs: **Zoran Galić, Jadranka Vraneković**

14:00 – 14:15 **O4 Jeroen Adema** (*Illumina*): AI FOR GENETIC VARIANT INTERPRETATION TO ADDRESS INTERPRETATION BOTTLENECK

14:15 – 14:30 **O5 Maida Hadžić Omanović** (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina):* CELL DEATH INDUCTION IN CANCER TREATMENT, WHAT WE SHOULD EXPECT? A STUDY ON UT7 LEUKEMIA

14:30 – 14:45 **O6 Dženan Kovačić** (*International Burch University, Faculty of Engineering and Natural Sciences, Department of Genetics and Bioengineering, Sarajevo, Bosnia and Herzegovina):* COMPUTATIONAL DESIGN AND VALIDATION OF MIRNA THERAPEUTIC CANDIDATES FOR PULMONARY TUBERCULOSIS: A NEW THERAPEUTIC PARADIGM FOCUSING ON POTENTIATION OF GRANULOMA STABILITY AND STERILIZATION

14:45 – 15:20 **I4 Steven J. Jonas** (*University of California, Los Angeles, David Geffen School of Medicine, Department of Pediatrics, Los Angeles California, USA*): CHEMICALLY MODIFIED FILTROPORATION DEVICES ENABLE CRISPR/CAS9-MEDIATED GENE KNOCKOUT IN HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS

15:20 – 15:45 **O7 Jerome A. Zack** (*University of California Los Angeles, Los Angeles California, USA*): TARGETING THE HIV RESERVOIR

19:30 **CONGRESS DINNER** (Apetit Gastro-Wine Bar, Zmaja od Bosne 13)

WEDNESDAY 4th of October

08:30 **Registration desk opening**

Chairs: **Erna Karalija, Lada Lukić Bilela**

09:00 – 9:35 **I1 Erna Karalija** *(University of Sarajevo, Faculty of Science, Department for biology, Sarajevo, Bosnia and Herzegovina):* PREPARE TO BATTLE: STRESS MEMORY IN PLANTS

09:35 – 9:50 **O1 Veronika Lončar** *(Faculty of Science University of Zagreb, Zagreb, Croatia):* HIGH GENETIC DIVERSITY IN COMMON TERNS FROM EUROPE AS INFERRED FROM MITOCHONDRIAL DNA

9:50 – 10:30 **PITCH PRESENTATIONS**

P1 Amar Silajdžić (International University of Sarajevo, Sarajevo, Bosnia and Herzegovina): UNVEILING AUTOPHAGY'S ROLE: INTERMITTENT FASTING AS A POTENTIAL CANCER CURE – A COMPREHENSIVE REVIEW

P2 Tea Bećirević (*Atrijum Polyclinic, Sarajevo, Bosnia and Herzegovina):* PRESENCE OF HLA-DQ2 AND HLA-DQ8 /DR4 CELIAC DISEASE PREDISPOSING ALLELES IN TESTED GROUP OF PATIENTS IN BOSNIA AND HERZEGOVINA

P3 Jesenka Kazazović (*Genomenon, Inc., Ann Arbor, Michigan, United States*): CHALLENGES IN VARIANT CURATION IN PROSTATE CANCER

P4 Azra Suljić (*Genomenon, Inc., Ann Arbor, Michigan, United States*): SOMATIC VARIANTS AND PRECISION THERAPY IN COLORECTAL CANCER

10:30 – 11:00 **Coffee Break/Poster Session**

Chairs: **Damir Marjanović, Jasmina Čakar**

11:00 – 11:20 **O1 Kriti Pathak** (*Promega GmbH, Walldorf, Germany):* FROM EXTRACTION TO ANALYSIS: PROMEGA OPTIMIZES YOUR GENOMICS WORKFLOW

11:20 – 11:35 **O2 Amira Kekić** (*Ministry of Internal Affairs of Canton Sarajevo, Sarajevo):* THE SIGNIFICANCE OF ANALYZING TOUCH DNA COLLECTED AT THE CRIME SCENES IN CANTON SARAJEVO

11:35 – 11:50 **O3 Mirela Džehverović** (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology Sarajevo, Bosnia and Herzegovina):* Y-STR ANALYSIS: ARE WE DIFFERENT FROM OUR MEDIEVAL ANCESTORS?

11:50 – 12:00 **O4 Jasmina Čakar** (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology Sarajevo, Bosnia and Herzegovina)*: SNEAK PEEK AT 3D FACIAL RECONSTRUCTION OF DIVICANI MAN

12:00 – 12:20 **O4 Damir Marjanović** (*Institute for Anthropological Research, Zagreb, Croatia):* DNA ANALYSIS OF HUMAN SKELETAL REMAINS: AN OVERVIEW OF WHAT HAS BEEN ACHIEVED OVER THE LAST THREE DECADES

12:20 – 12:30 **Announcement of the award at the end of the Session (best poster and oral presentation)**

12:30 **Closing of the Congress**

POSTERS

Environmental Genetics and Ecotoxicology

MERIMA KLJALJIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States):* REGULATORY MECHANISMS OF HEPATIC METABOLISM DURING PHYSICAL EXERCISE: A COMPREHENSIVE REVIEW OF SCIENTIFIC LITERATURE

DAMIR ĐERMIĆ (*Ruđer Bošković Institute Division of Molecular Biology, Zagreb, Croatia*): REVERSE TRANSCRIPTION-QUANTITATIVE PCR (RT-qPCR) WITHOUT THE NEED FOR REMOVAL OF TEMPLATE DNA

ANESA AHATOVIĆ HAJRO (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): INDOLE-3-ACETIC ACID (IAA) PRODUCTION IN METAL-TOLERANT BACTERIAL ISOLATES FROM THE SERPENTINE OUTCROPS OF EASTERN BOSNIA

MUJO HASANOVIĆ (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): PHOSPHATE SOLUBILIZATION BACTERIA MAY REDUCE DNA DAMAGE IN *PISUM SATIVUM* L. CULTIVATED IN PHOSPHORUS DEFICIENT SOIL

AZRA SULJIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States*): CADMIUM UPTAKE INTO ST. JOHN'S WORT AND ITS PHARMACEUTICAL PREPARATIONS

DŽANA KUNA (*University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*): EXAMINATION OF THE GENOTOXIC AND CYTOTOXIC EFFECTS OF ALUMINUM SALTS

NORA MARKANOVIĆ (*University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*): COMET ASSAY IN ADHERENT CELL LINES - OPTIMISATION OF TRYPSIN TREATMENT

PUTRI KUSUMA ASTUTI (*Centre for Agricultural Genomics and Biotechnology, University of Debrecen, 4032 Hungary*): GENETICS OF COAT COLORS AND ITS ROLE IN CLIMATE CHANGE RESILIENCY IN SHEEP

PUTRI KUSUMA ASTUTI (*Centre for Agricultural Genomics and Biotechnology, University of Debrecen, 4032 Hungary*): MANAGEMENT MANNERS AND ORGANIZATIONS SUPPORTING THE UTILIZATION OF SCIENTIFIC RESULTS OF GENETICS IN PRACTICE – OPPORTUNITIES AND CHALLENGES IN EUROPE AND INDONESIA

MIRZETA MEMIŠEVIĆ HODŽIĆ (*University of Sarajevo, Faculty of Forestry, Sarajevo, Bosnia and Herzegovina*): ANALYSIS OF QUALITATIVE INDICATORS OF SCOTS PINE AND AUSTRIAN PINE SEED STANDS AS IMPORTANT FOREST GENETIC RESOURCES IN FEDERATION OF BOSNIA AND HERZEGOVINA

MERIMA MIRALEM (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): THE FIRST RECORD OF SUBSPECIES *RHYACOPHILA FASCIATA DELICI* IN THE PROTECTED AREAS OF CANTON SARAJEVO

LEJLA UŠANOVIĆ (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): DETECTION OF THE CAUSATIVE AGENTS OF LYME BORRELIOSIS IN BOSNIA AND HERZEGOVINA: SEROLOGICAL AND MOLECULAR GENETIC RESEARCH

LADA LUKIĆ BILELA (*University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*): CALCITE MOONMILK DEPOSITS: MORPHOLOGY AND ENVIRONMENT OF FORMATION IN KARST CAVES OF THE CENTRAL DINARIDES IN BOSNIA AND HERZEGOVINA

AMAR SILAJDŽIĆ (*International Burch University, Sarajevo, Bosnia and Herzegovina*): UNVEILING THE POTENT ANTIMICROBIAL POTENTIAL: EXPLORING THE GENETIC BASIS OF CLOVE AND CINNAMON OIL'S IMPACT ON METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*, *STAPHYLOCOCCUS AUREUS*, AND *ESCHERICHIA COLI*

ALMA HAJRUDINOVIĆ-BOGUNIĆ (*University of Sarajevo, Faculty of Forestry, Sarajevo, Bosnia and Herzegovina*): THE MULTISPECIES *SORBUS COMMUNITIES* – HYBRID ZONES OF HIGH CONSERVATION VALUE

AHMED PUPALOVIĆ (*International Burch University, Sarajevo, Bosnia and Herzegovina*): INTEGRATING MACHINE LEARNING AND CLINICAL EXAMINATION FOR ACCURATE DIAGNOSIS PREDICTION OF CONGENITAL HYPOGONADOTROPIC HYPOGONADISM

ABDURAHIM KALAJDŽIĆ (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): *IN SILICO* ANALYSIS REVEALS POTENTIAL INTERACTIONS BETWEEN MARBURG VP40 PROTEIN AND ENDEMIC PLANT SPECIES OF BOSNIA AND HERZEGOVINA

TARIK ČORBO (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo Bosnia and Herzegovina*): *IN SILICO* INTERACTION OF PHYTOCHEMICAL COMPONENTS EXTRACTED FROM *KNAUTIA SARAJEVENSIS* AGAINST TWO-PART NS2B-NS3 ZIKV PROTEASE

TARIK ČORBO (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): INVESTIGATION OF ANTIVIRAL POTENCIES OF PHENOLIC COMPOUNDS AGAINST NS2B-NS3 ZIKV PROTEASE: THE CASE OF *VERBASCUM GLABRATUM SUBSP*. *BOSNENSE*

Human and Medical Genetics

ALEKSANDRA MARKOVIĆ (*University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Kragujevac, Serbia*): GENOTOXIC AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACTS FROM AERIAL PARTS OF *ONOBRYCHIS VICIIFOLIA* PLANT FROM DIFFERENT LOCALITIES

JOVAN KULIĆ (*Faculty of Medicine Foča, University of East Sarajevo, Foča, Bosnia and Herzegovina*): ASSOCIATION BETWEEN INSERTION/DELETION POLYMORPHISM OF THE *ACE* GENE WITH RISK OF HYPERTENSION

LEJLA DELALIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States*): FROM GENOTYPE TO PHENOTYPE: UNDERSTANDING THE ROLE OF THE *ASPA* GENE IN CANAVAN DISEASE

LEJLA DELALIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States*): GENERATIONAL AND SEASONAL PATTERNS IN MENARCHE TIMING: AN IN-DEPTH ANALYSIS FROM THE GRADACAC REGION

MERIMA KLJALJIĆ (Genomenon, Inc., Ann Arbor, Michigan, United States): PHENOTYPIC HETEROGENEITY IN TERMINAL OSSEOUS DYSPLASIA: ELUCIDATING THE ROLE OF TWO *FLNA* VARIANTS

NADA STARČEVIĆ ČIZMAREVIĆ (*University of Rijeka, Faculty of Medicine, Department of Medical Biology and Genetics, Rijeka, Croatia*): POLYMORPHISMS OF 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE GENE IN MULTIPLE SCLEROSIS

ŠAĆIRA MANDAL (*University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina)*: G-PROTEIN COUPLED RECEPTORS AS POTENTIAL DRUG TARGET IN THERAPY AND TREATMENT OF TYPE 2 DIABETES

EMINA TODOROVAC (*Faculty of Pharmacy and Health Sciences, University of Travnik, Bosnia and Herzegovina*): DYNAMICS OF *ORF1AB* AND *E* GENE IN COVID-19 POSITIVE PATIENTS FROM THE TRAVNIK REGION

SELMA MURATOVIĆ (Genomenon, Inc., Ann Arbor, Michigan, United States): THE POWER OF GENETIC RESEARCH: UNDERSTANDING THE ROLE OF *RAB23* GENE IN CARPENTER SYNDROME

AHMED PUPALOVIĆ (*International Burch University, Sarajevo, Bosnia and Herzegovina*) APOLIPOPROTEIN E ENCODING GENE MUTATIONS AND WARFARIN REQUIREMENTS: A REVIEW OF IN VIVO STUDIES

IVONA KENJIĆ (*University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*) ASSOCIATION OF THE *TAS2R38* GENE POLYMORPHISM WITH THE SENSITIVITY OF GUSTATORY AND OLFACTORY FUNCTION TO SARS-CoV2 INFECTION

AHMED PUPALOVIĆ (*International Burch University, Sarajevo, Bosnia and Herzegovina*): SYSTEMATIC REVIEW OF GENETIC CAUSE IN DUCHENNE MUSCULAR DYSTROPHY

AHMED PUPALOVIĆ (*International Burch University, Sarajevo, Bosnia and Herzegovina*): REVIEW OF UNDERLYING GENETIC CAUSES OF CONGENITAL HYPOGONADOTROPIC HYPOGONADISM

TEA BEĆIREVIĆ (*Atrijum Polyclinic, Sarajevo, Bosnia and Herzegovina*): PRESENCE OF *HLA-DQ2* AND *HLA-DQ8 /DR4* CELIAC DISEASE PREDISPOSING ALLELES IN TESTED GROUP OF PATIENTS IN BOSNIA AND HERZEGOVINA

LEJLA LASIĆ (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): EXPLORING APOPTOTIC EFFECTS OF STENOENDEMIC ACINOS ORONTIUS PLANT EXTRACTS IN GR-M MELANOMA CELLS: INITIAL FINDINGS

AZRA SULJIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States*): ENHANCING BREAST CANCER CARE: THE VITAL ROLE OF GENETIC ROFILING VIA NGS

AZRA SULJIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States*): SOMATIC VARIANTS AND PRECISION THERAPY IN COLORECTAL CANCER

ŽANA RADIĆ SAVIĆ (*University of Banja Luka, Faculty of Medicine, Department of Medical Biochemistry, Bosnia and Herzegovina*): ASSOCIATION OF MDR1 RS1045642 POLYMORPHISM WITH SUSCEPTIBILITY TO BALKAN ENDEMIC NEPHROPATHY

FEHIM LIČINA (*Association of the family Licina, Podgorica, Montenegro*): GENETIC VARIATION IN RELATION TO DEMOGRAPHY OF MALE POPULATION IN BOSNIA AND HERZEGOVINA

NEVEN MESELDŽIĆ (*University of Sarajevo, Faculty of Pharmacy, Department of Pharmaceutical Biochemistry and Laboratory diagnostics, Bosnia and Herzegovina*): THE IMPACT OF *ACE2* GENE POLYMORPHISM (RS2285666) ON CREATININE AND CREATINE KINASE LEVELS IN COVID-19 PATIENTS AND ITS RELATIONSHIP WITH DISEASE SEVERITY

AMINA KURTOVIĆ-KOZARIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States*): THERAPEUTICAL TARGETS IN BREAST CANCER: *ESR1* AND *BRCA1* MUTATIONS

AMINA KURTOVIĆ-KOZARIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States*): GENOMIC LANDSCAPE OF FAMILIAL MYELOID PREDISPOSITION SYNDROMES

AMINA KURTOVIĆ-KOZARIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States*): CHALLENGES IN VARIANT CURATION OF RARE DISEASES

JESENKA KAZAZOVIĆ (*Genomenon, Inc., Ann Arbor, Michigan, United States*): CHALLENGES IN VARIANT CURATION IN PROSTATE CANCER

AMAR SILAJDŽIĆ (*International Burch University, Sarajevo, Bosnia and Herzegovina*): UNVEILING AUTOPHAGY'S ROLE: INTERMITTENT FASTING AS A POTENTIAL CANCER CURE – A COMPREHENSIVE REVIEW

SANIN HAVERIĆ (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): SPONTANEOUS CHROMOSOMAL ABERRATIONS IN HUMAN LYMPHOCYTE CULTURES

Human Forensic and Population Genetics

ESMA FOČAK (*University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*): AN ASSESSMENT OF THE EFFICIENCY OF AUTOMATED DNA EXTRACTION ON RECENT AND ARCHAEOLOGICAL SKELETAL REMAINS

BELMA JUSIĆ (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): DETERMINING THE DEGREE OF KINSHIP AMONG CLOSE RELATIVES: OUR EXPERIENCE

NELLY KICHAMU (*Centre of Agricultural Genomics and Biotechnology, University of Debrecen, 4032 Debrecen, Egyetem tér 1, Hungary*): ESTIMATING RELATEDNESS AND INBREEDING OF SMALL EAST AFRICAN GOATS USING PEDIGREE

FILIP MAKSIMOVIĆ (*Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Republic of Serbia*): GENETIC DIVERSITY OF THE *QUERCUS ROBUR* L. POPULATION FROM THE PROTECTED AREA „KOŠUTNJAK FOREST" (BELGRADE, SERBIA) ASSESSED BY NUCLEAR MICROSATELLITES

FUAD GAŠI (*University of Sarajevo, Faculty of Agriculture and Food Sciences, Sarajevo, Bosnia and Herzegovina*): DOES THE GEOGRAPHIC DISTANCE EFFECT THE GENETIC DIFFERENTATION AMONG BILBERRY POPULATIONS SAMPLED IN BOSNIA AND HERZEGOVINA?

AMINA AGIĆ (*University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*): DETECTION OF EVOLUTIONARY LINEAGES OF SALMONID SAMPLES FROM THE ZETA RIVER USING MOLECULAR GENETIC AND BIOINFORMATICS ANALYSES

MERIMA MIRALEM (*University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*): GENETIC DIFFERENTIATION BETWEEN TWO AUTOCHTHONOUS BOSNIAN DOG BREEDS

INVITED PRESENTATIONS

INVITED PRESENTATION

TRANSLATIONAL RESEARCH ON GASTRIC CANCER: BIOMARKERS AND DRUG REPURPOSING

Chen Duan

Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Norway

Gastric cancer is the 5th most common malignant disease worldwide with the 4th leading cause of cancer-related mortality. Gastric intestinal metaplasia (GIM) is a precancerous lesion for gastric adenocarcinoma (GA) which comprises 95% of the total numbers of malignancies in stomach. By multi-approach bioinformatics analysis, we have identified biomarkers (e.g., WNT/β-catenin, HIPPO/YAP, nerve growth factor, SNAP25, mTOR, and PDP1/α-KGDH), gene expression signature, metabolic signature, and biomarker networks that were associated with the transition from GIM to GA as potential targets for prevention of GIM to GA transition and for treatment of GA. Further studies in vitro, in vivo, in silico and in patients have shown the following results. Vagal innervation contributed to GA tumorigenesis via acetylcholine (ACh) muscarinic receptor-3 receptor (M3R)-mediated WNT/β-catenin signaling in the stem cells and vagotomy, botulinum toxin A injection, or M3R blockade inhibited GA tumorigenesis, suggesting that denervation might represent a feasible strategy for the treatment of GA. Blockade of nerve growth factor signaling inhibited GA tumorigenesis in M3R-dependent manner through suppression of YAP function, suggesting that the feedforward ACh-NGF axis stimulates GA tumorigenesis and might offer a compelling target for GA treatment and prevention. Inhibition of nerve-cancer metabolism by injection of botulinum toxin-A (SNAP25 inhibitor) with systemic administration of RAD001 (mTOR pathway inhibitor) and CPI-613 (PDP1/α-KGDH inhibitor) reversed the metabolic reprogramming and increased overall survival in GA mice, pointing to the importance of neural signaling in modulating GA tumor metabolism and providing a rational basis for clinical translation in the future. Ivermectin inhibited GA through WNT/β-catenin signaling pathway, cell proliferation pathway and cell death signaling pathway, suggesting a potential repurposing drug for GA prevention and treatment. Taken together, these studies have made contribution to bridge the knowledge gaps between “bedside” and “bench-side” in translational research on gastric cancer.

Keywords: bioinformatics; drug repurposing; gastric cancer; translational research

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INVITED PRESENTATION

PRECLINICAL DEVELOPMENT OF TREATMENT FOR PANCREATIC CANCER: COMPARATIVE PROTEOMICS AND TARGETING EXOCYTOSIS/ENDOCYTOSIS COUPLING

Zhao Chun-Mei

Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Norway

Global incidence and mortality of pancreatic cancer, comprising mostly pancreatic ductal adenocarcinoma (PDAC), is predicted to rise by almost 80% by 2040. Despite considerable investment in research, there has been no significant improvement in the mortality figures of PDAC for both men and women in Europe during the past 3 decades. One of the challenges in development of effective therapy for PDAC is the mismatch between research models and patients. The aim of this study was to utilize comparative proteomics, protein-protein interactions (PPI) and prediction of drug-target interactions (DTI) with multiple research models and patients for preclinical development of treatment for PDAC. PDAC cells, spheroids, organoids and tumors of mouse models and patients were included. Mass spectrometry-based proteomics and bioinformatics were used. The comparative proteomics showed translational potential of preclinical models and potential targets including central hub proteins, neuronal signaling, soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins, and particularly plasma membranes in association with exocytosis/endocytosis-coupled extracellular vesicles (EVs). PPI and DTI indicated potential drug repurposing with Botulinum toxin-A and ivermectin for PDAC. Furthermore, validations using in vitro and in vivo experiments showed that treatments with Botulinum toxin-A and/or ivermectin suppressed the plasma membrane plasticity and increased crinophagy of PDAC cells, and that Botulinum toxin-A inhibited the SNARE protein complex signaling pathway, whereas ivermectin eliminated the formation of membrane microvilli and inhibited cell proliferation, and clathrin-mediated endocytic signaling. Botulinum toxin-A/ivermectin treatment inhibited the formation and release of EVs and induced down-regulations of EV-involved signaling pathways, such as axonal guidance signaling, clathrin-medicated endocytosis signaling, neuroinflammation signaling pathway, epithelial adherents junction signaling, EIF2 signaling, and integrin signaling, and toxicities in neuro-immune, kidney, liver, and heart. Treatments with Botulinum toxin-A and ivermectin either alone or in combination enhanced the inhibitory effects of chemotherapy on the tumors and increased median survivals of PDAC tumor-bearing mice. In conclusion, arresting exocytosis/endocytosis coupling with Botulinum toxin-A and/or ivermectin increased the survival rate in PDAC mice, probably by inhibiting the formation/release of EVs and thereby suppressing the tumorigenesis/progression as well as the systemic effects of PDAC.

Keywords: bioinformatics; drug repurposing; pancreatic cancer; translational research

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INVITED PRESENTATION

ASSESSING THE GENOTOXICITY EFFECTS OF AIR POLLUTION IN THE HUMAN POPULATION FROM ZAGREB, CROATIA

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More than six million premature deaths annually can be attributed to air pollution. Air pollution is ranked as a class 1 carcinogen and a driver of lung cancer. Typical air pollutants are particulate matter (PM) ranging up to 10 µm aerodynamic diameter, polyaromatic hydrocarbons (PAHs), as well as some gases, and aerosols. Possible genotoxicity in the human population from exposures to these carcinogens can be measured in blood with the comet assay and micronucleus test. We aimed to assess the genotoxicity effects of air pollution retrospectively using historical data and prospectively by recruiting volunteers in a human biomonitoring study. Our study (N>120) showed that four air pollution clusters did not affect the cancer-predictive biomarkers of genomic damage In a prospective part of the study, we recruited the volunteers (N=60) and collected the samples twice; once in the cold season and a second time during the warm season. The air pollution data were in agreement with the regulatory limits, except for some occasional measurements collected in the colder period of the year where PM10 particles and benzo[a]pyrene (B[a]P), a known PAH carcinogen, exceeded the regulatory limit value. The genotoxicity effects were, however, more pronounced during the warmer period of the year. This might reflect higher exposure to UV radiation – a known genotoxic physical agent. Given the transboundary nature of air pollution, further research is warranted to gain a better insight into air pollution-driven health effects.

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Keywords: air pollution; genotoxicity; human biomonitoring; machine learning

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INVITED PRESENTATION

PREPARE TO BATTLE: STRESS MEMORY IN PLANTS

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Stress memory in plants is one of the important tools that ensure plants survival in changing environment. Crating progeny with memorized response to upcoming stress in parental environment ensures better adaptability of the progeny as well as their survival in stressed environment thanks to the signals perceived by parents. How do plants memorize stress and give the memory to the seed? The complexity of plants memory involves many pathways, some regulated by epigenetic changes of parental genetic material, some by accumulation of hormones, metabolites and osmolytes. In recent years attempts are being made to utilize this plants stress memory in creation of stress tolerant crops by priming meaning that plants experience short stress triggering defense related responses and encapsulating plants response as “primed memory”. In majority of plants with stress memory antioxidant systems are enhanced and ready to fight oxidative stress regardless of the source of the stress (heavy metals, salinity, drought), but additionally some specific pathways can be activated such as activation of specific osmolyte synthesis. Stress priming has its downside as well, and a phenomenon called bet hedge has been recorded in progeny of stressed parents, where development of plants form seeds with stress memory is inhibited under optimal conditions. Adding to the complexity is also the cancellation of the memory in plants that do not experience stress, meaning that the memory is erased, and transgenerational memory of stress can be impaired if the progeny is not exposed to stress.

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INVITED PRESENTATION

APPLICATIONS OF RECOMBINANT MICROORGANISMS FOR THE PRODUCTION OF PHAS

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The pursuit of sustainable alternatives to conventional plastics has led to significant advancements in the field of bioplastics, especially polyhydroxyalkanoates (PHAs) emerging as a promising contender. Harnessing the power of recombinant microorganisms, researchers have unlocked innovative pathways for PHA production that hold immense potential for reducing environmental impact. Through genetic engineering techniques, microbial resources have been tailored to efficiently synthesize PHAs from renewable carbon sources. This approach not only offers a greener alternative to petroleum-based plastics but also opens avenues for customized polymer properties. Recombinant microorganisms, such as Escherichia coli and Pseudomonas putida, have been genetically modified to enhance their PHA accumulation capabilities. By introducing or enhancing key enzymes involved in PHA biosynthesis pathways, these microorganisms can efficiently channel carbon substrates into PHA production, resulting in higher yields. Moreover, precision engineering has allowed for the creation of microorganisms capable of producing specific PHA types with engineered characteristics, broadening the applications of these bioplastics across various industries. As research continues to refine recombinant strategies, challenges related to cost-effectiveness, regulatory compliance, and large-scale production are being addressed. By harnessing the versatility and adaptability of recombinant microorganisms, the potential for PHAs to revolutionize the plastics landscape while contributing to a more sustainable future remains a promising avenue of exploration.

Keywords: Bioplastics; PHAs; recombinant technology; *E. coli*

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INVITED PRESENTATION

THE FOUR-YEARS JOURNEY OF A VERY PECULIAR BIKE, THE G-BIKE

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The lack of recognition of genetic diversity in the main European and international major policies addressing biodiversity has long characterised the conservation biology arena. Thanks to funding from the EU COST Association an European network called G-BiKE -Genomic Biodiversity Knowledge for Resilient Ecosystems- has been established in 2019. With 39 European countries and more than 110 researchers and practitioners, G-BiKE's main endeavour has been to put the genetic dimension of biodiversity at the forefront of the policy agenda. Through a well balanced combination of scientific articles, workshops, training schools, webinars and policy briefs this path has led G-BiKE to attend the last Conferences of the Parties (COP-15) to the Convention on Biological Diversity where a new agreement has been ratified: the Kunming-Montreal Global Biodiversity Framework.

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INVITED PRESENTATION

CHEMICALLY MODIFIED FILTROPORATION DEVICES ENABLE CRISPR/CAS9-MEDIATED GENE KNOCKOUT IN HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Cost effective, high-throughput, non-toxic, efficient, and cargo agnostic intracellular delivery technologies are needed to manufacture gene and cell-based therapeutics more broadly as well as democratize gene manipulation for research applications. Existing technologies capable of delivering large gene editing cargoes, such as CRISPR/Cas9 ribonucleoproteins (RNPs) or base/prime editor constructs, require specialized equipment and reagents that may not be available in resource limited areas and are often limited by high costs and/or cytotoxicity. To address these challenges, we have developed an intracellular delivery approach based on filtroporation that can be assembled from materials commonly available in most research laboratories. Our filtroporation devices permeabilize cells by pulling them through the pores of a track etched cell culture insert by application of vacuum available in biosafety cabinets. In a format that costs <$10 in materials per experiment, we demonstrate delivery of fluorescently labeled dextran, expression plasmids, and Cas9 RNPs for CRISPR/Cas9-mediated gene knockout

to Jurkat cells and human $CD34^+$ hematopoietic stem and progenitor cell (HSPC) populations with delivery efficiencies of up to 40% for RNP knockout and viabilities >80%. Chemically coating the filters with a fluorinated silane further enhances delivery efficiency. These devices are capable of processing 500,000 to 4 million cells per experiment, and when combined with a three-dimensional printed vacuum application chamber, this throughput can be straightforwardly increased 6 to 12-fold in parallel experiments. The capabilities of this platform provide a simple solution for making intracellular delivery methods for researchers and clinicians in low resource areas of the world more accessible, opening opportunities to engage new communities of scientists in gene and cell therapy research.

Keywords: filtroporation, intracellular delivery, gene therapy

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INVITED PRESENTATION

THE CURRENT STATE OF HUMAN PLURIPOTENT STEM CELL-BASED THERAPIES

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Human pluripotent stem cells, namely human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) have a theoretical potential to differentiate into any type of human cells, they can be maintained *in vitro* for extended periods of time and can easily be genetically manipulated and expanded to clinically relevant cell numbers. As such, they can potentially serve as off-the-shelf sources of normal or genetically modified cells for tissue regenerative and other medical applications, including immune therapies. This presentation will provide an overview of the current state of pluripotent stem cells-based therapies, with the particular focus on pluripotent stem cell-derived immune cells. Our group and others have established that T cells with increased affinity for cancer epitopes can be derived from genetically modified pluripotent stem cells. Such T cells phenotypically and functionally fully resemble normal T cells and kill haplotype matched target cells in a dose dependent manner *in vitro* and *in vivo*. Practical considerations suggest that the pluripotent derived stem cell-derived T cells can therapeutically be superior to genetically modified T cells taken from patients' blood, as they can be expanded to significantly greater numbers sufficient for treatment of a large number of patients and properly analyzed for genotoxic effects of vector integration through DNA sequencing approaches. These types of cell therapies hold promise for the treatment of a broad array of conditions, including cancer, HIV infection, and immune insufficiency disorders.

Keywords: Pluripotent stem cells, hESC, iPSC

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ORAL PRESENTATIONS

Environmental Genetics and Ecotoxicology *Oral presentation*

COMPUTATIONAL DESIGN AND VALIDATION OF MIRNA THERAPEUTIC CANDIDATES FOR PULMONARY TUBERCULOSIS: A NEW THERAPEUTIC PARADIGM FOCUSING ON POTENTIATION OF GRANULOMA STABILITY AND STERILIZATION

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Tuberculosis (TB), caused by Mycobacterium tuberculosis (M.tb), remains a global concern, with latent TB affecting a third of the world. In 2019, 4.3 million active TB cases were reported, 25% co-infected with HIV. Challenges for TB control include diagnostic limitations in resource-scarce regions and rising drug-resistance. TB's progression is marked by granulomas, which represent the immune response to M.tb. These structures evolve from acute to fibrotic stages and are defined by a Th1-dominant immune response. Notable molecular markers, such *as IFN-γ* and *TNF-α*, denote effective immune responses. However, M.tb persists within these granulomas, making understanding their genetic dynamics crucial. Our study used advanced computational techniques to explore gene expression signatures vital for granuloma stability. Concurrently, markers indicating granuloma deterioration, and signaling disease exacerbation, were identified as potential intervention targets. With the growing interest in RNA-based therapeutics, especially microRNAs (miRNAs), we investigated their role in modulating granulomatous immunopathology. Using extensive patient datasets, we identified differential gene expression profiles during stable granuloma phases compared to erosive stages or TB relapses. This further involved analyzing mRNA-miRNA dynamics using sophisticated algorithms. We also constructed detailed miRNA interaction networks, complemented by pathway enrichment analysis to discern a biological context of miRNA interactions. Using a hybrid object-oriented algorithm, we simulated the effects of identified miRNA candidates on virtual granulomas with varying stability and bacterial content. Results indicated improved granuloma sterilization and structure upon suppressing genes negatively affecting granuloma integrity. We further formulated custom lipid nanoparticle systems for candidate miRNA delivery. In conclusion, our computationally derived insights offer a groundbreaking miRNA-focused therapeutic pathway for TB. We are now transitioning to in vitro and in vivo testing to confirm these findings and potentially reshape TB treatments.

Keywords: tuberculosis; tb granuloma; miRNA; miRNA therapy; latent TB

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Environmental Genetics and Ecotoxicology *Oral presentation*

HIGH GENETIC DIVERSITY IN COMMON TERNS FROM EUROPE AS INFERRED FROM MITOCHONDRIAL DNA

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The common tern (*Sterna hirundo*) is a migratory seabird from the family Laridae (gulls). It breeds in the Northern Hemisphere, including the European continent, whereas it migrates to the coastal areas of the South Hemisphere during the winter. Its breeding sites cover both freshwater and marine habitats. It is currently listed as Least Concern on the IUCN Red List, but several European countries have reported declines in breeding pair numbers. The primary disturbances are habitat destruction, flooding, human disturbances as well as competition and predation. Mitochondrial DNA is a genetic marker commonly used in population genetic studies. The control region is the fastest evolving region and a great tool for assessing intraspecies relationships. The aim of this study was to assess the mitochondrial DNA control region genetic diversity of several common tern colonies in Europe categorized into three groups: Northern, Southern Inland and Southern Marine. We used blood and feather samples of 319 individuals from 12 locations in Germany, Italy, Slovenia, Hungary, and Croatia and sequenced a 709 bp-long fragment of the mitochondrial DNA control region. We found 40 haplotypes, including three that had a nucleotide insertion. The most common haplotype, Stehi03, was found in 40.13% of all samples. Overall haplotype diversity was high (0.81), with

the highest value (0.86) found in the Southern Inland group, while the Northern and Southern Marine group showed somewhat lower values (0.74 and 0.77, respectively). Overall nucleotide diversity was 0.0023. The highest nucleotide diversity was found in the Southern Inland group (0.0027), followed by the Northern and Southern Marine group (0.0018 for both groups). The haplotype network constructed to visualize relationships between the samples showed no spatial association. This indicates high connectivity between the groups, and diversity indices suggest that the Southern Inland group is the most genetically diverse group. Future research should also incorporate nuclear markers, such as microsatellite loci, to assess the possible present-day genetic structure of European common terns.

Keywords: common tern; mitochondrial DNA; control region; genetic diversity; Europe

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Environmental Genetics and Ecotoxicology *Oral presentation*

BIOTECHNOLOGICAL POTENTIAL AND CONSERVATION OF MICROBIAL COMMUNITIES IN DINARIC KARST CAVES

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The Dinaric karst is a compact karst area with extremely thick carbonate rocks, mostly from the Mesozoic, which in some parts exceeds 8,000 meters. Characterized by exceptional gene, species and ecosystem diversity, Dinaric karst is also considered a world-renowned hotspot of subterranean biodiversity, including microorganisms. To overcome growth limiting factors, microbial communities in different types of biofilm create very complex, mutualistic networks. Depending on the intensity of light (or absence), these multispecies biofilms of varying thicknesses and morphologies consist of algae/cyanobacteria, bacteria and fungi that grow on speleothems and cave walls. Microbial colonization is a complex, multistep process that depends on substrates and environmental factors. It enables the survival of microorganisms, growth in unfavorable underground conditions and facilitates the exchange of genetic material through horizontal gene transfer (HGT). Microbes that inhabit subterranean ecosystems use different adaptation strategies and, by modifying their metabolic pathways, affect the other cave dwellers and the entire ecosystem inside the cave. Insight into their excellent survival strategies is crucial for the development of microbiotechnology, the discovery of new enzymes, bioactive molecules, and the development of new bioremediation techniques. Also, this surprising microbiodiversity could shed light on the resistome present in the isolates from a Dinaric karst caves. In particular, the cave moonmilk deposits contain an abundant and diverse actinobacterial population that has tremendous potential for producing novel natural bioactive compounds. Here we presented our multidisciplinary research focused on cultivable bacterial diversity of calcite moonmilk, which was sampled during the speleological expeditions "Ponor Kovači-Izvor Ričine" in August 2022 and 2023, from selected caves of the Dinaric karst in Bosnia and

Herzegovina. Mineralogical profiling by XRD (Bruker D8 ADVANCE) and morphological characterization of the different phases of moonmilk crystallization by SEM (JEOL JSM IT 200 LA) were carried out. Considering its biological origin, mainly from actinobacteria and/or fungi, systematic research is planned with the aim of metabarcoding and metagenomic moonmilk analysis from referent caves in the Duvanjsko polje area. Ultimately, we firmly believe that only an interdisciplinary approach in microbial ecology will enable the improvement of the protection of subterranean habitats and the conservation of biological diversity.

Keywords: Dinaric karst; Dinarides; moonmilk; microbiotechnology; conservation

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Environmental Genetics and Ecotoxicology *Oral presentation*

THE STATE OF GENETIC DIVERSITY CONSERVATION ACROSS NINE COUNTRIES FROM ALL CONTINENTS

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Of the three pillars of biodiversity- gene, species and ecosystem- the gene one has been historically ignored in biodiversity monitoring and conservation, mostly because measuring genetic diversity using DNA-based data remains resource intensive and time-consuming. However, new approaches were recently proposed to develop genetic diversity indicators for tracking and reporting genetic diversity status and trends for hundreds of species, without necessary relaying on genetic data. The indicators quantify two simple proxies of within-population and among-population genetic diversity and adaptive potential: small effective size (Ne<500) and loss of genetically distinct populations. The genetic diversity indicators were adopted in December 2022 in the Kunming-Montreal Global Biodiversity Framework of the Convention on Biological Diversity. Application of the genetic diversity indicators has been initiated in nine countries of all continents. We found that most species still maintain all their populations. However, most of these populations are too small to retain genetic diversity: in 70% of the species not even a quarter of their populations have an effective population size above 500. Here, I will detail the results of this ongoing effort, introduce the sources of data that can be leveraged to estimate the indicators and discuss how they could be implemented in more countries.

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Environmental Genetics and Ecotoxicology *Oral presentation*

PATHOGEN DETECTION USING THE DPCR MICROBIAL DNA DETECTION ASSAYS ON THE QIACUITY

Matulka Kamil

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Bacteria, fungi, viruses and parasitic metazoans are ubiquitous in the environment and are part of all aspects of human life, from human health to food production. Each microbe can trigger various harmful or beneficial effects on humans. This makes the specific detection and monitoring of microbes important for understanding their biological function, especially in infection or colonization of the human body. Digital PCR methods for detection and identification of microbial species and microbial genes will be presented, that enable rapid profiling and identification of microbial species, antibiotic resistance genes and virulence genes from diverse samples. These may include wastewater samples, infectious diseases, human pathogens, the human microbiome, multiple drug resistance, sepsis, food production or environmental samples.

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Human and Medical Genetics *Oral presentation*

THE ROLE OF GENETICS IN THE DISCOVERY OF CANCER TREATMENTS

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Cancer genetics and genomics has rapidly evolved in the last two decades with the discovery of new therapies and sequencing technologies. The simultaneous progress in genomic characterization of cancers and the emergence of targeted therapies and immunotherapies has increased treatment options for cancer patients, leading to better survival. Here, I will give 3 examples of drug discovery based on genetic and expression analysis: EGFR, BCR-ABL1, and PD-L1. These discoveries have served as the blueprint for the development of new treatments. The role of EGFR in cancer started early in the 1960s with the discovery of EGF and its receptor EGFR by Cohen and Levi-Montalchini, which subsequently led to the development of EGFR antibody by Sato and Mendelson. They showed the reduction of cell number after the application of EGFR antibody against epidermoid carcinoma cell line A-431, which was later branded as cetuximab for treatment of metastatic colorectal cancer. Besides cetuximab, a plethora of EGFR small-molecular inhibitors have been developed for EGFR-mutated non-small cell lung cancer. Another example of therapy guided by genomic aberration is BCR-ABL1, which was discovered in the 1960s, and further characterized in the 1970s as the first translocation associated with diagnosis of chronic myeloid leukemia. First BCR-ABL1 inhibitor, a tyrosine kinase inhibitor (TKI) was approved by the FDA in 2001, which revolutionized CML treatment. Subsequent second- and third- generation TKIs were developed not just for CML, but also other types of leukemia and lymphoma. A third example is the development of immunotherapy treatments. PD-L1 was identified in 1999 and to promote T cell invasion as a potential mechanism of immune evasion. A couple of years later, it was shown that PD-L1 can eliminate effector T cells through apoptosis, a process that can be blocked by anti-human PD-L1 antibodies. These studies served as the basis for development of anti-PD-L1 inhibitors, which is one of several different immunotherapy agents that have revolutionized cancer treatment in the last decade. These examples show some of key studies and discoveries that paved the way for plethora of different cancer modalities that are available for cancer patients today.

Keywords: genetics; targeted therapy; genomics; immunotherapy; cancer genomics

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Human and Medical Genetics *Oral presentation*

RARE RECIPROCAL TRANSLOCATION IN A PATIENT WITH ASTHENOZOOSPERMIA: CASE REPORT

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Reciprocal translocation is a type of structural chromosomal aberration that occurs when two non-homologous chromosomes exchange fragments. The frequency of reciprocal translocation is 0.16-0.2% in general population and about 1.3% in infertile men. It can occur both on autosomal and sex chromosomes. Depending on the location of the chromosomal break, the normal process of spermatogenesis may be consequently disturbed. Our patient was a 34-year old Caucasian male sent to our Department due to asthenozoospermia reported by semen analysis. G-banding revealed a reciprocal translocation between chromosomes 12 and 15. The karyotype was determined as 46,XY,t(12;15)(p13.2?;q15). To confirm the detected translocation, fluorescence in situ hybridisation (FISH) was performed with combined probes for chromosome 15 (Vysis Prader-Willi/Angelman region LSI SNRPN / CEP 15(D15Z1)/PML, PCP 15q, Vysis-Abott) and chromosome 12 (CEP 12 (D12Z3), TelVysion 12p Vysis-Abott). By means of multiplex-PCR, no Y-chromosome microdeletions were detected in the AZF regions. This report presents a rare chromosomal reciprocal translocation t(12;15)(p13.2?;q15), in a patient with asthenozoospermia. In the literature, the observed breakpoints have been linked to genomic regions associated with the various male reproductive problems. This case is particularly interesting because it represents an area that has not been fully explored or described. Therefore, it requires further detailed molecular analyses of the breakpoints, as well as detailed clinical evaluation, interpretation, and appropriate genetic counselling, respectively.

Keywords: asthenozoospermia; male infertility; reciprocal translocation

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Human and Medical Genetics *Oral presentation*

TARGETING THE HIV RESERVOIR

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Human immunodeficiency virus continues to be a global health concern that has claimed the lives of millions. Although anti-retroviral therapy slows disease progression, it is not curative due to certain reservoirs of replication-competent virus that persist during therapy. Therefore, if therapy is stopped, virus will emerge from these reservoirs and rapidly spread, causing renewed progression towards disease. One strategy for clearing the reservoir of latently infected cells is to use a kick and kill approach, in which latent cells are “kicked” or activated from latency, and then concurrently cleared or “killed”. Latency reversal agents can “kick” or induce HIV expression from latent cells, but do not necessarily cause the activated latent cells to die. Our group has been developing synthetic protein kinase C modulators based on natural products as latency reversing agents. We have shown these compounds activate latent virus in patient samples and in virus infected drug-treated humanized mice. Natural killer cells hold great promise as killing agents for virally infected cells as they re-emerge from latency due to their innate anti-viral recognition and cytotoxic function. We have recently shown that introduction of our best characterized latency reversing agent into infected humanized mice, followed by administration of allogeneic Natural Killer cells is superior in eliminating latent virus compared with either treatment alone. The use of a barcoded virus swarm to help quantitate effects of this “Kick and kill” strategy on the viral reservoir will be discussed.

Keywords: Human Immunodeficiency Virus; latency; reservoir; Natural Killer cells

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Human and Medical Genetics *Oral presentation*

CELL DEATH INDUCTION IN CANCER TREATMENT, WHAT WE SHOULD EXPECT? A STUDY ON UT7 LEUKEMIA

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Antitumor approaches attempt to inhibit the growth, differentiation and proliferation of cancer cells, which is a major challenge in defining appropriate therapeutic strategies. The development and efficacy of precision medicines intended to target cancerous but not healthy cells are preceded by a clear definition of the nature, mechanisms and consequences of their action, which depends on the type, sensitivity and specificity of cancer. A special approach is directed towards the discovery of antitumor mechanisms based on the cell death induction, mainly apoptosis, in different types of hematological cancers. We used a cell culture model of human acute myeloid leukemia UT-7 to analyse the apoptosis-based antitumor potential of halogenated boroxin (HB), at the cellular and molecular levels. Comparative treatments were done in peripheral blood mononuclear cells (PBMCs) obtained from tumor-healthy individuals. Methods for assessing the cell viability and cytotoxicity, apoptosis detection at the cellular level, relative expression analysis of 84 apoptosis-associated genes and expression of the anti-apoptotic BCL-2 were used. The results showed high HB potential in inhibition of cell viability, induction of cytotoxicity and apoptosis with measurable dose-dependent differences in reported effects between UT-7 and PBM cells. We observed that HB affected expression of 21 genes, primarily down-regulated anti-apoptotic genes in leukemia cells. Prediction of functional association of deregulated genes inferred HB impact on NF-κB signaling pathway inhibition, mostly active in different cancers. Significant decrease of BCL-2 expression ($p=0.04$) was found only in UT-7 cells that confirmed their pro-survival inhibition and active apoptotic process. This study provides a strong basis for further research of the HB selective antitumor activity, with a clear effect on cell death regulation in cancers via NF-κB signaling pathway inhibition. Our results warrant further study on HB use as precision apoptosis inducers in leukemia. Even though the underlying mechanism of HB action is still unknown, the elucidation of the induction process and types of cell death may lead to applied therapeutic solutions.

Keywords: apoptosis, halogenated boroxin, anti-cancer strategy

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Human and Medical Genetics *Oral presentation*

A VIEW FROM THE OUTSIDE: FLUORESCENT IMAGING AND FLOW CYTOMETRY IN VARIOUS RESEARCH AREAS

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Great number of applications based on molecular biology methods, like PCR, qPCR, capillary electrophoresis and next generation sequencing, are being used daily for genetic and biomedical research. These applications are widely used in areas like pathogen detection, pharmacogenomics and cell research, human and microbial identification, oncology and genetic disease research. Still, since they are mostly based on investigating DNA and RNA they are not entirely focused on investigating the bigger picture, cells. To enable sharper focus on cell research, innovation and progress has been made in microscopy and flow cytometry. To this end, Thermo Fisher Scientific has developed advanced EVOS imaging systems. These fully automated, multichannel fluorescence or brightfield microscopes can be used for the analysis of cell and tissue cultures, cell structures, viability, proliferation and death. EVOS XL Core is a basic transmitted light inverted system for routine cell culture analysis. EVOS FLoid is an inverted system for fast fluorescent imaging ideal for teaching and basic cell culture analysis. EVOS M5000 is a laboratory workhorse equipped with high-sensitivity camera for customizable fluorescent imaging and on-board analysis software. EVOS M7000 is an automated imaging system with monochrome and colour cameras which incorporates advanced imaging and data generation, including multiwell plate scanning and image stitching and tiling. Moreover, significant contribution to imaging has been made with the development of Attune Flow Cytometers. These cell-analyzers can be configured with up to four spatially-separated lasers and analyse panels of up to 14 colours. Implemented acoustic focusing technology reduces cell preparation and processing times, helps avoid clogging and allows the instrument to run 10x faster than conventional cytometers. Thus, the systems can perform sample through-put rates of 1,000 µL/min without compromising data integrity making it especially useful in research areas like immuno-oncology, cell biology and stem cell research. Furthermore, Attune CytPix model includes a high-speed brightfield camera to capture images of cell populations as one acquires flow data. This camera combined with integrated processing software which can measure over 25 image parameters, makes Attune CytPix especially useful for research of cell-cell interaction, cell death, autophagy, cell cycle, mitosis and in haematology, parasitology and microbiology.

Keywords: microscopy; flow cytometry; fluorescence imaging
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Human and Medical Genetics *Oral presentation*

AI FOR GENETIC VARIANT INTERPRETATION TO ADDRESS INTERPRETATION BOTTLENECK

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With the continued developments in the genomic field, labs are enabled to produce more and more genetic data. One of the key trends is the consolidation of various targeted genetic panels to Exomes and in some occasions genomes. Advances on the wet-lab side have removed major bottlenecks like price, turnaround times, hands-on times, increasing sample volumes. As a result of this efficiency gain the interpretation of results has become a major bottleneck. With advances and developments of informatic tools this interpretation burden is being addressed. In one of the latest Illumina interpretation tools we are leveraging Artificial intelligence to help with the interpretation of variants. By using the explainable AI a study on 180 subjects illustrated that in an overall 97% of subjects the AI identified the relevant variant in the top 10. This is a critical step to allow the genomics field to move forward to deploy genetics for more indications and scale up interpretation of larger parts of the genome.

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Human Forensic and Population Genetics *Oral presentation*

THE SIGNIFICANCE OF ANALYZING TOUCH DNA COLLECTED AT THE CRIME SCENES IN CANTON SARAJEVO

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Touch DNA, also known as trace DNA, represents one of the most frequent biological traces that are often found at crime scenes. It involves the transfer of biological material from a donor to an object or to another person during physical contact. Allows to be collected as evidence when there is a lack of visible DNA (blood, hair, semen, or saliva) or fingerprints are too stained or incomplete for fingerprint analysis. The collection and interpretation of the results of touch DNA contain very valuable information and represent a very important forensic tool that can lead to the identification of a criminal and help solve the crime. In the presented study, DNA analysis was done on samples collected at a crime scene investigation in Canton Sarajevo. Standard methods for DNA isolation, amplification, and DNA profile generation were used. From a total of 83 samples collected from different substrates at crime scenes, 6 samples with full DNA profiles and 8 with partial DNA profiles were generated. Additionally, 12 mixed DNA profiles were obtained. For 46 samples, no DNA profiles were generated. This research has shown that the accuracy of DNA profiling is significantly influenced by the proper collection of touch DNA from certain substrates. Although for half of the samples, DNA profiling was unsuccessful, this is the method of choice for this type of sample.

Keywords: Touch DNA; crime scene; DNA profile

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Human Forensic and Population Genetics *Oral presentation*

DNA ANALYSIS OF HUMAN SKELETAL REMAINS: AN OVERVIEW OF WHAT HAS BEEN ACHIEVED OVER THE LAST THREE DECADES

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It will soon be 25 years since the first identification of the skeletal remains of war victims from the territory of the former Yugoslavia. In those 25 years, domestic scientists in cooperation with their colleagues from other countries, processed several thousands of various skeletal remains. They achieved what many thought would not be possible. At the end of the 20th century, DNA analysis was a promising new method, but which still could not find its mass application in the identification of human skeletal remains. The first cases of the application of DNA analysis in individual plane crashes or the individual identification of American soldiers from the Gulf war and some other earlier wars served as a guiding idea that this method could be used in the identification of war victims found in mass graves found on the soil of BiH, Croatia and a little later Kosovo. After the initial difficulties and challenges encountered by this group of enthusiastic scientists, the entire process succeeded in alleviating the suffering of the local population, especially all those who were searching for the remains of their dearest family members. The scientific achievements resulting from these missions found their application years later in identification missions around the world, from victims of terrorist attacks in New York and Washington, over the victims of tsunamis and wars in Libya, Iraq and Syria, to the first identifications of civilian victims from mass graves. of the Second World War in Slovenia, Bosnia and Herzegovina. This presentation is brief reminder of previous scientific achievements and publications published on this topic within previous three decades.

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Human Forensic and Population Genetics *Oral presentation*

Y-STR ANALYSIS: ARE WE DIFFERENT FROM OUR MEDIEVAL ANCESTORS?

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Numerous archaeological sites on the territory of modern Bosnia and Herzegovina are witness to active and continuous life in the Middle Ages. The aim of this research was to determine the Y-haplotypes and predict Y-haplogroups, as well as the frequency of Y-haplogroups in the medieval population sample. A total of 42 male samples from 13 different medieval archaeological sites in Bosnia and Herzegovina were collected. These samples were selected to ensure that there was no kinship among the individuals, which is important for genetic analysis. DNA was extracted from bones and teeth by phenol-chloroform extraction, and Y-STR analysis was performed with the PowerPlex® Y23 System. Haplotypes were determined based on the allele variants detected in the Y-STR analysis. Y-haplogroups were predicted based on the obtained haplotypes using two available online software tools. The study compared the frequency of Y-haplogroups in the medieval Bosnian population with the recent population of Bosnia and Herzegovina. X^2 test based on the frequency of haplogroups was performed and the value of statistical significance is $p<0.05$. The most frequently detected haplogroups in the medieval Bosnian and recent B&H populations were I2a, R1a, R1b, and J2a. This suggests some continuity in the genetic composition of the region over time. Other haplogroups characteristic of European populations were also detected, albeit in slightly lower percentages. Haplogroup E1B1b is known to have a presence in various European populations and was also detected in the recent B&H population with a percentage of 12% but was not detected in the medieval population. It's assumed that the obtained results are a consequence of the insufficient number of successfully amplified Y-STR profiles, the small number of samples from the Middle Ages, as well as the possible stochastic effect. The results of the X^2 test indicate the absence of significant differences between the haplogroups in the two time periods. In conclusion, this research provides valuable insights into the genetic composition of the medieval Bosnian population and its comparison with the modern population of the region. It highlights the utility of genetic analysis in studying historical populations and understanding the dynamics of genetic lineages over time.

This research was supported by 2023 Grant of the Ministry of Education, Science and Youth of Canton Sarajevo.

Keywords: Ancient; DNA Y-STR markers; Medieval Bosnia; Archaeology
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Human Forensic and Population Genetics *Oral presentation*

SNEAK PEEK AT 3D FACIAL RECONSTRUCTION OF DIVIČANI MAN

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After learning about the genetic structure of our ancestral population, a 3D face reconstruction of medieval person excavated from the necropolis in Divičani near Jajce was done in order to find out what our ancestors looked like. Previous genetic research has revealed close kinship between three out of six people whose skeletal remains were buried in the necropolis, including mother and her two adult children. One child was buried in a sarcophagus that preserved skeletal remains well, so that skull was chosen for the reconstruction. The age at time of death was estimated using anthropological and dental methods while gender was confirmed by detection of Y chromosome at amelogenin locus. Additionally, DNA phenotyping revealed a high probability that the person had blue eyes, intermediate skin and light brown hair. Skull of a middle-age man named Divičani man was subjected to 3D scan, after which 3D reconstruction and 3D print of the reconstructed skull was done. Here we present the first results of 3D facial reconstruction of a medieval Bosnian man.

This research is part of the project "The face of medieval Bosnia – 3D reconstruction based on genetic information from archaeobiological samples" supported by Ministry of Education, Science and Youth of Canton Sarajevo (Grant no. 27-/02-11-41250-36/21).

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Human Forensic and Population Genetics *Oral presentation*

FROM EXTRACTION TO ANALYSIS: PROMEGA OPTIMIZES YOUR GENOMICS WORKFLOW

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As global biotechnology leader, Promega specializes in supporting scientists for more than 40 years with a wide range of innovative products including high quality reagents, enzymes, cell-based assays, and instruments. These products serve the scientists in basic research, applied research, drug discovery, agriculture, forensics, and molecular diagnostics. Focusing on genomic research, Promega can offer the full workflow from the extraction to data analysis. Our manual and automated systems promise efficient, rapid, and reliable extraction of DNA and RNA from a variety of samples. Our high performance GoTaq® taq polymerases which are available in different formulations covers the basic PCR, hot-start PCR, and long-range PCR applications. For applications that require fluorescence-based quantification, QuantusTM Fluorometer provides increased sensitivity compared to classical absorption-based methods. The last step of the workflow our Spectrum Compact capillary electrophoresis instrument supports scientists with fragment analysis application such as forensic and paternity testing, cell line authentication, mixed sample analysis, microsatellite instability testing, MLPA analysis, copy number variation. It also supports sanger sequencing applications with our ProDyeTM Terminator Sequencing system such as NGS confirmation, CRISPER/Cas9 mutation confirmation, Mitochondrial DNA analysis, HLA Typing, identification of SNPs and mutations, methylation sequencing, viral genotyping, microbial and fungal identification.

Keywords: DNA; RNA; extraction; genomics; sanger sequencing; fragment analysis

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POSTER PRESENTATIONS

Environmental Genetics and Ecotoxicology *Poster presentation*

REGULATORY MECHANISMS OF HEPATIC METABOLISM DURING PHYSICAL EXERCISE: A COMPREHENSIVE REVIEW OF SCIENTIFIC LITERATURE

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Fulfilling the heightened metabolic needs of active muscles during sustained exercise hinges upon a robust response from the liver. The increased output of glucose is essential to avoid hypoglycemia that threatens the body due to an inadequate breakdown of glycogen stores and recycling of metabolites through the gluconeogenic pathway. There is also the need to replenish the liver's glycogen stores after sustained exercise. This paper reviews the application of a global metabolomics approach to investigate the effect of exercise on substrate fluxes which offers the possibility to identify the metabolites exchanged between tissues and to elucidate group-specific differences in regulation. We also look at the known effects of the muscle-derived metabolites on hepatic metabolism and transcriptional regulation with the exercise-dependent regulation of hepatic metabolic pathways that reveal a surprising overlap. The results underline the essential function of the hepatic metabolism during exercise and support not only the relevance of the crosstalk of working muscles and the liver to exchange metabolite substrates but also the signaling molecules that support and mediate compensatory and adaptive processes during and after exercise.

Keywords: liver; exercise; muscle; metabolism; metabolites

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Environmental Genetics and Ecotoxicology *Poster presentation*

REVERSE TRANSCRIPTION-QUANTITATIVE PCR (RT-QPCR) WITHOUT THE NEED FOR REMOVAL OF TEMPLATE DNA

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One of the major problems in transcriptome analysis is inability to completely eliminate template DNA, which is indistinguishable from cDNA, thus resulting in false positive signals. We developed a novel method for transcriptome analysis by RT-qPCR (Reverse-Transcription quantitative Polymerase Chain Reaction), which circumvents the need for elimination of potential DNA contamination, therefore being more precise, simpler and more reproducible than the commonly used methods. The novel procedure involves the use of a modified specific primer during reverse transcription step, which contains mismatched bases, thus producing cDNA molecules not perfectly homologous to genomic DNA. By using the same modified primer in PCR amplification step, only cDNA template is amplified since genomic DNA template is not recognized by the primer. We determined the expression of *Escherichia coli recA* and *sulA* single-copy genes by RT-qPCR using either modified primers, or following the standard procedure. No *recA* and *sulA* sequence amplification was observed using our method unless cDNA was created by reverse transcription. The level of *recA* and sulA sequence amplification was unaffected by genomic DNA elimination from the sample. Conversely, the current method, which uses standard random/oligo-dT primers, showed a false positive signal even when reverse transcription step was skipped and the genomic DNA was (obviously incompletely) eliminated by DNase I treatment. Hence, our method of using a modified primer during cDNA synthesis produces a cDNA-specific PCR signal that is unaffected by genomic DNA and therefore quantifies gene expression much more accurately than the standard, commonly used method.

Keywords: *Escherichia coli*; improved transcriptome analysis; modified primers

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Environmental Genetics and Ecotoxicology *Poster presentation*

INDOLE-3-ACETIC ACID (IAA) PRODUCTION IN METAL-TOLERANT BACTERIAL ISOLATES FROM THE SERPENTINE OUTCROPS OF EASTERN BOSNIA

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Plant growth-promoting bacteria (PGPR) colonize the rhizosphere and improve plant growth through different mechanisms. Production of indole-3-acetic acid (IAA), one of the most physiologically active auxins, is an essential tool for rhizobacteria to stimulate plant growth. IAA supports root elongation and increases the number of root hairs and root laterals thus improving nutrients uptake. It also stimulates seed germination and cell division, delays leaf abscission, and induces flowering and fruiting. Some PGPR thrive in soil enriched with heavy metals and promote plant growth and heavy metal accumulation under otherwise toxic conditions. The bacterial strains inhabiting serpentine outcrops are adapted to high concentrations of heavy metals, partly due to their ability to promote the growth of plants from which they siphon off carbon and nitrogen. Such strains are of interest in agriculture as growth stimulators and in industrial biotechnology. The aim of this study was to isolate metal-tolerant rhizobacteria and quantify their production of IAA. The soil samples were collected from two serpentine sites in eastern Bosnia – Mioče and Gornje Cikote. Heavy metal concentrations in the soil samples were measured using flame atomic absorption spectroscopy. Bacterial isolates from the rhizosphere samples were screened for their Cu, Ni and Co tolerance. Rhizosphere samples showed high Cu, Ni and Cr concentrations. Isolates with the strongest heavy metal tolerance were tested for their propensity to produce IAA which was analyzed using a spectrophotometric method with Salkowski reagent. The exact IAA concentrations were calculated from the standard curve. IAA production ranged from 7.55 µg/ml to 901.36 µg/ml with the highest concentration in bacterial culture of isolate from Mioče. According to the DNA sequencing of the 16S rRNA gene, isolate with remarkable production of IAA was identified as *Pseudomonas putida*, while the isolate with the lowest IAA production showed the highest sequence similarity with the genus *Paenarthrobacter* (*P. nicotinovorans, P. aurescens*). Bacterial isolates which showed high IAA concentrations will be further screened for other PGP traits – nitrogen fixation, siderophore production, phosphate solubilization and ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity. These bacterial isolates will be also used for plant inoculation experiments to test their effects on plant growth and heavy metal tolerance.

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Keywords: IAA; rhizobacteria; PGPR; heavy metals; serpentines

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Environmental Genetics and Ecotoxicology *Poster presentation*

PHOSPHATE SOLUBILIZATION BACTERIA MAY REDUCE DNA DAMAGE IN *PISUM SATIVUM* L. CULTIVATED IN PHOSPHORUS DEFICIENT SOIL

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Phosphorus (P) is an essential macronutrient required for plant development and growth. It is a crucial component of numerous metabolic pathways and building molecules. Inadequate P or low bioavailability causes stunted development, oxidative stress, reduced yield, as well as DNA damage. In food production systems, P is supplemented within fertilizers with undesirable, long-term effects to the environment. Although present in significant quantities in the Earth's crust, P predominantly exists in insoluble organic and inorganic forms. Soil phosphate solubilizing bacteria play a substantial role in mineralization of organic and solubilization of inorganic P forms. To reduce the impact of P fertilizers, one ecologically acceptable solution is to exploit the subtle interactions between plants and rhizosphere bacteria with phosphate solubilizing properties. We applied plant comet assay to assess the ability of one phosphate solubilizing bacteria to mitigate DNA damage in *Pisum sativum* L. caused by P deficiency. *Acinetobacter sp*. strain with already known phosphate solubilizing properties was previously isolated from the topsoil at the Papratnica quarry, Bosnia and Herzegovina. The mixture of potting soil and sterilized vermiculite was prepared for the control and experimental pots and placed into the plant chamber. Surface sterilized *P. sativum* seeds were incubated in *Acinetobacter* sp. culture overnight. After inoculation, seeds were sown into the pots. The plants were watered with full nutrient solution and nutrient solution with $Ca_3(PO_4)_2$. The plant comet assay was performed on randomly selected fully-grown plant leaves. After staining the slides, the tail intensity (TI) of 50 cells was evaluated by Comet Assay IV. An Independent t-test was used for statistical analysis. Significant difference in TI ($p=0.014$) was observed between control samples and samples inoculated with bacterial strain watered with full nutrient solution. Similar result was observed ($p=0.0001$) for the samples watered with nutrient solution containing $Ca_3(PO_4)_2$. These results indicate that phosphate solubilizing bacteria have a significant role in mitigating DNA damage in P. sativum caused by P deficiency. It is possible that *Acinetobacter* sp. indirectly reduces DNA damage by improving nutrient availability, overall health and stress tolerance. To obtain conclusive data, further studies should focus on investigating plant physiology, plant-microbe interactions and targeted gene expression.

This research was supported by the Ministry of Science, Higher Education and Youth of Canton Sarajevo (Grant no. 27-02-11-41250-3/21).

Keywords: plant comet assay**;** DNA damage; phosphate solubilizing bacteria; *Pisum sativum*; *Acinetobacter*

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Environmental Genetics and Ecotoxicology *Poster presentation*

CADMIUM UPTAKE INTO ST. JOHN'S WORT AND ITS PHARMACEUTICAL PREPARATIONS

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Heavy metals can be toxic to living organisms due to their ability to accumulate in biological tissues and disrupt cellular processes. They can cause oxidative stress, DNA damage, and other genotoxic effects. Cadmium, as one of the toxic heavy metals, is a carcinogenic pollutant. Cadmium binds strongly to organic substances and is thus absorbed by plants. St. John's wort (*Hypericum perforatum* L.) is a native European plant widely used for its antioxidant, anti-inflammatory, and multiple other beneficial effects. Herbal preparations, due to their natural origin, can be contaminated with heavy metals, so it is important to determine their presence in those preparations. In this research, soil where the St. John's wort grows has been intentionally polluted with cadmium in order to study its uptake into this herb. The soil in the three pots with St. John's Wort was polluted with a maximum allowed concentration per gram of soil of cadmium. This was repeated twice, and samples of soil and herb were collected after each dosage. After the second cadmium dosage, the polluted herb was harvested, and then oil macerate was made of it. The cadmium concentration in every sample including soil, herb, and macerate, was measured using ICP-OES. Cadmium was not detected in the ground state of soil or herb. After the first cadmium dosage, it was present in the soil from all three pots, but it was absent from the herb itself. Then, after the second cadmium dosage, the concentrations in the soil samples from all three pots were increased, as expected. St. John's wort samples taken after the second cadmium treatment also contained cadmium. Despite this, in the oil macerate made from this contaminated St. John's wort, cadmium level was below the detection limit. The results show that higher concentrations and longer exposure to higher concentrations of cadmium in the soil are required for St. John's wort to absorb it. Even though oil macerate made from contaminated herbal drug in this study contained no cadmium, the possibility of of heavy metal intoxication in some other circumstances cannot not be ruled out.

Keywords: cadmium; Hypericum perforatum; St. John's wort

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Environmental Genetics and Ecotoxicology *Poster presentation*

C EXAMINATION OF THE GENOTOXIC AND CYTOTOXIC EFFECTS OF ALUMINUM SALTS

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Aluminum is a ubiquitous element that occurs naturally, and forms numerous compounds, including salts. Aluminium is used in various industries, including cosmetics, where it is used most often in the form of salt, as antiperspirant and disinfectant. This aim of this study was to evaluate the cytostatic, cytotoxic and genotoxic effects of aluminium (III) chloride hexahydrate in cultured human peripheral blood lymphocytes using the chromosome aberration test in vitro. A statistically significant deviation in the value of the mitotic index (cytostatic effects) was observed for the concentrations of 10, 20 and 25% aluminium (III) chloride hexahydrate, as compared to the control. Analysis of cytotoxicity markers revealed statistically significant differences in the frequency of apoptotic cells between 10 and 15% aluminium (III) chloride hexahydrate and the control group. Chromosome stickiness was observed in all metaphase cells at all tested concentrations. Based on the results, we can conclude that aluminium (III) chloride hexahydrate possess a certain proliferative, cytotoxic and genotoxic potential. It is important to emphasize that it is necessary to conduct further research to strengthen our results.

Keywords: Aluminum (III) chloride hexahydrate; human lymphocytes; chromosome aberations; genotoxicity; cytotoxicity

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Environmental Genetics and Ecotoxicology *Poster presentation*

COMET ASSAY IN ADHERENT CELL LINES - OPTIMISATION OF TRYPSIN TREATMENT

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The comet assay is a simple and versatile method widely used in the assessment of DNA damage, such as DNA single-strand breaks and alkali-labile sites in DNA nucleoids. Adherent cell lines are often used in assessment of cytotoxicity and genotoxicity, but detachment procedure may be confounder. Trypsin is the most common agent used for detachment; however it can damage cells and contribute to the elevated levels of DNA damage. In our studies, we assessed trypsin-induced DNA damage over different time periods (1, 2, 4 and 6 minutes) in two cell lines: MDBK (Madin-Darby bovine kidney) and human bladder carcinoma 5637. The aim was to determine the optimal period of trypsin treatment that would result in minimal DNA damage, and efficient detachment yield. Results revealed the highest trypsin-induced DNA damage in 1-minute treatment of MDBK cells; significantly increased compared to 2 and 4-minutes treatments. This suggests resistance of MDBK cells to trypsin-induced damage, and their efficient repair mechanisms. In 5637 cell line significant and positive association was found between the length of treatment period and the level of DNA damage, showing that this cell line is sensitive to trypsin treatment. Trypsin treatment optimization for each used cell line is highly needed for DNA damage analysis.

Keywords: cells detachment; DNA damage; genotoxicity

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Environmental Genetics and Ecotoxicology *Poster presentation*

GENETICS OF COAT COLORS AND ITS ROLE IN CLIMATE CHANGE RESILIENCY IN SHEEP

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Domestication resulted in a wide range of phenotypes due to adaptation to varied habitats and climate conditions, as well as different human preferences, including the phenotypic of coat colors in sheep. Numerous genes determining pigmentation and coat color-associated loci in sheep have been found, and they frequently influence either the generation or distribution of the pigment melanin by modifying the underlying processes. Under the recent climate change concern, as the barrier of animal's body from external factors, coat colors have become a promising aspect that can contribute to heat stress resiliency in sheep. Light-coated animals are preferred over dark-coated animals, according to the results of several studies conducted on other livestock; however, there are other, less consistent results available. The genetic mechanism of heat stress in various livestock has been extensively studied and determined to be a complicated trait, albeit no definitive theory has been established. Here, we develop the connection between coat color and thermal resistance in sheep based on the available literature. We discuss how genetics and genomics might be used to enhance our understanding of its mechanism.

This work was supported by the bilateral S&T cooperation programme, within the project "Effect of heat stress in Pramenka types of sheep using DNA and RNA based methods" from the National Development, Research and Innovation Fund (2021-1.2.4-TÉT-2021-00047).

Keywords: coat color, genetic and genomics tools, *Ovis aries*, thermal resistant

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Environmental Genetics and Ecotoxicology *Poster presentation*

MANAGEMENT MANNERS AND ORGANIZATIONS SUPPORTING THE UTILIZATION OF SCIENTIFIC RESULTS OF GENETICS IN PRACTICE – OPPORTUNITIES AND CHALLENGES IN EUROPE AND INDONESIA

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The growing population of the Earth, the fight against climate change, and the focus on environmental aspects pose complex tasks for both theorists and practitioners. It is necessary to ensure the nutrition of the population of countries with growing populations – mainly African and Asian – while reducing the impact on the environment. The science of genetics can make a major contribution to making agricultural production sustainable, identifying breeds that are better adapted to a changing climate and have less impact on the environment during breeding.However, for the practical application of modern results of genetics, it is necessary to familiarize them with producers in the agricultural sphere as soon as possible. Modern management methods and certain organizations that keep in touch with producers, livestock breeders, the government launching support projects, and academia can help in this case. In many European countries, there are formal contacts between breeding organizations, chambers representing the interests of agriculture, and public/state institutions. There are also examples of scientific results being disseminated to producers through this channel. These best practices are important for countries such as Indonesia to learn about, where organizing breeders and providing them with scientific results and effective management methods is still a challenge for the future.

Keywords: Agricultural policy; AnGR; Climate change; GFP; Livestock management

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Environmental Genetics and Ecotoxicology *Poster presentation*

ANALYSIS OF QUALITATIVE INDICATORS OF SCOTS PINE AND AUSTRIAN PINE SEED STANDS AS IMPORTANT FOREST GENETIC RESOURCES IN FEDERATION OF BOSNIA AND HERZEGOVINA

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Seed stands are very important objects for the production of selected seeds, which is used to achieve genetic gain in newly planted forests. The goal of the research is to determine the quantity and quality of Scots pine (*Pinus* sylvestris) and Austrian pine (*Pinus nigra*) seed facilities in Federation of Bosnia and Herzegovina, and to recommend measures for the improvement of these facilities for producing the highest quality reproduction material of these species for optimal use of forest habitats and realizing the ecological and economic role of these valuable forest tree species. For this research, four traits were measured, and nineteen evaluated on the trees of Scots pine in fifteen seed stands, and Austrian pine in eleven seed stands in the Federation of Bosnia and Herzegovina. According to the results of earlier research, some of these traits are highly heritable, and their negative expression must be avoided in seed objects. The results showed that the average age of the trees in the seed stands of both researched species was 100 years. The average tree diameter for Scots pine seed stands was 38 cm and Austrian pine 37 cm, while the average height of Scots pine trees was 24 m and Austrian pine 22 m. A sufficient number of full-wood trees and trees with excellent trunk straightness was determined in all stands examined. A small incidence of brittleness, and a large proportion of thin and medium-thick branches were determined. The number of branches in the vertebra was high in the seed stands of both species. A small number of damaged trees in the seed stands was also registered, and small number of trees with moderate twisting. Some of stands have shown very good quality, and individual selection should be made in them, while in other stands it is necessary to remove part of the trees. Considering the great ecological-vegetation diversity of Bosnia and Herzegovina, a greater number of Scots and Austrian pine seed facilities is needed. When selecting new seed plants of white and black pine, the focus should be on small stands that grow in extreme conditions.

Keywords: seed stands; Scots pine; Austrian pine

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Environmental Genetics and Ecotoxicology *Poster presentation*

THE FIRST RECORD OF SUBSPECIES RHYACOPHILA FASCIATA DELICI IN THE PROTECTED AREAS OF CANTON SARAJEVO

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The Balkan peninsula is widely known for its high biodiversity and endemism in freshwater ecosystems, with aquatic insects being the most recognizable group of macroinvertebrates. Caddisflies (Trichoptera) have been extensively studied since the late 19th century, and new species and subspecies are still being discovered and described. DNA barcoding is a widely used tool in biodiversity research and it is dependent on the availability of reference genetic sequence libraries. We collected larvae samples of aquatic insects and performed DNA barcoding using degenerated primers LCO1490-JJ and HCO2198-JJ. The sequence data for standard animal DNA barcode of specimens collected in two protected natural areas of the Sarajevo city's urban area, Dariva (river Miljacka) and Vrelo Bosne (river Bosna), were stored in the GenBank database. Based on our data a new subspecies *Rhyacophila fasciata delici* was discovered in the investigated areas (Valladolid et al. 2020). Among the nine GenBank enteries for this subspecies that we included in the database (accession numbers: MT765286, MT765287, MT765290, MT772015, MT772017, MT772020, MT772022, MT772028, MT772029), there are five different haplotypes. Three haplotypes were recorded for Dariva and two haplotypes for the Vrelo Bosne locality. The recorded subspecies and different haplotypes within it indicate that the diversity of Trichoptera species in Bosnia and Herzegovina is far from being fully explored. The use of DNA barcoding, in conjunction with morphological species identification, should be employed in the biomonitoring of protected areas in Canton Sarajevo. Our study once again emphasizes the importance of the existence and supplementation of publicly accessible genetic databases to improve species identification and bioassessment reliability. Further research with a larger sample size and in a wider area should shed more light on aquatic insects and their diversity in protected areas, not only in Canton Sarajevo but also throughout Bosnia and Herzegovina.

Keywords: biodiversity; water insects; Trichoptera; DNA barcoding; haplotype diversity

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Environmental Genetics and Ecotoxicology *Poster presentation*

DETECTION OF THE CAUSATIVE AGENTS OF LYME BORRELIOSIS IN BOSNIA AND HERZEGOVINA: SEROLOGICAL AND MOLECULAR GENETIC RESEARCH

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The causative agents of the most common vector-borne disease in Europe, Lyme borreliosis, are spirochetes from the complex *Borrelia burgdorferi* sensu lato. Detecting the causative agents is very important in both the clinical diagnosis of disease because of its complex etiology and monitoring the prevalence and prevention of disease development. In order to plan future research in this field in Bosnia and Herzegovina, it is necessary to know the current state, which we will present below. Until now, three serological and two genetic researches have been conducted in Bosnia and Herzegovina. ELISA, western blot, and indirect immunofluorescence were used in serological analysis. Out of 55 patients that were positive for borrelial presence, three were with clinical manifestations of cardioborreliosis, one with neuroborreliosis, and 51 patients with different clinical manifestations retrospectively analyzed at the Clinic for Infectious Diseases in Sarajevo in the period 1996-2006. For that period, just four cases were registered in the Institute for Public Health of the Federation of Bosnia and Herzegovina. On the other hand, traditional molecular genetic analysis, endpoint, and nested Polymerase Chain Reactions were used in detecting the causative agents in ticks as their vectors. In the first chronologically conducted research, there was no borrelial DNA detected. Three years later, the first molecular evidence *of Borrelia burgdorferi* sensu lato in Bosnia and Herzegovina was recorded. Both recorded species were pathogenic/potential pathogenic for Lyme borreliosis. The above-mentioned results indicate that in Bosnia and Herzegovina there is no recorded actual number of Lyme borreliosis patients in health institutions. Although a small number of sporadic studies have been conducted in the detection of the causative agent of Lyme borreliosis, their representation is large in relation to the sample size. Further research should go in the direction of subjecting all patients with a complex etiological picture of the disease which resembles to Lyme borreliosis to the serological or molecular-genetic analysis on borrelial presence and being recorded in the competent institutions. In order to prevent the development of the disease and determine the hotspots of possible ticks' infestations and borrelial infection, potential reservoirs of Borrelia burgdorferi sensu lato and their vectors in urban areas should be tested genetically with mapping of the analyzed area.

Keywords: vector-borne disease; *Borrelia burgdorferi* sensu lato; diagnosis; prevention

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Environmental Genetics and Ecotoxicology *Poster presentation*

CALCITE MOONMILK DEPOSITS: MORPHOLOGY AND ENVIRONMENT OF FORMATION IN KARST CAVES OF THE CENTRAL DINARIDES IN BOSNIA AND HERZEGOVINA

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Moonmilk is a secondary calcite deposit, mainly composed of fine calcium carbonate crystals (CaCO3), with different morphology and textures ranging from pasty, muddy, powdery to hard, depending on the water content. These karstic speleothems formed of calcite nanofibers in which the contribution of biotic rock-building processes is presumed to involve indigenous microorganisms. Although its biogenic origin is widely accepted, the mechanism of production of calcite nanofibers has not been fully elucidated. Cave moonmilk deposits found in karst caves host an abundant and diverse actinobacterial population that has a great potential for producing novel natural bioactive compounds. Microorganisms, mostly bacteria that are able to dissolve rock or induce mineral precipitation could be used in the construction industry and engineering. Crystallization and formation mechanism of calcium carbonate nanostructures are of high interest in different fields, such as geomicrobiology (rock-microbes interactions), astrobiology (biosignatures), medicine and pharmacy (antibiotics and bioactive compounds), nanotechnology (calcium carbonate nanofibers) but also archeology (necropoleis). Here we present the mineralogical profiling using X-ray powder diffraction (Bruker D8 ADVANCE) demonstrated that calcite was the dominant mineral in majority of speleothemes with a few variations in the elemental components. Morphological characterization of different phases of crystallization of the moonmilk was shown using scanning electron microscopy (JEOL JSM IT 200 LA). The content of nine heavy metals (Co, Mn, Ni, Cu, Fe, Zn, Cd, Cr and Pb), through two sample readings, was determined by flame atomic absorption spectrometry (Varian 240 FS). Considering the exceptional microbiological diversity of karst caves, intensive multidisciplinary research will contribute to the discovery of new bioactive molecules with a focus on antimicrobial compounds.

Keywords: moonmilk morphology; heavy metals; X-ray diffraction; SEM; Dinarides

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Environmental Genetics and Ecotoxicology *Poster presentation*

UNVEILING THE POTENT ANTIMICROBIAL POTENTIAL: EXPLORING THE GENETIC BASIS OF CLOVE AND CINNAMON OIL'S IMPACT ON *METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS*, *STAPHYLOCOCCUS AUREUS*, AND *ESCHERICHIA COLI*

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Antimicrobial resistance poses a significant challenge in medical therapies, urging exploration of alternative approaches. Essential oils, extracted from plants, possess high hydrophobicity that allows them to interact with bacterial cell membranes and mitochondria, inducing structural changes that enhance permeability. This study investigates the synergistic potential of combining essential oils, such as clove and cinnamon, with conventional antibiotics. Although these oils may not independently exhibit strong bactericidal effects, their collaboration with antibiotics demonstrates amplified antimicrobial activity. This synergy not only reduces antibiotic dosage requirements but also curtails adverse effects. In this experiment we evaluated the antimicrobial efficacy against *Methicillin-Resistant Staphylococcus aureus* ATCC 33591 (MRSA), *Staphylococcus aureus* NCTC 12493 (*S. aureus),* and *Escherichia coli* ATCC 25922 (E. coli). Results showed optimal antimicrobial impact at 25% cinnamon oil concentration for MRSA and S. aureus, and 50% concentration for E. coli. Similarly, 50% clove oil concentration exhibited the most potent antimicrobial effect across all three bacterial strains. Notably, cinnamaldehyde, the primary component of cinnamon oil, demonstrated antibacterial properties attributed to its natural antioxidant properties. To unravel the genetic basis of antibiotic resistance and strain-specific responses, genetic profiling and transcriptome analysis are desirable. Bacterial strains exhibit diverse genetic characteristics, influencing resistance mechanisms and virulence factors. Comparative genomics and molecular analyses are crucial for a comprehensive understanding. This experiment revealed that antibiotic resistance can be reduced while using natural sources of treatment. A combination of cinnamon and clove oil alone can have a significant antimicrobial effect, but in order to have complete treatment it is necessary to combine them with conventional antibiotics. This pathway can minimize the side effects of antibiotics and also be very effective in fighting bacteria.

Keywords: essential oils; antimicrobial resistance; synergistic effects; antibiotic dosage; natural resources

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Environmental Genetics and Ecotoxicology *Poster presentation*

THE MULTISPECIES *SORBUS* COMMUNITIES – HYBRID ZONES OF HIGH CONSERVATION VALUE

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Hybrid zones represent areas where two or more species coexist, where gene flow occurs generating hybrid derivates. In the woody genus *Sorbus* L., hybridization and polyploidy associated with apomixis are powerful factors in population structuring and divergence. Interspecific or intercytotype hybridization events are linked with unreduced and reduced gametes in *Sorbus* diploids and polyploids respectively, that produce heteroploid progeny. Newly formed polyploid derivates represent separate groups in relation to parental taxa due to the prevailing apomictic reproduction but they can subsequently participate in further hybridization events. The *Sorbus* communities harboring mixtures of different species, cytotypes and the interacting sexual and asexual lineages represent potential reservoirs of novel diversity and merit a special status of conservation concern. Previous research of different *Sorbus* complexes in Bosnia and Herzegovina revealed several such areas of exceptional scientific significance, namely High Conservation Values Forests (HCVF). The first steps to understand the processes that generate such complex diversity require comprehensive research based on the use of different molecular markers, cytometry, experiments with controlled pollination in the field and monitoring F1 derivatives. In the context of the global risk of biodiversity loss and pressing environmental challenges for trees and forests, such areas require urgent attention from local and regional decision makers to conserve the evolutionary potential generating novel tree diversity.

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Environmental Genetics and Ecotoxicology *Poster presentation*

INTEGRATING MACHINE LEARNING AND CLINICAL EXAMINATION FOR ACCURATE DIAGNOSIS PREDICTION OF CONGENITAL HYPOGONADOTROPIC HYPOGONADISM

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The integration of Machine Learning (ML) and Artificial Intelligence (AI) into medical diagnostics has emerged as a transformative paradigm, offering new dimensions of precision and efficiency. This research endeavors to harness the potential of ML and AI techniques to predict the diagnosis of Congenital Hypogonadotropic Hypogonadism (CHH) from comprehensive clinical examinations, advancing the landscape of personalized medicine. Our study engaged a curated cohort of CHH patients, subjecting them to a comprehensive round of clinical assessments, including hormone profiles, physical examination findings, and medical histories. Leveraging ML algorithms, we designed predictive models that extracted intricate patterns and relationships from the complex clinical data. Significantly, our results showcased the capacity of ML and AI to predict CHH diagnosis based on clinical features with ~80% accuracty. The developed models exhibited high sensitivity and specificity, empowering clinicians with an invaluable tool for early and accurate CHH identification. Notably, the predictive power of the models extended beyond traditional diagnostic markers, uncovering subtle patterns that elude human perception. The interpretability of our ML models was further enhanced through feature importance analysis, elucidating the crucial clinical parameters that contribute to accurate predictions. This insight provides a window into the pathophysiological mechanisms underlying CHH, guiding future research and enhancing clinical decision-making. Moreover, our study presents a novel framework for integrating genetic and omics data into the predictive models, enabling a holistic approach that captures the multifaceted nature of CHH. In summation, this research underscores the transformative potential of ML and AI in revolutionizing CHH diagnosis. By bridging the gap between clinical examination and precise disease classification, our study paves the way for enhanced patient care and therapeutic interventions in the realm of CHH diagnosis.

Keywords: Machine Learning (ML); Artificial Intelligence (AI); Congenital Hypogonadotropic Hypogonadism (CHH); prediction

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Environmental Genetics and Ecotoxicology *Poster presentation*

IN SILICO ANALYSIS REVEALS POTENTIAL INTERACTIONS BETWEEN MARBURG VP40 PROTEIN AND ENDEMIC PLANT SPECIES OF BOSNIA AND HERZEGOVINA

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The Marburg virus, a highly virulent pathogen, can induce infectious diseases and hemorrhagic fever in humans. Taxonomically, it is classified within the Marburg virus genus and is a member of the Filoviridae family, sharing both structural and functional similarities with the Ebola virus. Notably, there is currently no available medicine for managing this disease. Medicinal plants, on the other hand, contain a multitude of therapeutic phytochemicals, some of which exhibit potent antiviral properties. These properties render them promising candidates for drug development against various viral illnesses. In this ongoing study, we employ an in silico approach to identify potential phyto-derived remedies sourced from indigenous Bosnian plants for the treatment of Marburg virus infections. The three-dimensional structure of the VP40 protein was obtained from the RCSB Protein Data Bank, while the 3D structures of the isolated components from the native plant were sourced from the PubChem database. For active site prediction, we employed PrankWeb, and the preparation of VP40 and phytochemical compounds was conducted using AutoDock Tools and OpenBabel. Subsequently, molecular docking was carried out using AutoDock Vina, and the resulting structural interactions were visualized and analyzed with PyMOL. Our findings revealed that hesperidin and luteolin exhibited the highest binding affinity for the VP40 protein. Hesperidin established a total of seven significant hydrogen bonds (including interactions with Ile88 - 2.8 Å, Arg136 - 2.8 Å, Gln143 - 2.7 Å, and four interactions with Arg180 - 2.2 Å, 2.3 Å, 2.4 Å, and 3.3 Å) and exhibited a substantial binding affinity of -10.6 kcal/mol. On the other hand, luteolin displayed a binding affinity of -8.8 kcal/mol and formed six hydrogen bonds (including interactions with Pro134 - 2.0 Å, Arg136 - 2.9 Å, two with Arg139 - 2.1 Å and 2.1 Å, one with Gln143 - 2.7 Å, and one with Gln276 - 3.9 Å). Our results suggest that these compounds may have the potential to exert effects against the VP40 protein. Nonetheless, it is imperative to undertake further comprehensive *in vitro* and *in vivo* investigations to confirm their therapeutic utility in treating infected patients.

Keywords: Marburg virus; VP40; hesperidin; luteolin

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Environmental Genetics and Ecotoxicology *Poster presentation*

IN SILICO INTERACTION OF PHYTOCHEMICAL COMPONENTS EXTRACTED FROM *KNAUTIA SARAJEVENSIS* AGAINST TWO-PART NS2B-NS3 ZIKV PROTEASE

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The Zika virus (ZIKV) is a severe health concern with a lack of treatment options, that can cause Guillain-Barré syndrome in adults and microcephaly in newborns. *In silico* drug screening greatly contributed to rationalisation of drug design process. Since the two-part ZIKV protease is essential for viral replication and is a great candidate for drug design studies, we performed molecular docking analysis of structural interactions of compounds isolated from endemic plant *Knautia sarajevensis* against the NS2B-NS3 ZIKV protease. The 3D crystal structure of ZIKV protease was retrieved from the RCSB Protein Data Bank and the 3D structures of compounds isolated from the endemic plant were obtained from the PubChem database. PrankWeb was used for the active site prediction, while AutoDock Tools and OpenBabel were used for the preparation of the protein and phytochemical compounds. AutoDock Vina was used for molecular docking and the structural interactions were visualised and analyzed using PyMOL. Chlorogenic acid, myricetin and quercetin showed the highest binding affinity for two-part ZIKV protease. Chlorogenic acid formed eight significant hydrogen bonds in total (one with Ser 81 – 2.4 Å, one with His51 – 2.4 Å, one with Ser135 – 2.3 Å, one with Gly151 - 2.2 Å, one with Asn152 – 3.3 Å, two with Gly153 – 2.2 Å and 3.5 Å, and one with Tyr161 – 3.1 Å), and had a binding affinity for NS2B-NS3 at −7.2 kcal/mol. Myricetin had a binding affinity at -6.9 kcal/mol and formed six significant hydrogen bonds (one with Phe84 – 3.3 Å, one with Gly151 – 2.3 Å, two with Gly153 – 2.5 and 2.5 Å, and two with Tyr161 – 2.2 and 3.0 Å). Quercetin had the same binding affinity as myricetin and it formed significant hydrogen bonds with Gly82 – 3.2 Å, His51 – 3.5 Å, Tyr130 – 2.5 Å and Pro131 – 2.8 Å. Results obtained in this study showed that these compounds potentially have effects against NS2B-NS3 protease. Potential therapeutic use of this plant in the treatment of infected patients should be further evaluated with in vitro and in vivo studies in order to confirm their effect.

Keywords: Molecular docking; phenolic compounds; chlorogenic acid; myricetin; quercetin

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Environmental Genetics and Ecotoxicology *Poster presentation*

INVESTIGATION OF ANTIVIRAL POTENCIES OF PHENOLIC COMPOUNDS AGAINST NS2B-NS3 ZIKV PROTEASE: THE CASE OF *VERBASCUM GLABRATUM* SUBSP. *BOSNENSE*

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Worldwide, healthcare systems are strugling with the increased rates of morbidity and mortality that are caused by viral infections. World Health Organization declared the Zika virus (ZIKV) as Public Health Emergency of International Concern. The virus can cause microcephaly in newborns and Guillain-Barré syndrome in adults. Currently, there are no drugs that are effective against ZIKV. Computational tools are widely used for drug design, as a first step in the identification process of components with potential activity against, e.g., viruses. Plant derived metabolites are one of the prime focuses in new drug discovery. Potential medicinal properties of species from *Verbascum* L. (mullein) genus were reported in previous research, so the aim of the present study was to investigate bioactive potential of the phenolic compounds isolated from *Verbascum glabratum* subsp. *bosnense* (K. Maly) Murb. (an endemic plant of Bosnia and Herzegovina, Albania and Northern Greece) against the two-part ZIKV NS2B-NS3 protease using protein-ligand docking method. Protein's active site was predicted and confirmed using PrankWeb and the literature review. AutoDock Vina was used for the molecular docking, and PyMOL for the visualisation of structural interactions. Rutin and quercitrin had the highest binding affinity and forming of significant hydrogen bonds with NS2B-NS3 protease. Rutin reacted with Ser81 and Asp83 of NS2B part and Val72, Asp75, Tyr130, Pro131, Ala132, Ser135, Gly151, Asn152, Gly153, Val154 of NS3 part, forming four significant hydrogen bonds in total (one with Gly151 - 2.2 Å, two with Gly153 – 2.5 Å and 3.1 Å, and one with Tyr161 – 1.9 Å), with a binding affinity for NS2B-NS3 at −8.2 kcal/mol. Quercitrin had a binding affinity at -7.8 kcal/mol and reacted with Gly82 and Asp83 of NS2B part and His51, Asp75, Tyr130, Pro131, Ala132, Ser135, Gly151, Asn152, Gly153, Val155 of NS3 part, forming six significant hydrogen bonds (with His51 – 3.3 Å, Asp75 – 2.5 Å, Tyr130 – 3.0 Å, Pro131 – 2.2 Å, Ser135 – 3.0 Å and Asn152 – 2.3 Å). The results showed that *V. glabratum* subsp. *bosnense* could potentially be used in the treatment of Zika virus infection. Further *in vitro* and *in vivo* studies are needed in order to confirm the present findings.

Keywords: Zika virus; molecular docking; *in silico*; rutin; quercitrin

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Human and Medical Genetics *Poster presentation*

GENOTOXIC AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACTS FROM AERIAL PARTS OF *ONOBRYCHIS VICIIFOLIA* PLANT FROM DIFFERENT LOCALITIES

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The present study aimed to investigate biological activities (genotoxic and antioxidant), total phenolic/flavonoid content, and heavy metal concentrations in methanolic extracts from aerial parts of the *Onobrichys viciifolia* plant, which were gathered from different localities: uncontaminated (Gornji Milanovac) and a tailing site (Žitkovac, Kosovska Mitrovica). The genotoxic activity was investigated using the single-cell gel electrophoresis (comet assay) on human peripheral blood lymphocytes *in vitro*. The antioxidant activity was evaluated by a DPPH free radical scavenging assay, while the total phenolic and flavonoid contents were measured by spectrophotometry. The heavy metal analysis was conducted using atomic absorption spectrophotometry. All tested concentrations (125, 250, 500, and 1000 µg/mL) of the extract from the uncontaminated locality significantly increased the genetic damage index (GDI), ($p<0.0005$). The average GDIs in treatments ranged from 1.44±0.05 to 2.27±0.07, while the value in the negative control (untreated cells) was 0.32±0.08. Also, the extract from the tailing site exhibited significant genotoxicity across all tested concentrations, resulting in an increased genetic damage index (from 2.10±0.67 to 3.16±0.04) compared to the controls ($p<0.0005$). The Pearson correlation coefficient demonstrated that all tested concentrations of both extracts exhibited a dose-dependent increase in the GDI ($r=0.921$ and $r=0.963$, $p<0.0005$, respectively). The plant extract obtained from the tailing site demonstrated higher antioxidant activity compared to the extract from the uncontaminated locality, with an IC_{50} value of 65.40 µg/mL versus 74.40 µg/mL. Conversely, the higher phenolic and flavonoid content exhibited the plant extract from the uncontaminated locality in comparison to the tailing site (137.83 versus 86.16 mg GA/g; 66.64 versus 25.41 mg RU/g). Analysis of heavy metals revealed the presence of Mn, Ni, Ca, Mg, Fe, Zn, Cr, Pb, and Cu in both localities of plants, although their concentrations were notably higher in plant from the tailing site. In conclusion, the study suggests that the methanolic extracts of the aerial parts of *Onobrichys viciifolia* regardless of the locality have a genotoxic activity in all tested concentrations and a high amount of heavy metals that cause concern and may cause serious clinical complications when using this plant.

Keywords: *Onobrichys viciifolia*; human lymphocytes; genotoxicity; antioxidant activity; heavy metals

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Human and Medical Genetics *Poster presentation*

ASSOCIATION BETWEEN INSERTION/DELETION POLYMORPHISM OF THE *ACE* GENE WITH RISK OF HYPERTENSION

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Angiotensin-I-converting enzyme (*ACE*) gene I/D polymorphisms have been identified as potential genetic markers for hypertension. The aim of this study was to determine distribution of the genotypes and alleles of *ACE* insertion/deletion polymorphisms (rs1799752) and to compare them between groups divided by gender and presence of hypertension in the population of Republic of Srpska, Bosnia and Herzegovina.The study included two groups, 100 hypertension patiens and 100 normotensive control individuals. Among them, there were 101 men and 99 women. DNA was isolated from whole blood, amplified with PCR, and visualized using 2% agarosis gel electrophoresis. Distribution of DD genotype and D allele was higher in hypertension group (DD 45.0%; D 63.5%) compare to control group (DD 32.0%; D 55.5%), but statistically significant difference was not detected. In hypertension and control groups II genotype distribution was 18% and 21%, respectively. A statistically significant difference of allele I was observed among groups (p = 0.004), while no significant difference was observed in *ACE* alleles between genders. Genotype odds ratio, (DD + ID) on the II, was 1.2110 (0.6006 to 2.4418; 95% CI; p = 0.5927). Statistically significant difference was not detected between genders and *ACE* genotypes and alleles. Allele D and DD genotype were more frequent in hypertension group, however this association was not statistically significant in examined population.

Keywords: angiotensin-I-converting enzyme; *ACE* gene polymorphism; hypertension

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Human and Medical Genetics *Poster presentation*

FROM GENOTYPE TO PHENOTYPE: UNDERSTANDING THE ROLE OF THE *ASPA* GENE IN CANAVAN DISEASE

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Aspartoacylase is a hydrolytic enzyme encoded by the *ASPA* gene, and carriers of defective *ASPA* genes are affected by Canavan disease. Canavan disease is characterized by a myriad of symptoms, but the salient clinical features of the affected individuals are early onset, neurodevelopmental delay, macrocephaly, optic atrophy, and leukodystrophy. Some patients also present with seizures and elevated N-acetyl-L-aspartic acid (NAA) in urine. We conducted a comprehensive review of existing literature and assessed functional studies with the aim of collecting data on phenotype-genotype correlations and examining the gene-disease relation validity between the *ASPA* gene and Canavan disease.

Keywords: aspartoacylase; *ASPA* gene; Canavan disease; phenotype-genotype correlation

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Human and Medical Genetics *Poster presentation*

GENERATIONAL AND SEASONAL PATTERNS IN MENARCHE TIMING: AN IN-DEPTH ANALYSIS FROM THE GRADACAC REGION

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Menarche is the most reliable sign of puberty and is preceded by a period of intense physical growth. It represents a significant turning point in a series of events in the life of the female organism, and it most often occurs two years after the first signs of puberty. This research covered 7 primary schools in the Gradacac area. The survey collected basic data on the date of birth of girls and mothers, as well as data on the first appearance of menarche and the physical activity of the respondents. The study included a total of 608 female participants (304 girls and 304 mothers). The research method used was a survey, statistical analysis of the data was performed using an independent t-test. The aim of this study was to determine the median age of onset of menarche in girls of primary school age in Gradacac (chronological age from 10 to 15 years). Additionally, the study aimed to establish whether there is a correlation between the occurrence of menarche in mothers and their daughters. Another goal was to determine whether the mother's age at the birth of a girl affects the occurrence of the girl's menarche, and to assess the seasonal impact on the occurrence of menarche in the study area (seasonal variations). We found a significant relationship between the age of menarche in girls and their mothers (r= 0.030). In relation to the seasons, we saw the highest frequency of menarche in the summer, lowest during the autumn, with similar frequencies in winter and spring. We also established an acceleration trend, in keeping with the Western European statistics.

Keywords: menarche; puberty; physical growth; female organism

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Human and Medical Genetics *Poster presentation*

PHENOTYPIC HETEROGENEITY IN TERMINAL OSSEOUS DYSPLASIA: ELUCIDATING THE ROLE OF TWO *FLNA* VARIANTS

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Terminal osseous dysplasia with pigmentary defects (TODPD) is an extremely rare condition characterized by skeletal dysplasia of the limbs, recurrent digital fibroma, pigmentary changes and dysmorphic facial features. The condition is inherited through an X-linked dominant pattern, only presented in females, while causing early prenatal death in affected males. This condition has been associated with a singular *FLNA* variant, c.5217G>A. Recently, a second variant (c.5217+5G>C) has been described in affected individuals. Here we performed an extensive scientific literature review and comparative analysis of phenotypic features reported in affected individuals.

Keywords: terminal osseous dysplasia with pigmentary defects; *FLNA* variants; X-linked dominant; phenotype comparison; TODPD

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Human and Medical Genetics *Poster presentation*

POLYMORPHISMS OF 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE GENE IN MULTIPLE SCLEROSIS

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Multiple sclerosis is an inflammatory, demyelinating disease of the central nervous system in which both environmental and multiple genetic factors play a role in the etiology with moderate effects. Homocysteine-induced neurotoxicity may be a factor influencing inflammation, and 5,10-methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme involved in homocysteine metabolism. The association between *MTHFR* polymorphisms and multiple sclerosis has been studied in different ethnic groups.Therefore, we tested the hypothesis that polymorphisms in *MTHFR* (*C677T* and *A1298C*) influence the predisposition to multiple sclerosis and its clinical expression in the Croatian population.Two hundred patients and 200 healthy controls were genotyped by polymerase chain reaction-restriction fragment length polymorphism method.No significant differences ($p > 0.05$) in genotype distribution or allele frequency of the *MTHFR C677T* polymorphism were found between patients and controls. Regarding the *MTHFR A1298C* polymorphism, there were no significant differences ($p>0.05$) in allele frequencies between patients and controls, although the MTHFR 1298C/C genotype was underrepresented in patients ($p=0.01$; OR =0.16; 95% CI: 0.05-0.57). The study showed no statistically significant differences in predisposition between men and women, nor between familial and non-familial cases of multiple sclerosis with respect to either *MTHFR* polymorphism. We found no correlation between the *MTHFR A1298C* polymorphism and disease behavior ($p>0.05$). However, we observed a statistically significant association ($p=0.04$) between carrier status of the *MTHFR 677T* allele and disease progression. Our results suggest that in the study populations, the MTHFR 1298C/C genotype may be a protective factor for multiple sclerosis susceptibility, while the *MTHFR 677T* allele may be a good predictor of disease progression.

Keywords: MTHFR; gene polymorphism; multiple sclerosis

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Human and Medical Genetics *Poster presentation*

G-PROTEIN COUPLED RECEPTORS AS POTENTIAL DRUG TARGET IN THERAPY AND TREATMENT OF TYPE 2 DIABETES

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Type 2 diabetes (T2D) as the most common form of diabetes mellitus is condition that characterized by hyperglycemia and disturbances in lipid and protein metabolism. There are many risk factors involved in the development of T2D such as nutrition, the environment and genetics. Recent studies suggested that lipid changes may not only be a consequence of impaired glucose metabolism but also a causative factor. Fatty acids as an energy fuel in the body, are important as biomolecules, particular as free fatty acids (FFAs) and play a key role in various metabolic functions by acting as signaling molecules and regulators or stimulators of biological functions. Fatty acids influence translocation of glucose transporters and insulin receptor binding and signaling, in addition to cell membrane fluidity and permeability. It is thus suggested that FAs may have an essential role in the development of IR and T2D. Specific FFAs (short-, medium- and long-chain fatty acids) activated several G-protein coupled receptors (GPCR) and represent important receptors of FFAs name as FFA1–4. These FFA receptors (FFARs) are mediate various physiological functions, such as peptide hormone secretion and inflammation related conditions and thereby contribute energy homeostasis. Since imbalance in energy homeostasis lead to metabolic disorders, such as obesity and T2D, FFARs are considered to be a potential drug and therapeutic targets in these diseases. Novel findings have been shown that the administration of selective agonists of FFAR1 and FFAR4 improved glucose metabolism and ameliorated systematic metabolic disorders by increasing glucose-stimulated insulin secretion (GSIS) as well a direct positive effect on GSIS, while activation of FFAR2 and FFAR3 are linked with metabolic function of saturated fatty acids (SFAs) in anti-inflammation and energy metabolism by reducing inflammation and improvement in insulin sensitivity. This work, presented recent findings of FFARs physiological as well biological functions and their potential as selective and specific ligands for development of novel drugs to treatment obesity and T2D as metabolic disorders and inflammation related conditions.

Keywords: free fatty acid receptors, Type 2 diabetes, inflammation, therapy

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Human and Medical Genetics *Poster presentation*

DYNAMICS OF ORF1AB AND E GENE IN COVID-19 POSITIVE PATIENTS FROM THE TRAVNIK REGION

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Quantitative reverse transcription polymerase chain reaction (RT-qPCR) is an essential diagnostic tool used to accurately identify and confirm SARS-CoV-2 infection in patients who are showing symptoms or are suspected of being infected. The WHO recommends a combination of viral-specific genes, including the Envelope (E), the RdRP/Helicase (Hel), the spike protein-encoding gene (S), and the *ORF1ab* gene, as molecular targets for detection. Among the possible PCR targets, the *E* gene of SARS-CoV 2 is considered to be the least specific and it shows significant sequence homology to other common coronaviruses. The present study aimed at investigating the dynamics of *ORF1ab* and *E* gene from COVID–19 positive patients considering the Ct values of both genes. The study included population of 130 patients who showed symptoms of COVID-19 between November 2021 and February 2022 in Central Bosnia and Herzegovina. Out of these patients, 86 tested positive for the virus. For molecular confirmation of SARS-CoV-2, the RT-qPCR protocol was performed. Average Ct values were automatically generated with values ≤Ct30 reported as positive. Average Ct value for ORF1ab was 26.43 (S.D.± 3.37) and for *E* gene was 27.45 (S.D.±2.29). Study also revealed that the prevalence of COVID-19 was 54.5% in males and 46.5% in females, showing that males had an increase of 8% positive cases than females. The Average Ct value for ORF1ab is lower than for E gene which is in correlation with recent studies. Studies suggested that *ORF1ab* exists in higher quantities than *E*, thus, as patients recover, the *E*-gene RNA is the first to become undetectable. The current findings, as far as infectivity is concerned, indicates that men are more vulnerable than women to COVID-19.

Keywords: Ct value; SARS-CoV-2; qRT-PCR; genes

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Human and Medical Genetics *Poster presentation*

THE POWER OF GENETIC RESEARCH: UNDERSTANDING THE ROLE OF RAB23 GENE IN CARPENTER SYNDROME

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The RAB23 gene encodes a protein that belongs to the RAB family of guanosine triphosphate enzymes and acts as a negative regulator of hedgehog signaling. Genetic variations of this particular gene have been linked to Carpenter syndrome. Carpenter syndrome is an extremely rare autosomal recessive disorder with only about 70 cases reported in the medical literature. Patients with this condition have been reported to experience craniosynostosis, which is the premature fusion of certain skull bones, as well as abnormalities of the fingers and toes, and other developmental issues. In this study, based on our thorough analysis of relevant literature and functional studies, we have gathered valuable data regarding the correlation between phenotype and genotype, as well as the validity of the gene-disease relationship associated with the RAB23 gene and Carpenter syndrome.

Keywords: RAB GTPase; RAB23 gene; Carpenter syndrome; phenotype-genotype correlation

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Human and Medical Genetics *Poster presentation*

APOLIPOPROTEIN E ENCODING GENE MUTATIONS AND WARFARIN REQUIREMENTS: A REVIEW OF IN VIVO STUDIES

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Apolipoprotein E is encoded by *APOE* gene and is known to participate in warfarin clearance by the liver using receptor – specific uptake, as well as acts as a ligand for receptors that mediate vitamin K uptake by cells. Its three alleles ε2, ε3, and ε4 are single – base changes causing missense mutations at amino acid positions 112 and 158 and have different metabolic consequences, including differential warfarin requirements. Warfarin is a vitamin K antagonist and probably the most widely used oral anticoagulant drug worldwide. Changes on chromosome 19q13.2 can cause different mutation depending on which allele it happens. Mutations on allele ε2 may be a great risk of early vascular disease and genetic disorder type III hyperlipoproteinemia, and ε2 is also responsible for Parkinson's disease. Some mutations on ε4 are: Alzheimer's disease, cognitive function, multiple sclerosis, traumatic brain injury, HIV, etc. Thrombophilia can be defined as an inherited disorder that increase a patient's risk of developing thrombosis (abnormal blood clotting) in the veins or arteries. Personalized medicine is a medical model that divides patients into different groups – with medical decisions, practices, interventions based on their predicted response or risk of disease. It is used to examine and determine time duration and warfarin dose requirement for Apolipoprotein E mutations. The effect of APOE mutations on warfarin metabolism in different populations is reviewed in this topic.

Keywords: ApoE; warfarin; anticoagulant; thrombophilia; personalized medicine

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Human and Medical Genetics *Poster presentation*

ASSOCIATION OF THE TAS2R38 GENE POLYMORPHISM WITH THE SENSITIVITY OF GUSTATORY AND OLFACTORY FUNCTION TO SARS-CoV2 INFECTION

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One of the early symptoms of the COVID-19 virus is the loss of the sense of taste and smell, and how and why this virus affects the senses is still being researched. Various molecules from food and the environment that we introduce into the body bind to the receptors of the olfactory and gustatory system, and through a cascade of activated physiological processes we receive information about the taste and aroma of the ingested food. One of the important receptors associated with bitter taste is *TAS2R38*, and it is known as an oral marker for individual differences in taste perception, general food preferences and eating behavior, with consequent links to body mass and other physiological mechanisms. This study examines the association of polymorphisms in the *TAS2R38* gene with infection-induced loss of sense. 96 individuals who recovered from SARS-CoV2 infection were included in the research. The subjects were divided into two groups, those who lost their senses and those who did not, and a sample of the buccal mucosa was taken from all of them, DNA was isolated and new generation sequencing was performed on the gene panel. The data were used to calculate the allelic and genotypic frequencies for the five loci of the *TAS2R38* gene. Applying Chi-square and Fisher Exact tests to check the significance of differences in variation between the two examined groups, no statistically significant association was observed between the *TAS2R38* gene responsible for bitter taste and sensory loss accompanied by SARS-CoV2 infection.

Keywords: TAS2R38; SARS-CoV2; dysgeusia; dysosmia

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Human and Medical Genetics *Poster presentation*

SYSTEMATIC REVIEW OF GENETIC CAUSE IN DUCHENNE MUSCULAR DYSTROPHY

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Duchenne Muscular Dystrophy (DMD) is a manifestation of how our genes may have an impact on our health. By examining the function of these genes and how they affect the development of the illness and potential therapies, this comprehensive review seeks to help us comprehend the genetic components behind DMD. When we examine scientific studies, we discover several alterations in the dystrophin (*DMD*) gene. These modifications, such as deletions, copies, and mutations, disrupt the normal synthesis of the crucial protein dystrophin. Over time, this causes muscles to become weaker. Researchers have found a variety of modifications in the dystrophin gene, with deletions being the most prevalent, thanks to improved methods for sequencing genes. Additionally, scientists now know more about sarcoglycans and dystroglycans, two additional proteins related to dystrophin. Muscles become weaker as a result of issues with these proteins, and the compromised cell communication makes the muscle fibres even more brittle. Understanding how certain genes are regulated by small molecules known as non-coding RNAs is a novel concept in DMD genetics. These molecules control the production of dystrophin and have an impact on several cellular processes. This could be crucial for developing medicines specifically for DMD. Doctors can forecast how a patient's DMD may worsen by having an understanding of the genetics of the condition. This aids them in making the greatest patient care decisions. Additionally, there is an exciting prospect to correct specific genetic issues with modern gene-editing techniques like CRISPR-Cas9, which might result in novel therapies for DMD. This review guides us through the complex genetics of DMD and demonstrates how understanding genes is essential for understanding the condition and developing effective new therapies.

Keywords: Duchenne Muscular Dystrophy (DMD); dystrophin; CRISPR – Cas9; muscle weakness

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Human and Medical Genetics *Poster presentation*

REVIEW OF UNDERLYING GENETIC CAUSES OF CONGENITAL HYPOGONADOTROPIC HYPOGONADISM

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Congenital Hypogonadotropic Hypogonadism (CHH) stands as a multifaceted and heterogeneous ailment, characterized by an insufficiency in the secretion of gonadotropin-releasing hormone (GnRH). This deficiency culminates in compromised gonadal maturation and the onset of hypogonadism. The past several decades have witnessed substantial advancements in the unraveling of the intricate genetic substrates underpinning CHH. This comprehensive exposition seeks to meticulously assess the present breadth of comprehension regarding the genetics governing CHH, accentuating pivotal genetic determinants implicated in its etiology. A methodical scrutiny of the scholarly corpus brings to the fore a manifold of diverse genetic etiologies contributing to the manifestation of CHH. Pertinent to note are mutations transpiring within the genetic loci that encode indispensable constituents of the GnRH neuronal network, prominently inclusive of *KISS1R*, *GNRHR*, and *TAC3/TACR3*, all of which have undergone exhaustive documentation. Moreover, the advancements in sequencing methodologies have led to the revelation of uncommon genetic variations and alterations in gene copy numbers linked to CHH, thus broadening our knowledge of its genetic framework. Furthermore, emerging evidence hypothesizes a complex interplay between genetic susceptibility and environmental factors in the genesis of CHH. Key genes involved in the regulation of GnRH have been shown to be modulated by epigenetic changes and non-coding RNA molecules. The complex web of regulatory networks underlying reproductive neuroendocrinology is being revealed by integrative strategies that combine genomic, epigenomic, and transcriptome research. Additionally, the development of precision medicine offers the possibility of individualised therapy approaches based on a person's genetic profile. As a whole, the genetics of CHH constitute an emerging and forward-looking field with significant implications for both basic research and therapeutic use. A thorough understanding of the genetic causes of CHH not only broadens our understanding of reproductive physiology but also prepares the path for revolutionary treatment approaches.

Keywords: Congenital Hypogonadotropic Hypogonadism (CHH); gonadotropin-releasing hormone (GnRH); hypogonadism; mutations

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Human and Medical Genetics *Poster presentation*

PRESENCE OF HLA-DQ2 AND HLA-DQ8 /DR4 CELIAC DISEASE PREDISPOSING ALLELES IN TESTED GROUP OF PATIENTS IN BOSNIA AND HERZEGOVINA

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Celiac disease (CD) is an autoimmune disease characterized by gluten intolerance. The main cause of this immune - mediated enteropathy is gliadin, a protein mainly present in wheat, rye, barley and spelt. The predisposition to celiac disease is determined by HLA class II genes encoding MHC II heterodimer molecules, more specifically *HLA-DQ2* and *HLA-DQ8*. Up to date the exact number of people suffering from celiac disease in Bosnia and Herzegovina is still not known because there is no public registry for this disease. The aim of this study was to evaluate the *HLA-DQ2* and *HLA-DQ8/DR4* presence in tested group of patients referred to Polyclinic Atrijum in Sarajevo in period from August 2022. to August 2023. *HLA-DQ2* and *DQ8/DR4* allele identification was performed using Real-time PCR technique. According to our preliminary results, approximately 20% of tested patients were positive for *HLA-DQ2* and/or *HLA-DQ8/DR4* haplotype. To our knowledge this is the first study evaluating the presence of HLA-DQ2 and *HLA-DQ8* genotypes in tested population in Bosnia and Herzegovina. However, for better understanding of *HLA-DQ2* and *HLA-DQ8* genotype association with celiac disease and their distribution in Bosnian and Herzegovinian population, a larger study with higher number of tested patients with clinical data should be conducted.

Keywords: Celiac disease; *HLA-DQ2*; *HLA-DQ8*

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Human and Medical Genetics *Poster presentation*

EXPLORING APOPTOTIC EFFECTS OF STENOENDEMIC *ACINOS ORONTIUS* PLANT EXTRACTS IN GR-M MELANOMA CELLS: INITIAL FINDINGS

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The plant world is a rich source of bioactive compounds with therapeutic potential. Plant-derived chemicals have long been utilized for cancer treatment or prevention. More than 25% of anti-tumor drugs are directly derived from plants, and 25% are synthetic analogs. However, out of approximately 250,000 higher plants, only 5-15% have been studied in in vitro and in vivo studies, with only about thirty in clinical trials. Species within the genus Acinos are known for containing high levels of flavonoids and linolenic acid in their essential oils. These compounds have antioxidant properties, which make them potentially valuable natural sources of antioxidants. Currently, there's a lack of comprehensive data on their effects on cytotoxicity, apoptosis, and antiproliferative activity. The primary aim of the study is to investigate the impact of *Acinos orontius* extracts on gene expression related to apoptosis and apoptosis signal pathways in GR-M melanoma cells. Fresh plant material was collected from five locations in Herzegovina. Both aqueous and DMSO extracts were prepared. Cultured GR-M cells were treated with different concentrations (0.01, 0.05, 0.1, and 0.2 mg/mL) of the extracts. The NucleoSpin® RNA extraction kit was used for RNA extraction. The SALSA RT-MLPA R011-C1 Apoptosis assay (MRC Holland) was used to measure the relative expression of apoptotic genes. Electrophoresis was performed on Genetic Analyzer 3500 (Applied Biosystems). Initial results indicate the potential of the extracts to regulate pro- and anti-apoptotic genes, with upregulation of pro-apoptotic genes *BOK*, *MOAP1*, and *BBC3*, and downregulation of anti-apoptotic gene *BIRC7*. Notably, Cyclin-Dependent Kinase Inhibitor 1A (*CDKN1A*) was overexpressed upon treatment, suggesting a pro-apoptotic effect. This finding is associated with *CDKN1A*'s role in DNA damage response and cell cycle arrest, orchestrated by tumor suppressor protein p53. However, apoptosis involves a network of genes, and the observed effects shouldn't be attributed solely to a single gene. Besides that, the extracts consistently downregulated *BIRC7*, an anti-apoptotic gene, implying a mild apoptotic effect. *BIRC7* inhibits caspases, crucial players in apoptosis. Extracts seem to counteract its function. In conclusion, *Acinos orontius* extracts may hold promise as anti-tumor agents, particularly against melanoma, potentially acting through the *TP53* gene. Nevertheless, further studies are needed to validate these findings and unravel the underlying mechanisms.

Keywords: Gene expression; apoptosis; plant extracts

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Human and Medical Genetics *Poster presentation*

ENHANCING BREAST CANCER CARE: THE VITAL ROLE OF GENETIC PROFILING VIA NGS

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Breast cancer is one of the most prevalent fatal malignancies in females. Latest discoveries in precision medicine put the breast cancer genetic landscape at the spotlight. This review underscores the crucial importance of genetic profiling in breast cancer management, specifically through Next-Generation Sequencing (NGS). We emphasize how NGS reveals the unique genetic makeup of each patient's tumor, enabling tailored treatment approaches. This precision not only optimizes therapy but also identifies actionable mutations and informs ongoing monitoring. Beyond treatment, genetic profiling contributes to risk assessment, familial screening, and drug target discovery. Integrating NGS into routine practice is a transformative step towards more effective and patient-centered breast cancer care.

Keywords: breast; cancer; NGS

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Human and Medical Genetics *Poster presentation*

SOMATIC VARIANTS AND PRECISION THERAPY IN COLORECTAL CANCER

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Colorectal cancer (CRC) remains a major global health challenge, necessitating innovative approaches for more effective treatment strategies. In recent years, precision medicine has emerged as a promising avenue, driven by advancements in understanding the genetic landscape with aberrations in RAS genes, which serve as markers for targeted therapy for CRC. The RAS-MAPK pathway is regulated by many proteins but its activation is one of the most common triggers for CRC. This review paper presents a comprehensive overview of somatic variants in CRC, focusing on variants in the RAS family of genes (KRAS, NRAS, HRAS) and their critical role in shaping the landscape of precision therapy, particularly through targeted immunotherapy. The review also encompasses the recent advances in molecular profiling techniques, including next-generation sequencing (NGS) and liquid biopsies, which enable precise identification of somatic variants. These techniques are pivotal in tailoring therapy choices for individual CRC patients, optimizing treatment response, and minimizing adverse effects.

Keywords: colorectal cancer; RAS mutation; immunotherapy

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Human and Medical Genetics *Poster presentation*

ASSOCIATION OF MDR1 rs1045642 POLYMORPHISM WITH SUSCEPTIBILITY TO BALKAN ENDEMIC NEPHROPATHY

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Balkan endemic nephropathy (BEN), chronic tubulointerstitial disease, occurs only in clusters of villages in alluvial valleys of tributaries of the Danube River in Bosnia, Serbia, Croatia, Bulgaria, and Romania. One of its most peculiar characteristics is a strong association with upper tract urothelial carcinoma. Despite decades of intensive research into BEN's aetiology, which still remains unclear, aristolochic acid (AA) was shown to be the true culprit. The biotransformation of AA includes complex metabolic activation, associated with the production of reactive oxygen species, resulting in oxidative distress. As a part of the Phase III detoxification system, MDR1 as an ATP-dependent exporter is involved in the detoxification of various xenobiotics from cells. Moreover, experimental evidence shows that members of ABC transporters could also be involved in AA metabolism. *MDR1* rs1045642 SNP results in altered protein expression and lower MDR1 (P-glycoprotein) expression in the kidneys. Considering the potential influence of altering MDR1 activity, we hypothesized that *MDR1* rs1045642 polymorphism may modify individual susceptibility to BEN. Therefore, we evaluated the effect of this SNP on the risk for BEN development and BEN-associated urothelial carcinoma. The case-control study comprised 202 BEN patients and 140 healthy individuals, residents of endemic settlements. The *MDR1* genotypes were determined by qPCR using Applied Biosystem Taqman Drug Metabolism Genotyping assays. We found a significant association between *MDR1* rs1045642 polymorphism and risk for BEN development. Carriers of variant *MDR1*TT* genotype were at almost 2-fold increased risk of BEN development (OR3=1.93; 95%CI=1.00-3.73; p=0.048). (OR3=1.93; 95%CI=1.00-3.73; p=0.048). There was no significant individual impact of this polymorphism on BEN-associated tumors ($p > 0.05$). In Kaplan-Meier survival analysis *MDR1* (rs1045642) polymorphism did not show an effect on time to dialysis in BEN patients ($p > 0.05$).

Regarding *MDR1* rs8177412 polymorphism, the gene variant that confers lower expression is associated with a significant increase in BEN risk. More extensive research in this field on BEN patients are needed.

Keywords: Balkan endemic nephropathy; upper tract urothelial carcinoma, aristolochic acid, *MDR1* rs1045642 polymorphism

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Human and Medical Genetics *Poster presentation*

GENETIC VARIATION IN RELATION TO DEMOGRAPHY OF MALE POPULATION IN BOSNIA AND HERZEGOVINA

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Genetic studies in relation to demography of the appropriate population structure in Bosnia and Herzegovina have a relatively long history and are becoming increasingly popular with the development of commercial genetic tests. In recently time, the results of the tests can be found in the databases of projects in Bosnia and Herzegovina but also in the neighbor countries. The goal of projects is the development of demographic genetic studies, and the data are publicly available. The paper presents the results of data obtained by testing specific markers on the Y chromosomes of the male population with the indicated place of residence in Bosnia and Herzegovina. In total, the data obtained by testing 2501 samples were analyzed. The results of research point out that there are 8 Y haplogroups in BiH. The most represented is haplogroup I with prevalence of the 52% of the tested population belongs to this haplogroup (I1 - 8% and I2 - 44%). The second most represented is haplogroup R with 21% (R1a-5% and R1b-16%). Then follow haplogroups E-13%, J-9% (J1-1% and J2-8%), N-3%, G-2%, and haplogroups T (3 tested, 0%) and Q are very slightly represented (1 tested, 0%). Four main haplogroups: I, R, E and J are represented in Bosnia and Herzegovina with 94%. The analysis of the representation of haplogroup I as well as its sub-branches I1 and I2 among the three constituent peoples in Bosnia and Herzegovina showed that there is no significant difference.

Keywords: Population Genetics; Demography; Y haplogroup; Bosnia and Herzegovina

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Human and Medical Genetics *Poster presentation*

THE IMPACT OF ACE2 GENE POLYMORPHISM (RS2285666) ON CREATININE AND CREATINE KINASE LEVELS IN COVID-19 PATIENTS AND ITS RELATIONSHIP WITH DISEASE SEVERITY

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COVID-19 manifests with a spectrum of symptoms, varying in intensity, and can lead to fatal outcomes in certain instances. Within the human genetic makeup, the *ACE2* (angiotensin-converting enzyme 2) gene governs the production of the ACE2 protein—a receptor situated on the surface of human cells. This receptor holds a pivotal role in the control of blood pressure and cardiovascular functions. This study aimed to investigate the potential impact of the *ACE2* gene polymorphism (rs2285666) on serum creatinine and creatine kinase (CK) levels among COVID-19 patients and to assess its association with disease severity. The research encompassed 750 individuals diagnosed with COVID-19, all of whom were enrolled at General Hospital Tešanj. These patients were categorized into three groups based on the severity of their condition—mild, moderate, and severe. Genomic DNA was isolated from whole blood samples. The genotyping process was carried out using the Applied Biosystems QuantStudio5 RT-PCR System. Our findings revealed noteworthy associations within the group of patients exhibiting mild disease severity. Specifically, individuals carrying the *CC* (93.59±3.02) and *TT* genotypes (93.59±3.02) demonstrated significantly elevated creatinine levels in comparison to those with the *CT* genotype (79.32±3.27), with p-values of <0.001 and 0.011, respectively. Additionally, among patients with a mild clinical outcomes, those with the *CC* genotype (322.98 ± 59.04) displayed significantly higher creatine kinase levels (p=0.043) when contrasted with individuals carrying the *CT* genotype (151.87 ± 38.07). However, within the groups of patients with moderate and severe clinical picture, our results did not reveal statistically significant associations between different genotypes and creatinine and CK levels. *ACE2* gene polymorphism (rs2285666) appears to influence creatinine and creatine kinase levels specifically in COVID-19 patients with mild disease severity, suggesting a potential genetic basis for variations in renal and muscle function within this subgroup. However, no statistically significant associations were found in patients with moderate and severe clinical outcomes.

Keywords: COVID-19; *ACE2*; rs2285666; creatinine; creatine kinase

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Human and Medical Genetics *Poster presentation*

THERAPEUTICAL TARGETS IN BREAST CANCER: ESR1 AND BRCA1 MUTATIONS

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Breast cancer (BC) is the most common cancer type in women with ~2.3 million new cases annually worldwide, where most patients are diagnosed after the age of 50. Survival rate depends on the molecular subtype and stage of the disease. Genetic testing and curation help identify patients who will benefit from the targeted therapy and immunotherapy. Genomic landscape includes aberrations in PIK3CA, TP53, BRCA1, BRCA2, and CDH1 genes. In this paper, we will review variant interpretations in ESR1 and BRCA1 genes. ESR1::CCDC170 fusion can be found in ~6–8% of the luminal B BC and is typically the result of tandem duplications. The ESR1 5′ UTR is fused to the coding region of CCDC170, leading to N-terminally truncated CCDC170 proteins, which results in reduced sensitivity to endocrine therapy. Thus, the detection of ESR1 fusions in patient samples will select patients who should not receive endocrine therapy. Resistance to endocrine therapy in patients with ER-positive BC is a challenge because it is also associated with disease recurrence and progression to metastatic disease. A more commonly mutated gene in BC is BRCA1. Functional BRCA1 gene is involved in regulation of cell development, as well as the repair of DNA double-strand breaks. Germline mutations in BRCA1 are associated with hereditary breast and ovarian cancer syndrome (HBOC). Patients with early or metastatic HER2-negative BC with germline mutations could be eligible for treatment with olaparib, a PARP inhibitor. For example, patients with germline BRCA1 c.5324T>G (p.Met1775Arg) mutation, a pathogenic mutation associated with reduced protein stability and aberrant DNA repair, should undergo genetic counseling. Genetic counseling is important for patients with BOCS for therapy options and prognosis.

Keywords: breast cancer; ESR1 fusion; BRCA1; PARP inhibitor; endocrine therapy

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Human and Medical Genetics *Poster presentation*

GENOMIC LANDSCAPE OF FAMILIAL MYELOID PREDISPOSITION SYNDROMES

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Myeloid neoplasms (MN) include myelodysplastic syndromes and acute myeloid leukemia, usually affecting the elderly population, while familial cases are reported to occur in 4-10% of patients. Genes associated with germline predisposition to myeloid malignancies are: *CEBPA, DDX41, TP53, RUNX1, ANKRD26, ETV6, GATA2, SAMD9, SAMD9L, BLM, TERC, TERT, SRP72, ACD, BRCA1, MSH6, XPCdelTG, ERCC6L2,* and *LIG-4.* Overall 10-year survival in familial AML with germline CEBPA mutations was significantly higher compared to both single and double-mutation CEBPA sporadic AML. Rate of relapse is higher in familial vs. sporadic AML. In terms of mutational burden, there were no differences in familial and sporadic MN. Germline mutations are more associated with therapy-related AML than sporadic disease. Familial MN tend to be diagnosed in younger patients, but can be found at older age, such as in patients with DDX41 germline mutation. The management of patients with familial myeloid predisposition syndromes is different from sporadic cases, especially in decisions regarding the allogeneic stem cell transplantation. Genetic counseling is crucial in patients with suspected familial myeloid predisposition. Patients who should undergo genetic testing are: individuals with ≥2 cancers, 1 of which is a hematological malignancy (HM) OR individuals with a history of HM and positive family history of HM / another hematopoietic abnormality / solid tumor at a young age OR individuals whose tumor-based molecular profiling identified a deleterious variant with a VAF consistent with germline status OR individuals with HM and a specific molecular and cytogenetic aberrations OR individuals with HM diagnosis at an unusually young age. Germline confirmation should ideally be performed on DNA from cultured skin fibroblasts. Recently, authors demonstrated that many individuals with myeloid neoplasms could be identified years in advance. Even so, challenges to clinical testing include: insufficient training of clinicians, rapid increase in susceptibility genes, high proportion of variants of uncertain significance, distinguishing germline from somatic mutations, and a lack of standardization in patient selection. The field studying genetic predisposition to myeloid neoplasms is continuously evolving. Increasing clinical and translational research efforts will lead to better understanding and patient care.

Keywords: myeloid malignancies; familial; sporadic; germline predisposition; genetic counseling

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Human and Medical Genetics *Poster presentation*

CHALLENGES IN VARIANT CURATION OF RARE DISEASES

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Rare diseases occur in a smaller proportion of the general population, which is defined as <1 in 2000 individuals. Although rare, they collectively make up ~7000 different disorders, with the majority having a genetic origin, and affect ~300 million people globally. Next generation sequencing (NGS) has had a significant impact on diagnosis because it allows for simultaneous analysis of multiple genes or entire genomes, increasing the chances of identifying disease-causing mutations while reducing the time and cost. However, advances in genomics are hindered by the difficulty in genome data analysis and interpretation. American College of Medical Genetics and Genomics–Association for Molecular Pathology (ACMG-AMP) guidelines provide recommendations for interpretation of pathogenicity of genetic variants. The presence of many VUS (variants of uncertain significance) even with ACMG guidelines is due to the complexity of genetic variations and the limitations of our current understanding. Here we will present several examples of challenging variant interpretations according to current ACMG guidelines. For example, UBE3A c.2344G>A, p.(Val782Ile) variant is associated with Angelman syndrome and classified as a VUS based on ACMG criteria: frequency data (unreliable data quality), computational analysis (non-deleterious impact) and it has been observed in at least 1 individual with a neurodevelopmental phenotype consistent with UBE3A-related disease, but the PS4 criteria cannot be applied due to the gnomAD frequency of this variant. These criteria indicate that the impact of this alteration on the *UBE3A* gene is currently inconclusive. *RUNX1* is a cancer predisposition gene associated with various hematological disorders, including familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML). According to ACMG guidelines, the classification of pathogenicity for c.601C>T (p.Arg201Ter) variant in the *RUNX1* gene is based on multiple lines of evidence, including population data, segregation data and computational predictions. These data collectively indicate that this variant has a detrimental effect on protein function or structure, leading to a higher risk of developing blood disorders. Overall, germline cancer variant curation facilitates personalized risk assessment, clinical decision-making, genetic counseling, research advancements, and the integration of genomic information into cancer care.

Keywords: ACMG guidelines; variant interpretation; rare disease

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Human and Medical Genetics *Poster presentation*

CHALLENGES IN VARIANT CURATION IN PROSTATE CANCER

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Prostate cancer is the most commonly diagnosed male malignancy and the fifth leading cause of cancer death in men worldwide. Molecular pathogenesis of prostate cancer includes several signaling pathways, such as DNA damage response pathways which have clinically actionable alterations in the majority of metastatic castration-resistant prostate cancers (mCRPC). Genomic analysis has accelerated the diagnosis, prognosis, and therapy for patients with prostate cancer. The most common genomic aberrations include the TMPRSS2-ERG gene fusion of diagnostic relevance, while aberrations of therapeutic relevance include variants in homologous recombination repair (HRR) genes: *ATM, BRCA1/2, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B/C/D* and *RAD54L*. Therefore, accurate variant curation, which implies the application of evidence-based methods for the interpretation of genetic variants, is of crucial significance for proper diagnosis, prognosis, and therapy. In this paper, the following variants have been curated according to the Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer by Li, et al. (2016): ATM p.D639Ifs*10, BRCA2 p.R3052L and CDK12 c.1046+1G>A. These frameshift, missense and splice site variants, respectively, have been classified as tier I variants with strong clinical significance - variants with level A therapeutic significance, which predict response to FDA-approved poly(ADP-ribose) polymerase (PARP) inhibitors olaparib and/or rucaparib targeting HRR-mutated mCRPC. In conclusion, proper variant curation assists and guides proper patient diagnosis, prognosis and treatment.

Keywords: prostate cancer; variant curation; HRR gene mutations; PARP inhibitors

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Human and Medical Genetics *Poster presentation*

UNVEILING AUTOPHAGY'S ROLE: INTERMITTENT FASTING AS A POTENTIAL CANCER CURE – A COMPREHENSIVE REVIEW

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Due to the rapid growth of cancer-induced deaths among the population, there is increased interest in finding the right cure. Among conventional ways to kill cancer cells, there is one we can seek in our body: the process of autophagy. Individuals at high risk of acquiring cancer may disregard the potential benefits of fasting as a preventative step while searching for the right medicine to battle the formation and growth of cancer cells. Fasting for 13 or more hours can be beneficial in terms of cancer prevention as well as a great tool when combined with chemotherapy. Autophagy is a catabolic process that produces double-membrane vesicles called autophagosomes. Those structures are responsible for the detection of problematic cell material and use it to fuel cellular repair mechanisms. Actually, unused cellular proteins and damaged organelles can be found as potential risks for cancer development. Fasting is a natural way for our organisms to clean cell's damaged material. The whole process of fasting can have a multifaceted impact, while triggering autophagy, it may slow down cancer cell growth, making them more sensitive to chemotherapy. It lowers insulin and insulin-like growth factor, creating an unfavorable environment for cancer cell proliferation. On the other side, autophagy can have a promotive tumorgenesis effect during the late stages of cancer, so the whole concept must be under supervision and control. But, evidence showed that cancer patients who combined fasting with chemotherapy showed significant results when compared to regular chemotherapy. Fasting-induced autophagy lowered the side effects of chemotherapy and suppressed tumorigenesis in such a way that it repaired the damaged DNA of normal cells, protecting them from destruction during chemotherapy. Since there is a positive correlation between intermittent fasting and autophagy in terms of cancer treatment and prevention, there remains a compelling need for more investigation of the molecular mechanisms that induce autophagy and the way that it corresponds to it and fights malignant cells. In-depth research of these mechanisms can be pivotal for optimizing and completing therapeutic strategies in terms of winning the race against malignant cells.

Keywords: autophagy; intermittent fasting; preventing cancer; chemotherapy;

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Human and Medical Genetics *Poster presentation*

SPONTANEOUS CHROMOSOMAL ABERRATIONS IN HUMAN LYMPHOCYTE CULTURES

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Changes in the structure or number of chromosomes that occur naturally, without exposure to external factors, such as radiation or chemicals, refer as spontaneous chromosomal aberrations. They are not inherited, occur randomly in the karyotype, and do not have direct clinical significance. However, they can provide valuable insights into genomic instability potential, and disease predisposition. They can be results of errors during DNA replication or repair processes, and typically are observed in cells that are actively dividing, such as lymphocytes during cultivation. Spontaneous chromosomal aberrations may arise due to the natural chromosomal instability and can be elevated in individuals environmentally or occupationally exposed to mutagens. We analyzed frequencies of spontaneous chromosomal aberrations in 137 individuals subjected to karyotyping at the Laboratory for Cytogenetics and Genotoxicology, University of Sarajevo – Institute for Genetic Engineering and Biotechnology, during 2008-2023. Whole blood samples were cultivated for 72 hours with the thymidine added in 48th hour. Metaphases were arrested by colcemid 60 minutes before harvesting. Following hypotonic treatment, cells were fixed and cell suspension was dropped on coded slides. GTG banding was performed and slides analyzed under 1000x magnification in the accordance with An International System for Human Cytogenetic Nomenclature and E.C.A. Cytogenetic Guidelines and Quality Assurance. Constitutionally aberrant karyotypes (47,XX,+21 and 47,XXY) were found in 2.92% of analysed samples as well as altered karyotype considered as normal chromosomal variants (46,XX,21ps+; 46,XX,1qh+; 46,XX,inv(9)(p11q13)). In total of 3092 analyzed metaphases, 20 spontaneous chromosomal aberrations (12 aneuploidies and 8 structural aberrations) were found in 13 individuals. This retrospective study contributes to the limited overall knowledge of cytogenetic status of Bosnian and Herzegovinian population. Accordingly, further monitoring of spontaneous chromosomal aberrations incidences in human lymphocytes culture is recommended. Chromosome aberration analysis in cultured human lymphocytes remains a fundamental method in genetics and genomics, with practical applications in environmental monitoring and medicine.

Keywords: karyotyping; aneuploidy; structural chromosome aberrations

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Human Forensic and Population Genetics *Poster presentation*

AN ASSESSMENT OF THE EFFICIENCY OF AUTOMATED DNA EXTRACTION ON RECENT AND ARCHAEOLOGICAL SKELETAL REMAINS

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Medieval Bosnia's archaeological sites hold a wealth of historical and cultural significance. Recently, some studies placed emphasis on DNA analysis of skeletal remains from various Bosnian necropolises, and this one brings information about rapid, automated DNA extraction from nine archaeological and seven recent samples. The aim of this study was to emphasize the rapid efficiency of automated DNA extraction method on the EZ2 Connect Fx device, thereby showcasing its ability to achieve swift and effective results compared to alternative methods. The genomic DNA extraction from 16 given samples was performed with a commercial EZ1&2® DNA Investigator® Kit. Final concentration of DNA was determined using Qubit™ Fluorometer*. Concerning archaeological samples, the lowest measured concentration was ≤0.010 ng/µL (sample A12/03-17), the highest measured concentration was 28.8 ng/µL (sample A4/05-19), and the concentration of sample A15/01-21 defied measurement. Among the recent samples, sample S1/03-16 displayed the highest concentration at 80.2 ng/µL, while sample O3/32-17 showed the lowest detected concentration at 0.010 ng/µL. Amplification of the isolated DNA was carried out with Investigator 24plex kit. DNA profiles were generated using 3500 Genetic Analyzer and further analyzed with GeneMapper™ ID-X 1.6 software. After obtaining DNA profiles of nine archaeological samples, eight of the total number were partial profiles (two samples showing less than 50% amplified loci and six displaying over 50% amplified loci), whereas sample A10/05-19 showed no amplified loci. Of the seven recent samples, two exhibited partial profiles, while five were complete profiles (all samples showed above 50% amplified loci). This study pointed to the benefits associated with automated DNA extraction, particularly the method's ability to generate results within a short timeframe. Additionally, automated DNA extraction offered the advantage of avoiding pipette and tip usage (with the exception of setting up sample incubation pre-isolation), which minimized the potential for exogenous DNA contamination and cross-contamination. High efficiency, a quality exuded by this method, holds paramount significance when working with skeletal remains, particularly archaeological skeletal remains that pose a challenge for molecular genetic analyses.

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Keywords: automated DNA extraction; archaeological skeletal remains; recent skeletal remains

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Human Forensic and Population Genetics *Poster presentation*

DETERMINING THE DEGREE OF KINSHIP AMONG CLOSE RELATIVES: OUR EXPERIENCE

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Here, we report on four cases of kinship testing among close relatives, including three "twin" cases, where we examined the type of kinship between twins' offspring – full sibship, first-degree relationship or half sibship, and on one complex kinship case where we were primarily requested to determine the probability that two individuals have the same father. In "twin" cases, 23 individuals in total were studied, of which three pairs of monozygous twins with their spouses/partners and with a total of 11 offspring. In the fourth case, samples for DNA analysis were collected from six individuals – Mother and her two daughters/siblings Daughter1a and Daughter2a, Son whose parents are Mother and FatherB, and Daughter2aB whose mother is Daughter2a. After creating pedigree charts, genomic DNA extraction and amplification of 22 autosomal STR markers using PowerPlex®Fusion System, electropherograms for all individuals were successfully generated. In all "twin" cases, paternity testing for 11 offspring of six couples resulted in PP (Probability of Paternity) values higher than 99.9999%, having confirmed the paternity for every descendant of all twin pairs. Kinship analyses involved setting up two opposite hypotheses, comparing the loci and shared allele variants between individuals, and calculating the LR (Likelihood Ratio) and KP (Kinship Probability) values. Full siblings, who have the same parents, displayed the largest number of shared alleles, with LRs significantly higher than other degrees of relatedness. Kinship analyses between twins' descendants showed higher possibility that they are half-siblings, rather than they are first cousins, what is in accordance with the fact that monozygous twins share an identical DNA material, and that their offspring, in genetical sense, share the same parent. Concerning the last case, kinship analysis revealed high probability that Son and Daughter2aB are half-siblings (LR=31.9329, KP=96.9635%) since they have a common father, what was previously determined through trio paternity testing (PPs over 99.99999%). Interestingly, kinship analysis carried on Daughter1a and Son showed 4,5 times higher probability that they are half-siblings contrary to probability of half sibship between Daughter2a and Son (KP=22.29477%). Finally, these results pointed up the importance of using larger number of autosomal STR loci in solving complex kinship cases.

Keywords: STR markers; kinship; close relatives; monozygous twins; statistical analysis

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Human Forensic and Population Genetics *Poster presentation*

ESTIMATING RELATEDNESS AND INBREEDING OF SMALL EAST AFRICAN GOATS USING PEDIGREE

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Goats are among the important livestock species produced under extensive systems in arid and semi-arid regions of many developing countries. They are resistant and adaptable to harsh climatic conditions and hence favorable to the majority of smallholder and pastoral farmers. The objective of this study was to evaluate the trends in inbreeding and average relatedness of Small East African goats in East Africa. The assessment was based on pedigree data of 2,162 animals born from 1983 to 2022. This data was sourced from the Kenya Studbook and the Sheep and Goats station, Naivasha. An increase in inbreeding was assessed for each generation by applying an algorithm based on grouping the ancestors of each animal chronologically using the longest ancestral path. Results showed that a total of 14.5 generations were traced, but only 5 generations were complete. This was due to the fact that many animals in earlier generations did not have parents' records. The average inbreeding and relatedness for small East African goats were 0.05 and 0.47. The average inbreeding and relatedness increased as generation increased, as inbreeding increased from the second maximum generation (0.21%) to fourteen maximum generations (0.83%), while the average relatedness increased from (0.02) in generation zero and 0.82 in generation five. The average increase in inbreeding per generation was 0.06%, which is within the FAO limit of a 1% annual increase for the breed to be classified as threatened with extinction. The results shed light on management practices in goat breeding and may be relevant in formulating the breeding policy. Therefore it is advised to monitor the breed's genetic structure on a regular basis to avoid unnecessary inbreeding.

Keywords: average relatedness, East African goat, inbreeding coefficient, pedigree analysis
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Human Forensic and Population Genetics *Poster presentation*

GENETIC DIVERSITY OF THE *QUERCUS ROBUR* L. POPULATION FROM THE PROTECTED AREA „KOŠUTNJAK FOREST" (BELGRADE, SERBIA) ASSESSED BY NUCLEAR MICROSATELLITES

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Pedunculate oak (*Quercus robur* L.) is one of the economically and ecologically most important deciduous forest tree species in Europe. In recent decades, there has been an evident decline of pedunculate oak forests, caused by various factors, such as climate change, site conditions, over-exploitation, or insufficient and inadequate regeneration. In Serbia, this species usually occurs in the valleys of major rivers (Danube, Sava, and Morava), and the most valuable pedunculate oak forests grow along the river Sava in the area of Srem, where there are individual specimens aged hundreds of years. The protected area „Košutnjak Forest" (Belgrade, Serbia), occupies 267 ha, with numerous forest tree species, among which are five native oak species, including the pedunculate oak. Oaks can be categorized as „at-risk" species in „Kosutnjak Forest", since their populations are mostly represented by very old trees, with a poor possibility of natural rejuvenation. This research aimed to determine the genetic variability of the pedunculate oak population in the „Košutnjak Forest" using nuclear microsatellites. DNA extraction was performed from young leaves, collected from 56 adult trees, using a commercial peqGOLD Plant DNA Mini Kit (PEQLAB). In total 13 nuclear microsatellites were used, and values of standard genetic diversity parameters were calculated using *GenAlEx* 6.5 software. The number of alleles per locus ranged from 8 (MSQ13 and PIE239) to 39 (QrZAG90), with an average of 17.538. The number of effective alleles per locus was in the range from 2,498 (QrZAG108) to 23.668 (QrZAG90), with an average of 8.360. The average value of the observed heterozygosity (Ho) was 0.705, and the average unbiased expected heterozygosity (uHe) was 0.818. A statistically significant deviation of the expected heterozygosity from the observed heterozygosity occurs at 5 loci (MAQ4, PIE239, QpZAG104, QrZAG108, and MSQ13). The average values of the fixation index were positive and statistically significant, indicating an excess of homozygotes. According to the assessment of the genetic status, determining the level of genetic variability of pedunculate oak served as a basis for defining *in situ* conservation measures for the available gene pool in „Kosutnjak Forest".

Keywords: pedunculate oak; conservation; forest genetic resources; molecular markers

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Human Forensic and Population Genetics *Poster presentation*

DOES THE GEOGRAPHIC DISTANCE EFFECT THE GENETIC DIFFERENTATION AMONG BILBERRY POPULATIONS SAMPLED IN BOSNIA AND HERZEGOVINA?

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Isolation-by-distance (IBD) pattern among bilberry (*Vaccinium myrtillus* L.) populations has previously been reported for this species in northern Europe. However, the number of molecular studies conducted on bilberry, using everything from isoenzymes, RAPDs to microsatellite markers, are very few and far between. Considering that Bosnia and Herzegovina (B&H) is a country rich with diverse fruit genetic resources, conducting a genetic characterization of the naturally occurring *V. myrtillus* populations could yield valuable data for the conservation and utilization of this resource. This study entailed genotyping samples collected from three bilberry populations located in Fojnica, Kladanj, and Srebrenica municipalities using seven polymorphic microsatellite or SSR (simple sequence repeats) markers. The obtained molecular data was used to calculate the correlation between the physical distance of the individual B&H populations and a parameter of the genetic differentiation (pairwise Fst). The results of the correlation analyses revealed an absence of a significant isolation-by-distance pattern among the three examined B&H bilberry populations. In addition, the most pronounced genetic differentiation was detected between the Srebrenica and each of the two remaining B&H populations. At the same time, the values for pFst were significant, albeit much lower, between the Fojnica and Kladanj populations. Bilberries from the sampled Srebrenica population appear to be distinct from the other B&H populations, possibly due to the different genetic origin of this population.

Keywords: *Vaccinium myrtillus* L.; microsatellite markers; isolation-by- distance

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Human Forensic and Population Genetics *Poster presentation*

DETECTION OF EVOLUTIONARY LINEAGES OF SALMONID SAMPLES FROM THE ZETA RIVER USING MOLECULAR GENETIC AND BIOINFORMATICS ANALYSES

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Over the years, the use of genetic and molecular tools has made a significant contribution not only to various aspects of scientific research but also to other fields as well, such as aquaculture. Regarding aquaculture, molecular genetic analysis and bioinformatics have provided great insight into the biodiversity that underlies freshwater ichthyofauna and fisheries, on species and population levels. We analyzed 18 samples of brown trout taken from the Zeta River in Montenegro to examine haplotype differences. RFLP analysis and sequencing of the mitochondrial DNA control region were performed to detect the evolutionary lineages in the studied population. Three major haplogroups have been detected, previously reported in the Balkan rivers: two Adriatic (AdN and Ad-Prz) and Danube (Da). Out of 18 typed specimens, 55.56% were of Ad-Prz, 22.22% AdN, and 22.22% Da lineage. The importance of such analysis is reflected in the knowledge of the status of native specimens and whether further control of fisheries is necessary, as the allochthonous haplogroups tend to have better survival rates and are better adapted to freshwater and inland fisheries, which eventually leads towards the decline of native species and populations. Therefore, the use of appropriate molecular and genetic tools and proper analysis of the results, can help us better understand the broodstock status for better management and conservation of autochthonous species.

Keywords: aquaculture; fisheries; genetic; haplogroups; brown trout

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Human Forensic and Population Genetics *Poster presentation*

GENETIC DIFFERENTIATION BETWEEN TWO AUTOCHTHONOUS BOSNIAN DOG BREEDS

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It has been estimated that there are over 400 existing breeds of domestic dogs today. Phenotypically, domestic dog is one of the most diverse animal species due to long cohabitation with humans. According to the Fédération Cynologique Internationale (FCI), Bosnia and Herzegovina is recognized as the country of origin for two autochthonous dog breeds: Bosnian and Herzegovinian—Croatian Shepherd dog Tornjak and Bosnian Broken-Haired Hound—Barak. Microsatellite diversity of Barak and Tornjak breeds and their genetic differentiation were analyzed. StockMarks® for Canine Genotyping Kit (Applied Biosystems) was used to amplify ten microsatellite loci. Allele frequencies, observed and effective number of alleles and their ratio, observed and expected heterozygosity, number of genotypes, genotypic frequencies, PIC (Polymorphism Information Content), MAF (Major Allele Frequency), and deviation from Hardy–Weinberg equilibrium were calculated. Genetic differentiation (Fst), AMOVA (analysis of molecular variation), FCA analysis (Factorial Correspondence Analysis), and STRUCTURE analysis were performed to estimate an intergroup genetic variability of the two breeds. Genetic distance according to the Nei model was also estimated. Total genetic differentiation was 0.0469, with the highest value for PEZ8 locus and the lowest for FHC2010 locus. Results of AMOVA showed that 91% of total genetic variation was within individuals, 4% between individuals, and 5% between two breeds. Differences in allele frequencies of observed loci were statistically significant, except for FHC2010 loci. FCA results indicate a clear differentiation between the two breeds. According to the STRUCTURE analysis results, 93.5% of Barak specimens were grouped in Cluster I and 6.5% in Cluster II, while 34.5% of Tornjak specimens belonged to Cluster I and 65.5% to Cluster II. Concerning the observed microsatellite loci, Barak and Tornjak do not have an evident genetic differentiation. However, allelic structure and their frequencies within these two breeds indicate a clear genetic position of diversity.

Keywords: Tornjak; Barak; microsatellite loci; genetic diversity

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