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Welcoming address,

Dear colleagues,

It is my great fortune and privilege to be able to address this forum in my role as a Chairman of Genetic Association in Bosnia and Herzegovina (GENuB&H) and Organizing Committee of the Congress. I am proud to be your host, here in Sarajevo, at the 1st Congress of Geneticists in Bosnia and Herzegovina with International Participation that has gathered significant assembly of researchers working in various fields of genetics.

GENuB&H successfully organized The First (2011 in Sarajevo) and The Second Symposium of BH Geneticists (2015 in Banja Luka) that included noteworthy presentations by international authors and bh. diaspora. With this Congress, we enlarge our perspectives and diversity of affiliations and countries included. With this special edition of Genetics and Application journal the Congress has obtained support from the University of Sarajevo - Institute for Genetic Engineering and Biotechnology as its publisher but also contributed to the promotion of this young scientific journal.

We hope that the Congress will provide a platform where some important topics in contemporary genetics may be addressed. We expect fruitful Round table discussion that will identify changes in conducting and presenting science that need to be made if science is to assume its role as driving force of society.

Significant interest in the Congress was sparked through COST Action hCOMET - The *comet* assay as a human biomonitoring tool (CA15132) COST action. University of Sarajevo – Institute for Genetic Engineering and Biotechnology and GENuB&H joined their efforts in organizing international „Comet workshop – Basic comet assay techniques“, as an auxiliary event.

I extend my gratitude to all my colleagues from Organizing and Scientific Committee as well as all the young colleagues that invested their knowledge, effort and time and turned an idea of a congress into a real life event.

This gathering and our work would not be possible without support of Ministry of Civil Affairs of Bosnia and Herzegovina and Federal Ministry of Science and Education. We are also proud that BH Futures Foundation gladly provided support for the youngest scientists thus addressing an upsetting and growing brain drain trend. Equally important are contributions of all our sponsors and supporters. And final gratitude goes to all our colleagues and friends that supported GENuB&H throughout the preparations phase be it with an advice, an idea or simple word of encouragement.

Kasim Bajrović

SCIENTIFIC PROGRAMME

1st Congress of Geneticists in Bosnia and Herzegovina with International Participation
Sarajevo, Bosnia and Herzegovina, 02nd - 04th October, 2019.

WEDNESDAY 2nd October

14:00 **Registration desk opening and poster mounting**

15:00 **Opening ceremony: Welcome note**

Chairs: **Kasim Bajrović, Stojko Vidović, Andrew Collins**

15:15 – 15:50 **PL1 Adaleta Durmić-Pašić** *(University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina):* A SHORT HISTORY OF GENETICS IN BOSNIA AND HERZEGOVINA

15:50 – 16:25 **PL2 Andrew Collins** *(University of Oslo; Department of Nutrition, Oslo, Norway):* THE COMET ASSAY; STATE OF THE ART

16:25 – 17:00 **PL3 Matthew R. Willmann** *(Cornell University, Plant Transformation Facility, Ithaca, New York, United States of America):* THE IMPACT OF GENOME EDITING ON CROP IMPROVEMENT

17:00 – 17:40 **Coffee Break**

17:40 – 18:15 **PL4 Joao Teixeira** *(National Institute of Health, Environmental Health Department, Porto, Portugal):* GENETIC DAMAGE OF OCCUPATIONAL EXPOSURE TO STYRENE

18:20 – 18:55 **PL5 Zvonimir Marelja** (Institut Imagine, Paris, France): LYSOSOMAL CYSTINE EFFLUX OPPOSES MTORC1 REACTIVATION UPON FASTING THROUGH THE TCA CYCLE

19:00 – 19:35 **PL6 Zoran Galić** *(UCLA, David Geffen School of Medicine, Department of Medicine, Division of Hematology/Oncology, Los Angeles, United States of America):* DERIVATION OF GENETICALLY MODIFIED T CELLS FROM HUMAN EMBRYONIC STEM CELLS

19:40 **Vocal choir ensemble „Corona“ performance**

20:00 **GENuB&H Awards Presentation**

THURSDAY 3th of October

Invited lectures

Chairs: **Lejla Kapur Pojskić, Irena Drmić Hofman, Duan Chen**

09:00 **I1 Dijana Plašeska Karanfilska** *(Macedonian Academy of Sciences and Arts, Research Center for Genetic Engineering and Biotechnology, Skopje, Macedonia):* THE COMING AGE OF POLYGENIC RISK SCORES: TOWARDS PERSONALIZED BREAST CANCER RISK ASSESSMENT AND PREVENTION

09:30 **I2 Irena Drmić Hofman** *(University of Split, School of Medicine Split, Croatia):* DETECTION OF BRCA1 AND BRCA2 MUTATIONS IN OVARIAN CANCER BY NEXT- GENERATION SEQUENCING

10:00 **I3 Borut Peterlin** *(Clinical Institute of Medical Genetics, Ljubljana Slovenia):* NEW GENOMIC TECHNOLOGIES FOR IMPROVEMENT OF NATIONAL HEALTH SYSTEM

10:30 -11:00 **Coffee Break**

Oral presentations

11:00 **O1. Branka Zukić** *(University of Belgrade, Institute of Molecular Genetics and*

Genetic Engineering, Belgrade, Serbia): NEXT-GENERATION SEQUENCING AS A TOOL TO STUDY RARE DISEASES

11:20 **O2. Mensuda Hasanhodžić** *(University Clinical Centre Tuzla, Policlinic of Medical Genetics with Genetic Counselling for Out-Patient Care, Department of Paediatrics, Tuzla, Bosnia and Herzegovina):* THE GENETIC CAUSES OF THE HEARING IMPAIRMENT IN BOSNIAN POPULATION

11:40 **Ivo Barić** *(University Hospital Centre Zagreb, Croatia)*

12:10 **O3. Rijad Konjhodžić** *(ALEA Genetic Center, Sarajevo, Bosnia and Herzegovina):* PATHOGENIC MUTATIONS ASSOCIATED WITH COLORECTAL CANCER AND NON-SMALL CELL LUNG CANCER IN BOSNIA AND HERZEGOVINA POPULATION

12:30 **Poster Session 1 (Odd poster numbers)**

13:00 – 13:40 **Lunch Break**

Oral presentations

Chairs: **Borut Peterlin, Mensuda Hasanhodžić, Branka Zukić**

13:40 **O4. Duan Chen** *(Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine, Trondheim, Norway):* PROTEOMICS-BASED DEVELOPMENT OF NEW TREATMENT FOR PANCREATIC CANCER

14:00 **O5. Chun-Mei Zhao** *(Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine, Trondheim, Norway):* TARGETING NERVE-CANCER METABOLISM IMPROVED OVERALL SURVIVAL IN AGED MICE WITH GASTRIC CANCER

14:20 **O6. Mirella Perruccio** (*Qiagen GmbH, Hilden, Germany):* A BRIEF STORY OF QIAGEN IN THE FORENSIC FIELD

14:40 – 15:10 **Coffee Break**

Oral presentations

15:10 **O7. Matea Zajc Petranović** *(Institute for Anthropological Research, Zagreb, Croatia):* THE VARIABILITY OF DETOXIFYING *GSTP1* GENE POLYMORPHISMS IN THE ROMA POPULATION FROM CROATIA

15:30 **O8. Süreyya Bozkurt** *(Istinye University, Faculty of Medicine, Department of Medical Biology, Istanbul,Turkey):* MONOSOMAL KARYOTYPE IN MYELODISPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA PATIENTS

15:50 **O9. Josip Brajković** *(Labena d.o.o, Zagreb, Croatia):* ddPCR – NEW GENERATION OF PCR TECHNOLOGY

16:10-18:10 **Round table discussion**

Chair: Lejla Kapur Pojskić

Genetic testing for diagnosis and treatment of rare disorders / Networking event for genetic testing laboratories, medical specialists, geneticists and regulatory representatives involved in the topic

(All interested participants should register for the meeting. Separate agenda for the meeting will be provided)

19:30 **CONGRESS DINNER**

FRIDAY 4th of October

Invited lectures

Chairs: Emina Kiseljaković, Nataša Golić, Joao Teixeira

09:00 **I4 Nataša Golić** *(University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Laboratory for Molecular Microbiology, Belgrade, Serbia):* GUT MICROBIAL-HOST CROSS TALK IN HUMAN HEALTH AND WELLBEING

09:30 **I5 Jadranka Vraneković** *(University of Rijeka, School of Medicine, Department of Medical Biology and Genetics, Rijeka, Croatia):* POLYMORPHISMS IN FOLATE PATHWAY GENES AS RISK FACTORS FOR CONGENITAL HEART DEFECTS IN DOWN SYNDROME

10:00 **I6 Nermin Gözukirmizi** *(Istinye University, Department of Molecular Biology and Genetics, Istanbul, Turkey):* lncRNAs

10:30 – 11:00 **Coffee Break**

11:00 **I7 Amaya Azqueta** *(University of Navarra Department of Pharmacology and Toxicology, IdiSNA, Navarra Institute for Health Research):* MODIFICATIONS OF THE COMET ASSAY FOR DETECTING DIFFERENT DNA LESIONS

11:30 **I8 Pourrut Bertrand** *(Université de Toulouse; UPS, INP; EcoLab (Laboratoire d'écologie fonctionelle et environnement); ENSAT, Toulouse, France):* PLANT COMET ASSAY IN ENVIRONMENTAL STUDIES: USES, LIMITS AND PERSPECTIVES

Oral presentations

12:00 **O10. Milena Janković** *(University of Belgrade, School of Medicine, Neurology Clinic, Clinical Centre of Serbia, Belgrade, Serbia):* ANGIOGENIN GENE MUTATIONS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS FROM TERTIARY CENTER IN BELGRADE

12:20 **O11. Nurşen Başaran** *(Hacettepe University, Faculty of Pharmacy Department of Pharmaceutical Toxicology, Ankara, Turkey):* DOES CINNAMIC ACID HAVE BENEFICIAL EFFECTS ON DIABETES INDUCED GENOTOXICITY?

12:40 **O12. Mirta Milić** *(Institute for Medical Research and Occupational Health, Mutagenesis Unit, Zagreb, Croatia):* THE INFLUENCE OF A THREE-WEEK HYPOCALORIC DIET ON DNA DAMAGE PARAMETERS MEASURED BY ALKALINE COMET ASSAY AND CYTOCHALASIN B-BLOCKED MICRONUCLEUS ASSAY IN OBESE PATIENTS FROM THE SPECIAL HOSPITAL FOR EXTENDED TREATMENT OF DUGA RESA, CROATIA – PRELIMINARY RESULTS

13:00 – 14:00 **Lunch Break**

14:00 **Poster Session 2 (Even poster numbers)**

14:30 **PhD Students Session – Oral and poster presentations**

Chairs: **Jadranka Vraneković, Marija Vuković, Lada Lukić Bilela**

14:30 **OS1. Ivana Šolić** *(University of Split, School of Medicine, Department of Anatomy, Histology and Embryology, Split, Croatia):* GENE EXPRESSION OF α-TUBULIN, INVERSIN AND DISHVELLED-1 IN POSTNATAL KIDNEY TISSUE

14:40 **OS2. Sumejja Baljević** *(University of Sarajevo, Clinical Center, Department of Clinical Pathology, Cytology and Human Genetics, Sarajevo, Bosnia and Herzegovina):* ROLE OF FLOW CYTOMETRY IN DIAGNOSIS OF LARGE GRANULAR LYMPHOCYTE LEUKEMIA

14:50 **OS3. Anesa Ahatović** *(University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Zmaja od Bosne 8, Kampus, Sarajevo, Bosnia and Herzegovina):* EVALUATION OF HEAVY METAL TOLERANCE IN SERPENTINE RHIZOBACTERIA ASSOCIATED WITH *MEDICAGO LUPULINA* L.

Graduate Students Session – Poster presentations

Undergraduate Students Session – Poster presentations

15:10 **Announcement of the award at the end of the Session**

15:30 **Sightseeing Tour (Closing of the Congress)**

5th and 6th of October

"COMET Workshop – Basic Comet assay techniques"
Sarajevo, Bosnia and Herzegovina

Coorganized and cohosted by:
Genetic Association in Bosnia and Herzegovina
CA15132 hCOMET Cost Action
University of Sarajevo – Institute for Genetic Engineering and Biotechnology

Posters

P01. Grażyna Adler (Pomeranian Medical University, Department of Studies in Antropogenetics and Biogerontology, Szczecin, Poland): RELATIONSHIP BETWEEN *H1* AND *H2* HAPLOTYPES OF THE 17q21 INVERSION AND PREGNANCY LOSS IN BOSNIAN POPULATION: A CASE-CONTROL STUDY

P02. Aida Ćatić (Institute for Gynecology, Perinatology and Infertility "Mehmedbašić", Sarajevo, Bosnia and Herzegovina): DECLINING RATE OF INVASIVE PROCEDURES FOR PRENATAL DIAGNOSIS OBSERVED IN A PRIVATE PERINATAL CLINIC "MEHMEDBAŠIĆ"

P03. Branko Tomić (University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia): FREQUENCY OF FV LEIDEN AND FII G20210A HOMOZYGOTES AND COMBINED CARRIERS IN LARGE COHORT OF PATIENTS FROM SERBIA

P04. Sajra Komić (University of Sarajevo, Faculty of Pharmacy, Department of Biochemistry and Clinical Analysis, Sarajevo, Bosnia and Herzegovina)*:* MICRO RNA AS A NOVEL BIOMARKER FOR DIAGNOSIS, PROGNOSIS AND MANAGEMENT FOR PATIENTS WITH *DIABETES MELLITUS*

P05. Šaćira Mandal (University of Sarajevo, Faculty of Pharmacy, Department of Natural Sciences in Pharmacy, Sarajevo, Bosnia and Herzegovina): ASSOCIATION OF *FADS1* GENE POLYMORPHISM WITH DESATURASE ACTIVITY IN TYPE 2 DIABETES

P06. Irina Milovac (University of Banja Luka, Faculty of Medicine, Department of Human Genetics, Banja Luka, Bosnia and Herzegovina): *DAT* POLYMORPHISM AND ITS ASSOCIATION WITH IRITTABLE BOWEL SYNDROME

P07. Višnja Tomac (Osijek University Hospital, Department of neurology, genetic, metabolic disease and endocrinology Osijek, Croatia; University of Osijek, Faculty of Medicine, Osijek, Croatia): A RARE MICRODELETION OF CHROMOSOME 19q13.43 SYNDROME – PSYCHOMOTOR DELAYED, INTELECTUAL DISABILLITY, EPILEPSY - CASE REPORT TWO PATIENTS

P08. Višnja Tomac (Osijek University Hospital, Department of neurology, genetic, metabolic disease and endocrinology Osijek, Croatia; University of Osijek, Faculty of Medicine, Osijek, Croatia): A RARE DE NOVO DUPLICATION 21q22.3 SYNDROME - CASE REPORT

P09. Anita Skakić (University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia): GENOMIC PROFILING OF GLYCOGEN STORAGE DISEASES

P10. Josip Brajković (Labena d.o.o, Zagreb, Croatia): ddPCR, SIMPLE, ACURATE AND RELIABLE TOOL FOR PRECISION DIAGNOSTICS

P11. Marina Anđelković (University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia): DIFFERENTIAL DIAGNOSIS OF PATIENTS WITH PEDIATRIC LUNG DISEASES AND DISCOVERY OF NOVEL DISEASE-CAUSING GENES AND GENETIC VARIANTS BY USING NEXT GENERATION SEQUENCING TECHNOLOGY

P12. Marija Vuković (University Clinical Center of Republic of Srpska, Banja Luka, Bosnia and Hercegovina): THE INFLUENCE OF *UGT1A1* PROMOTER VARIANTS ON THE BILIRUBIN LEVEL IN SERUM OF PATIENTS WITH β-THALASSEMIA MINOR AND CHRONIC HEPATITIS C

P13. Mirjana Berić (University Clinical Center of the Republic of Srpska, Department of Medical Genetics, Banja Luka, Bosnia and Herzegovina): INFLUENCE OF PRENATAL CYTOGENETIC DIAGNOSTICS IN REDUCTION OF NUMBER OF NEWBORN CHILDREN WITH DOWN SYNDROME AT THE UNIVERSITY CLINICAL CENTER OF THE REPUBLIC OF SRPSKA FROM 2009-2019 YEAR

P14. Svjetlana Đajić Uletilović (University Clinical Center of Republic of Srpska, Department of Medical Genetics, Banja Luka, Bosnia and Herzegovina): TWO CASES REPORT OF UNBALANCED KARYOTYPE 45,XX,der(4)t(4;22)(p16;q11.2),-22

P15. Emina Kiseljaković (University of Sarajevo, Faculty of Medicine, Department of Medical Biochemistry): METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM (MTHFR) AS A NON-TRADITIONAL RISK FACTOR OF CARDIOVASCULAR DISEASES IN PATIENTS ON HEMODIALYSIS

P16. Adisa Ahmić (University of Tuzla, Faculty of Science, Department of Biology, Tuzla, Bosnia and Herzegovina): Y-CHROMOSOME HAPLOGROUP DIVERSITY OF THE ROMA POPULATION OF NORTH-EASTERN BOSNIA AND HERZEGOVINA

P17. Abdurahim Kalajdžić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, B&H): PREDICTION OF Y HAPLOGROUP IN ANALYSIS OF HUMAN SKELETAL REMAINS FROM ARCHAEOLOGICAL SITES IN BOSNIA AND HERZEGOVINA

P18. Abdurahim Kalajdžić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, B&H): DNA ANALYSIS OF SKELETAL REMAINS: CASE OF DISPUTABLE KINSHIP TESTING

P19. Amira Kekić (Ministry of Internal Affairs of Canton Sarajevo, Sarajevo, Bosna and Herzegovina): DNA ANALYSIS OF BLOODSTAINS FROM CRIME SCENE: CASEWORKS EXPERIENCE

P20. Zsófia Szilágyi (National Public Health Center, Division of Radiobiology and Radiohygiene, Department of Non-ionizing Radiation): COMBINED EXPOSURE OF RADIOFREQUENCY AND UV RADIATION INDUCE ADAPTIVE RESPONSE IN KERATINOCYTE CELLS *IN VITRO*

P21. Anna Sáfár (National Public Health Center, Department of Non-ionizing Radiation, Budapest, Hungary): A HIGH-THROUGHPUT COMET ASSAY METHOD FOR THE JOINED PROCESSING OF SAMPLES TAKEN SEPARATELY

P22. Anja Haverić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): ANALYSIS OF DNA DAMAGE IN PERIPHERAL BLOOD CULTURES TREATED WITH CURCUMIN OR SUNSET YELLOW

P23. Nevenka Veličkova (University "Goce Delcev", Faculty of Medical Sciences, Stip, Republic of North Macedonia): THE IMPORTANCE OF NUCLEAR DIVISION INDEX IN BIOMONITORING HUMAN STUDIES USING THE MICRONUCLEUS ASSAY

P24. Nurşen Başaran (Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkey): GENOTOXICITY EVALUATION OF URSOLIC ACID ON MAMMALIAN CELL LINES BY MICRONUCLEUS ASSAY

P25. Edvina Ibradžić (The Public Institution Primary School "Harmani I", Bihać, Bosnia and Herzegovina): EVALUATION OF THE GENOTOXICITY POTENTIAL OF WATER FROM THE LAKE HAZNA FROM BOSNIA AND HERZEGOVINA BY MICRONUCLEUS ASSAY IN ERYTHROCYTES OF *CARASSIUS GIBELIO* AND *ALLIUM CEPA* L. ASSAY

P26. Ajla Smajlović (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): THE INFLUENCE OF HALOGENATED BOROXINE ON THE DEREGULATION OF THE GENES CAUSED BY IMIQUIMOD INDUCED INFLAMMATORY DERMATOSIS IN RATS

P27. Nikolina Elez-Burnjaković (University of East Sarajevo, Medical Faculty, Foča, Bosnia and Herzegovina): HALOGENATED BOROXINE INFLUENCE ON BASAL LEVEL AUTOPHAGY IN HUMAN MELANOMA GR-M CELL LINE

P28. Maida Hadžić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, 71000 Sarajevo, Bosnia and Herzegovina): CYTOTOXICITY OF 1-SUBSTITUTED 1,2,3,4-TETRAHYDROISOQUINOLINES IN 5637 HUMAN BLADDER CARCINOMA CELL LINE

P29. Tamara Ćetković (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): CYTOTOXICITY EVALUATION OF HIGHLY OXIDIZED GRAPHENE ON PERIPHERAL BLOOD MONONUCLEAR CELLS

P30. Dunja Rukavina (University of Sarajevo, Veterinary Faculty, Department of Biology, Sarajevo, Bosnia and Herzegovina): THE GENETIC STRUCTURE OF POTENTIAL BOSNIAN MOUNTAIN HORSES BASED ON MICROSATELLITE MARKERS

P31. Jasna Hanjalić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): DNA BARCODING OF FOUR BUTTERFLY SPECIES OF GENUS *ARGYNNIS* FABRICIUS, 1807 (NYMPHALIDAE: HELICONIINAE) FROM BOSNIA AND HERZEGOVINA

P32. Lejla Lasić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): OPTIMIZATION OF NESTED POLYMERASE CHAIN REACTION CONDITIONS FOR MOLECULAR-GENETIC DETECTION OF *BORRELIA BURGDORFERI* SENSU LATO IN *IXODES RICINUS* TICKS

P33. Faruk Bogunić (University of Sarajevo, Faculty of Forestry, Sarajevo, Bosnia and Herzegovina; University of Sarajevo, Faculty of Science, Department of Biology, Laboratory for research and protection of endemic resources, Sarajevo, Bosnia and Herzegovina): DIVERSITY OF REPRODUCTIVE PATHWAYS IN *COTONEASTER INTEGERRIMUS* (ROSACEAE) IS DRIVEN BY HETEROPLOID CROSSES AND APOMIXIS

P34. Marie Florence Sandrine Ngo Ngwe (National Herbarium of Cameroon, Institute of Agricultural Research for Development, Yaoundé, Cameroon): HETEROCHROMATIN AND rDNA PATTERN REVEALED HETEROMORPHIC SEX CHROMOSOMES IN *DIOSCOREA DUMETORUM*

P35. Mirzeta Memišević Hodžić (University of Sarajevo, Faculty of Forestry, Sarajevo, Bosnia and Herzegovina): HETEROZYGOSIS AS A MEASURE OF THE GENETIC VARIABILITY OF PEDUNCULATE OAK (*QUERCUS ROBUR*, L.) IN THE BOSNIAN-HERZEGOVINIAN PROVENANCE TEST

P36. Paulina Šaravanja (University of Mostar, Faculty of Agriculture and Food Technology, Mostar Bosnia and Herzegovina): INVESTIGATION OF THE GENETIC DIVERSITY OF POMEGRANATE IN HERZEGOVINA

P37. Jasmin Ramić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): BACTERIAL GENOME IDENTIFICATION FROM FECAL SAMPLES OF SUBJECTS WITH IRRITABLE BOWEL SYNDROME (IBS)

P38. Kamelija Madacki-Todorović (University Sarajevo School of Science and Technology, Sarajevo Medical School, Sarajevo, Bosnia and Herzegovina): DRUG-INDUCED MODULATORS OF *ESHERICHIA COLI* VIRULENCE GENE EXPRESSION

P39. Naida Mulahuseinović (General Hospital, Tešanj, Bosnia and Herzegovina): PREVALENCE OF *MYCOPLASMA HOMINIS, UREAPLASMA SPP.* AND *CHLAMYDIA TRACHOMATIS* IN ROUTINE GYNECOLOGICAL EXAMINATION IN TEŠANJ AREA

P40. Mujo Hasanović (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): ANTIMICROBIAL SUSCEPTIBILITY OF BIOFILM-PRODUCING AND NON-PRODUCING *PSEUDOMONAS AERUGINOSA* ISOLATES IN THE NON-HOSPITAL ENVIRONMENT

PhD Students Session

PS01. Vanja Vidović (University of Banja Luka, Faculty of Medicine, Department of Human Genetics, Banja Luka, Bosnia and Herzegovina): FINDINGS FROM ACGH IN PATIENT WITH PSYCHOMOTOR DELAY-CASE REPORT

PS02. Maja Barbarić (University of Mostar, School of Medicine, Laboratory of Morphology, Department of Histology and Embryology, Mostar, Bosnia and Herzegovina; University Hospital in Mostar, Department of Pathology, Cytology and Forensic Medicine, Mostar, Bosnia and Herzegovina): IMP3 EXPRESSION IS DECREASED IN TROPHOBLAST CELLS FROM PREGNANCIES WITH SEVERE AND NON-SEVERE PREECLAMPSIA

PS03. Anita Kolobarić (University of Mostar, School of Medicine, Laboratory of Morphology, Department of Histology and Embryology, Mostar, Bosnia and Herzegovina; University Hospital in Mostar, Department of Pediatrics, Mostar, Bosnia and Herzegovina): EXPRESSION AND DISTRIBUTION OF FIBROBLAST GROWTH FACTOR RECEPTOR 1, 2 AND OTHER SIGNAL PROTEIN IN HUMAN FETAL LUNG

PS04. Anita Racetin (University of Split, School of Medicine, Department of Anatomy, Histology and Embryology, Split, Croatia; University of Mostar, School of Medicine, Mostar, Bosnia and Herzegovina): CRKL GENE EXPRESSION IN KIDNEYS OF *YOTARI* MICE

PS05. Marija Jurić (University of Split, School of Medicine, Department of Anatomy, Histology and Embryology, Split, Croatia): AIFM3 GENE EXPRESSION IN KIDNEYS OF *YOTARI* MICE

PS06. Ivona Kosović (University of Split, School of Medicine, Department of Anatomy, Histology and Embryology, Split, Croatia): CX37, CX40, CX43 AND CX45 GENE EXPRESSION IN DEVELOPING, POSTNATAL AND NEPHROTIC HUMAN KIDNEYS

PS07. Sumejja Baljević (University of Sarajevo, Clinical Center, Department of Clinical Pathology, Cytology and Human Genetics Sarajevo, Bosnia and Herzegovina): PRESENCE OF COMPLEX COMBINED HEMATOLOGIC NEOPLASMS – CASE REPORT

PS08. Sanja Ćakić (University of Belgrade, Institute for Medical Research, Department for Medical Entomology, Belgrade, Serbia): MULTILOCUS SEQUENCE TYPING ANALYSIS OF *BORRELIA AFZELII* STRAINS ISOLATED FROM *IXODES RICINUS* TICKS FROM SERBIA

PS09. Dijana Topalović (University of Belgrade, Faculty of Pharmacy, Department of Pathobiology, Belgrade, Serbia): ANTIGENOTOXIC EFFECT OF QUERCETIN ON THYROXINE-INDUCED DNA DAMAGE IN HUMAN WHOLE BLOOD CELLS *IN VITRO*

PS10. Marija Bruić (University of Belgrade, Faculty of Pharmacy, Department of Pathobiology, Belgrade, Serbia): ASSESSMENT OF DNA DAMAGE IN BLOOD, LIVER AND KIDNEY CELLS IN A HYPERTENSIVE RAT MODEL USING COMET ASSAY

PS11. Ana Huđek (University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia): GENOTOXIC EFFECT OF IRINOTECAN ON HUMAN LIVER AND COLON TUMOR CELLS

PS12. Maida Hadžić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): $K_2[B_3O_3F_4OH]$ INDUCED APOPTOSIS AND REDUCED CELL VIABILITY IN HUMAN ACUTE MYELOID *LEUKEMIA* CELL LINE UT-7

PS13. Jelena Petković (University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia): CELLULAR FACTORS INVOLVED IN RECONSTITUTION OF OXIDATIVE DAMAGED POPULATION OF *USTILAGO MAYDIS*

PS14. Dragana Bosnić (University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Laboratory for Plant Molecular Biology, Belgrade, Serbia): SILICON ALLEVIATES COPPER TOXICITY IN CUCUMBER BY INCREASED CU-BINDING CAPACITY AND ENHANCED ANTIOXIDATIVE DEFENSE

PS15. Ivana Nikolić (University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Laboratory for Plant Molecular Biology, Belgrade, Serbia): GENERATION OF STABILE *ARABIDOPSIS DSSI* MUTANTS USING CRISPR-CAS9 TECHNOLOGY

PS16. Jasna Hasanbegović (University Džemal Bijedić, Agromediterranean Faculty, Mostar, Bosnia and Herzegovina): GENETIC CHARACTERISATION OF ALMOND (*PRUNUS AMYGDALUS L.)* USING MICROSATELLITE MARKERS

Graduate Students Session

PS17. Nikolina Tomić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): MODULATORY EFFECTS OF DELPHINIDIN AND HALOGENATED BOROXINE ON *CAT* GENE EXPRESSION IN CULTURED LYMPHOCYTES

PS18. Azra Ličina Sinanović (University of Tuzla, Faculty of Technology, Tuzla, Bosnia and Herzegovina): BIHOR DNA PROJECT - RESULTS OF RESEARCH

PS19. Merima Miralem (University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina): DNA ANALYSIS SUGGEST POTENTIAL KIN RELATIONSHIP BETWEEN TWO PERSONS FROM DISTINCT MEDIEVAL ARCHAEOLOGICAL SITES

PS20. Dženana Klepo (University of Sarajevo, Faculty of Natural Science and Mathematics, Sarajevo, Bosnia and Herzegovina): EVALUATION OF THIOMERSAL AND PARACETAMOL GENOTOXICITY IN HUMAN LYMPHOCYTE CULTURE

PS21. Irma Durmišević (University of Sarajevo, Faculty of Science, Department of Biology, Sarajevo, Bosnia and Herzegovina): CYTOGENOTOXIC POTENTIAL OF THREE DIFFERENT PARABENS *IN VITRO*

PS22. Mahira Mehanović (University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina): COMPARATIVE CYTOTOXICITY ANALYSIS OF EXTRACTS OF *THYMUS BRACTEOSUS* VIS EX BENTHAM AND *ACINOS ORONTIUS* (K.MALY) ŠILIĆ

Undergraduate Students Session

PS23. Nora Pušeljić (University of J.J.Strossmayer, Faculty of Medicine, Osijek, Croatia): TURNER SYNDROME WITH HAPLOINSUFFICIENCY IN XP22.3 AND XQ28 MICRODELETIO

PS24. Nika Pušeljić (University of Osijek, Faculty of Medicine, Osijek, Croatia): CONGENITAL HYPOTONIA AND EPILEPSY AS MAIN CLINICAL SIGNS OF WOLF-HIRSCHHORN SYNDROME

PS25. Emina Huseinbegović (International University of Sarajevo, Genetics and Bioengineering Department, Sarajevo, Bosnia and Herzegovina): URINE qRT-PCR ASSAY AS A SCREENING TOOL FOR THE DETECTION OF CONGENITAL HUMAN CYTOMEGALOVIRUS INFECTION OF INFANTS IN SARAJEVO CANTON

PS26. Tamara Lukić (University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina): DNA ANALYSIS OF ABORTED FETAL TISSUES IN FORENSIC CASES

PS27. Alen Džaferspahić (University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina): CRITICAL POINTS IN PERFORMING ALKALINE COMET ASSAY ON ORAL LEUKOCYTES

PS28. Dženan Kovačić (International Burch University, Department of Genetics & Bioengineering, Sarajevo, Bosnia and Herzegovina): TWO-COMPONENT VAULT NANOPARTICLE SYSTEM FOR EFFICIENT TREATMENT OF LATENT AND ACTIVE TUBERCULOSIS

PLENARY PRESENTATIONS

Abstract number: **PL1**

A SHORT HISTORY OF GENETICS IN BOSNIA AND HERZEGOVINA

Adaleta Durmić-Pašić, Anja Haverić, Belma Kalamujić Stroil, Jasmina Čakar, Kasim Bajrović, Lejla Pojskić, Naris Pojskić, Sanin Haverić, Rifat Hadžiselimović

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The history of genetics in Bosnia and Hercegovina is a four-chapter story that began with bio-anthropological analyses of local population at the end of the 19th century. The opening chapter started with Himmel, the Austro-Hungarian military physician, who analyzed the frequencies of heritable traits (height, weight, eye color, hair color, and other anthropological variables) in a group of Herzegovinian young Army recruits. Weissbach, also Austrian physician, built on the Himmel's pioneering work and introduced population genetics by comparing population attributes among major ethnic groups and other regional Slavic populations. The second chapter was marked by the introduction of cytogenetic markers that expanded the specter of traits that were studied during the second half of the 20th century. However, the most prominent advances during this phase were made in the fields of genealogy and genetic distance analysis in B&H human population, based on morphological, biochemical and physiological traits. Cytogenetic methods were mostly employed in animal and plant systematics, and genotoxicity testing *in vitro*. The following chapter is associated with the progress of modern technology, (bio)informatics and molecular biology. In the postwar period all major Universities in the country developed laboratories and study programs that addressed topics in molecular genetics. B&H had an unfortunate opportunity to serve as the first site of application of molecular markers in human identification on large-scale. The experiences gained through the process of DNA identification of war victims led towards DNA characterization of isolated and representative human populations and other biological resources but were also instrumental to the introduction of genetic testing in the applied sciences as well. By entering the seductive world of omics and machine learning we unlocked the fourth chapter. The story will go on with basic research firmly connected with applications and strongly supported by international networking and research activities.

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Abstract number: **PL2**

THE COMET ASSAY; STATE OF THE ART

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It is now many years since lesion-specific enzymes were introduced into the comet assay protocol, but the approach is only now widely accepted. Detecting lesions other than strand breaks enhances the sensitivity and versatility of the assay, and provides valuable additional information in both biomonitoring and genotoxicological contexts. The assay can be applied to the study of DNA repair, either by challenging cells with a defined damaging agent and following the decrease in lesions with time, or by a more biochemical approach in which a cell extract is incubated with a defined DNA substrate. A limitation of the comet assay is the number of samples that can be run in one experiment. The capacity is greatly increased by reducing the size of gel; 12 minigels on one slide, or 96 on a GelBond® film. Inter-experimental variation is less of a problem, but scoring of gels then becomes a bottleneck; the need for simple automated scoring is acute. Certain critical parameters demand attention: agarose concentration, times of lysis, alkaline unwinding and electrophoresis, and voltage gradient. Reference standards (cells with a known amount of damage) should be included in every experiment, to check on assay performance, and allow normalisation of results between experiments. The comet assay is the method of choice for examining genotoxicity of nanomaterials. There are advantages from a regulatory point of view in adopting the assay, since it is quick, economical, sensitive, can be used on virtually any kind of cell and many tissues, and is applicable to the 3D model systems currently being developed.

I gratefully acknowledge the support of COST: the Action hCOMET, now in its final year, has provided a valuable forum for discussing these issues, exploring new ideas, training young researchers, and consolidating an international network of comet assay enthusiasts.

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Abstract number: **PL3**

THE IMPACT OF GENOME EDITING ON CROP IMPROVEMENT

Matthew R. Willmann

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Plant scientists are constantly striving to improve crop plants in ways that directly benefit consumers, farmers, and food processors. These efforts are guided by a need to address a set of fundamental problems, including a rapidly growing world population, a changing climate, an ongoing battle against pathogens and insects, worsening soils, the negative effects of agriculture on the environment, and the need for tastier and more nutritious foods. Researchers have used traditional plant breeding and transgenesis to address these issues, but both have significant disadvantages. Recently, genome editing has become reliable in plants, and this advance has the potential to dramatically speed research and crop improvement. Genome editing is a tool for making a specific genetic change at a targeted location in a genome. This technology, also called targeted mutagenesis, has been possible in bacteria, yeast, and mammalian systems for many years, but genome editing has only recently become efficient and widespread in plant research. The most wide-ranging breakthrough for genome editing in plant science came in 2013, when the highly versatile CRISPR/Cas9 genome-editing system was first applied to plants. This method involves the use of the Cas9 nuclease, which is targeted to a specific site in a genome by binding a guide RNA (gRNA). The gRNA has a specific 20 nt sequence within it that binds to complementary places in the genome. Once this binding occurs, Cas9 creates a double-stranded break at the binding site. While the normal DNA repair systems usually repair such breaks without fail, errors can occur, resulting in sequence insertions and deletions at the target site. Further modifications of the system allow for targeted insertions and deletions of various sizes, single base pair changes, allelic swaps, and even epigenetic changes. Here we will discuss the technology and how it can be used for plant improvement.

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Abstract number: **PL4**

GENETIC DAMAGE OF OCCUPATIONAL EXPOSURE TO STYRENE

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Styrene is a commercially important chemical widely used in the manufacture of synthetic rubber, resins, polyesters, and plastics. Occupational exposure to styrene occurs in the styrene-butadiene rubber, styrene monomer and polymerisation, and reinforced plastics industries. Although the genotoxic potential of styrene is known, very limited and inconclusive information is available regarding its dose-dependent genotoxic effects in humans. The objective of this work was to study occupational exposure to styrene in a multistage approach, in order to integrate the following end-points studied: styrene in workplace air, mandelic and phenylglyoxylic acids (MA+PGA) in urine, haemoglobin (Hb) adducts, sister-chromatid exchanges (SCE), micronuclei (MN), DNA damage (comet assay) and genotypes of polymorphic genes of some metabolising enzymes. Seventy-five workers from a fibreglass-reinforced plastics factory and seventy-seven unexposed controls took part in the study. The mean air concentration of styrene in the breathing zone of workers (30.4ppm) was higher than the threshold limit value of 20ppm recommended by the ACGIH, and the biological exposure index adopted by the ACGIH for exposure to styrene prior to the next shift (MA+PGA=400mg/g cr) was exceeded, indicating that styrene exposure for this group of workers was higher than recommended. The level of Hb adducts and SCE in exposed workers were significantly increased as compared with controls. The DNA damage was higher among styrene-exposed workers than in controls. Concerning the effect of the genetic polymorphisms on the different exposure and effect biomarkers studied, we observed the effect of microsomal epoxide hydrolase activity on Hb adducts of highly exposed individuals and on the levels of SCE of exposed workers. The present results suggest the importance of individual susceptibility factors in modulating genotoxicity, although cautious interpretations are required since the size of the study population limits the power of many of the analyses.

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Abstract number: **PL5**

LYSOSOMAL CYSTINE EFFLUX OPPOSES MTORC1 REACTIVATION UPON FASTING THROUGH THE TCA CYCLE

Zvonimir Marelja[1], Patrick Jouandin[2], Andrey A Parkhitko[2], Miriam Dambowsky[1], John M Asara[3], Ivan Nemazanyy[4], Matias Simons[1], Norbert Perrimon[2]

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The highly regulated process of adapting to cellular nutritional status depends on lysosomal mTORC1 (mechanistic Target of Rapamycin Complex 1) that integrates nutrients availability via the sensing of amino acids to promote growth and anabolism[1]. Nutrient restriction inhibits mTORC1 activity, which in turn induces autophagy, a crucial adaptive process that recycles internal nutrient stores to promote survival[2]. However, as successful amino acid recycling through autophagic degradation reactivates mTORC1 signalling over time[3,4], it is unclear how autophagy can be maintained during prolonged starvation. Our study shows that one particular amino acid, cysteine, limits mTORC1 reactivation *in vivo*. We provide evidence that the lysosomal export of cystine through cystinosin[5], a cystine transporter associated with the rare inherited lysosomal storage disorder cystinosis, fuels a metabolic pathway that suppresses mTORC1 signaling and maintains autophagy during starvation. This pathway involves cystine/cysteine catabolism to generate acetyl-CoA that enters the TCA cycle to promote anaplerosis and concomitant increase in the levels of TCA cycle intermediates thereby limiting mTORC1 reactivation. We propose that cysteine mediates a communication between lysosomes and mitochondria to control mTORC1 signalling under prolonged starvation, highlighting how changes in nutrient availability divert the fate of an amino acid into a growth suppressive program to maintain the balance between nutrient supply and consumption.

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Abstract number: **PL6**

DERIVATION OF GENETICALLY MODIFIED T CELLS FROM HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESC) have the ability to form any type of cells, including T cells, as demonstrated by our group and others. hESC can be easily genetically manipulated and expanded *in vitro* and therefore can be used as a source of genetically modified T cells. We previously documented the stable expression of a reporter gene in T cells derived from genetically modified hESC. In our current studies, we have cloned the alpha and beta chains of a T cell receptor (TCR) specific for the MART-1 melanoma-associated antigen, introduced this TCR into hESC using a lentiviral vector and established cell lines which stably express this transgene. Subsequently, we differentiated these transgenic hESC into T cells progenitors, which when introduced into haplotype-matched HLA-A0201+ human thymic implants in SCID-hu mice gave rise to MART TCR-expressing cells of T lineage. These cells are developmentally skewed toward CD8 lineage, as expected given that the transgenic TCR is restricted to MHC class I. Upon activation, the hESC-derived MART-1 TCR expressing T cells produce interferon gamma and kill haplotype-matched target cells in a dose dependent manner *in vitro*. Furthermore, our time lapse studies point to the fact that a single transgenic T cells is sufficient to kill a melanoma cell and that the whole process takes less than three hours. Our work provides a proof-of-principle that hESC can be developed into melanoma fighting cytotoxic T lymphocytes. However, these studies will also be relevant to other tumors expressing defined tumor antigens.

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INVITED PRESENTATIONS

Abstract number: **I1**

THE COMING AGE OF POLYGENIC RISK SCORES: TOWARDS PERSONALIZED BREAST CANCER RISK ASSESSMENT AND PREVENTION

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Breast cancer (BC) is the most prevalent malignancy and the second leading cause of death in women. The etiology behind developing BC is multifactorial, with many risk factors including lifestyle, diet, hormonal status, reproductive factors, mammographic density and genetic predisposition. Twin studies estimated that the heritability of breast cancer ranges from 20 to 30%. However, only 5–10% of BC cases have a strong inherited component identified in a form of rare genetic variants in BRCA1, BRCA2 and around 20 additional high and intermediate penetrance genes, indicating that in addition there should be a considerable polygenic component in BC heritability. This was also supported by the results of large genome-wide association studies (GWAS) that identified around 180 low penetrance alleles as being associated with BC, explaining around 18% of BC heritability. Individually the low penetrance alleles confer small risk for BC, but their combined effect, summarized as polygenic risk score (PRS) can be substantial. Since early diagnosis of BC could lead to successful treatment and good prognosis for recovery, efficient risk prediction algorithms to identify high-risk individuals are needed. Recent studies showed that incorporation of PRS to the currently available risk prediction models that include classical risk factors, improves the accuracy of the prediction. PRS helps to better stratify women according to their risk to develop BC and allows tailoring screening and preventive strategies in accordance with the risk. PRS is also useful in further stratifying the risk of BC among women who are carriers of BRCA1 and BRCA2 pathogenic mutations. In this talk I will give brief introduction on the BC susceptibility genes and alleles, with the main focus on low-risk genetic variants and will review the evidence supporting personal and clinical utility of PRS for breast cancer and other common heritable conditions.

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Abstract number: **I2**

DETECTION OF BRCA1 AND BRCA2 MUTATIONS IN OVARIAN CANCER BY NEXT-GENERATION SEQUENCING

Irena Drmić Hofman[1,3], Miljenko Katunarić[2], Sendi Kuret[1], Snježana Tomić[1,3], Blaženka Grahovac[4]

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BRCA1 and BRCA2 genes play a major role in a normal process of DNA damage repair and alterations in these genes confer a higher risk for breast/ovarian cancer development. The most common form of epithelial ovarian cancer is high-grade serous ovarian cancer (HGSOC), with the frequency of BRCA germline and somatic mutations of about 11-18% and 6-8%, respectively. Identification of BRCA1/2 mutations could facilitate the implementation of targeted therapy with selective poly(ADP-ribose) polymerase (PARP) inhibitors. The study included patients from University Hospital of Split (n=121) and Clinical Hospital Center Rijeka (n=111), who underwent HGSOC surgical resection between 2013 and 2018. Analyses were performed using the next generation sequencing (NGS) platforms (Illumina MiniSeq and PGM sequencers). Overall, pathogenic BRCA 1/2 mutations were found in 28,4 % patients (66/232), 53 were located in BRCA1 and 13 in BRCA2 gene. The mutations were SNV and short indel with introducing frameshift or stop codon. No large deletions or insertions were detected. All detected BRCA mutations are described in relevant data bases as germline. Pathogenic relevance of BRCA1/2 genes has been improved in diagnostics, therapy and prognosis of ovarian cancer. Highly sensitive and specific NGS method allowing simultaneous sequencing of multiple cancer susceptibility genes to test the cancer patients is a feasible approach.

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Abstract number: **I3**

NEW GENOMIC TECHNOLOGIES FOR IMPROVEMENT OF NATIONAL HEALTH SYSTEM

Borut Peterlin

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New genomic technologies, especially next generation sequencing (NGS) has a significant impact on the recognition of etiology of hundreds of human diseases. Recognition of etiology is however of paramount importance for timely diagnosis, treatment, and prevention. Moreover, identification of people at an increased risk for developing diseases or transmitting them to their offspring is a key step in futuristic medical concepts like personalized and preventive medicine. Identification of highly penetrant genetic variants might be therefore considered as an important public health issue and relevant goal of public health genomics.Health systems are usually resistant to rapid integration of novel technologies due to several barriers including lack of clinical guidelines and pathways as well as professional standards for genomic applications, limited access to expertise, limited evidence of benefit/value and finally lack of reimbursement. Consequently, the potential of new technologies remains currently relatively poorly exploited in the health systems worldwide, but especially so in economically deprivileged countries. We suggest that timely implementation of new innovative genomic technologies in the framework of new generation health services might significantly improve the efficacy and quality of health systems with favourable economic effects. The Slovene model of translation of genomic technologies into public health system will be presented.

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Abstract number: **I4**

GUT MICROBIAL-HOST CROSS TALK IN HUMAN HEALTH AND WELLBEING

Nataša Golić, Maja Tolinački, Katarina Veljović, Milica Živković, Jelena Djokić, Miroslav Dinić, Svetlana Soković, Nikola Popović, Jovanka Lukić, Amarela Terzić-Vidojević, Dušan Radojević, Emilija Brdarić, Dušanka Popović

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The factors and mechanisms staying behind gut microbiota-host communication are still largely unexplored. The aim of our research is to decipher the role of gut microbiota in various infectious and non-communicable diseases (obesity, diabetes, hepatotoxicity), gut-brain axis (multiple sclerosis, autism, depression, inflammatory pain), gut-lung axis (*Aspergillus* infections, chronic obstructive pulmonary disease). Our recent results revealed some genes/molecules and mechanisms involved in health-promoting properties of lactic acid bacteria. For example, GABA-producing *Lactobacillus brevis* BGZLS10-17 have strong immunosuppressive effect downregulating the production of IFN-γ and IL-17 and the expression of MHCII and CD80 on antigen presenting cells, while stimulating the expression of immunosuppressive molecules such as $Foxp3^+$, IL-10, TGF-β, CTLA4 and SIRP. *L. brevis* BGZLS10-17 induces autophagy in different types of MLNC, such as $CD4^+$ and $CD8^+$ T lymphocytes, NK and NKT cells, as well as DC, Mf and B cells. Besides, antihyperalgesic and antiedematous effects of the exopolysaccharides produced by *Lactobacillus paraplantarum* BGCG11 were showed. These effects were followed by a decreased expression of IL-1β and iNOS mRNAs in rat's paw tissue suggesting that the antihyperalgesic and antiedematous effects of the EPS CG11 are related to the suppression of inflammatory response. Moreover, the probiotic strain *Enterococcus faecium* BGPAS1-3 can alleviate detrimental the effects of *Listeria monocytogenes* on Caco-2 cells, by increasing mRNA expression of claudin, TGF-β, TLR2, TLR4, and Myd88 and decreasing mRNA expression of IL-8. On the other hand, the antibiotic use in early life have subsequent unfavourable effects on the diversity and composition of gut microbiota and regulation of the immune system. In particular, the antibiotic treatment decreased proportions of *Clostridia* and *Bacilli* classes, *Firmicutes, Actinobacteria*, *Helicobacteraceae, Spirochaetaceae* and *Turicibacteriaceae* and increased proportions of *Proteobacteria* and *Bacteroidetes*, leading to aggravation of clinical symptoms of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis.

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Abstract number: **I5**

POLYMORPHISMS IN FOLATE PATHWAY GENES AS RISK FACTORS FOR CONGENITAL HEART DEFECTS IN DOWN SYNDROME

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Congenital heart defects (CHD) are one of the most common abnormalities occurring in 40% - 60% of Down Syndrome (DS) cases. Most commonly, these are septal defects, including atrial septal defect, ventricular septal defect and complete atrioventricular canal. Studies have shown that phenotypic variability in a individuals with DS may contribute to various genetic factors. The polymorphisms of genes involved in folate metabolism are the most significant candidate genes for CHD. However, the correlations between genes and CHD were inconsistent in various reports. The study aimed to determine the spectrum of CHD among DS and to evaluate the effects of polymorphisms of 5-methyltetrahydrofolate homocysteine methyltransferase reductase (rs1801394) and 5,10-methylenetetrahydrofolate reductase (rs1801133 and rs1801131) genes on CHD in DS. A total of 155 individuals with DS have been enrolled in this study. Genotyping of polymorphisms was performed by PCR-RFLP. Statistical significance was considered at $P<0.05$. CHDs was present in 50% of participants and higher frequencies of CHDs was observed among females than males (54% vs. 46%, P=0.077). Atrial septal defect was most common presents in females as well in males (32% vs. 22%). No significant differences in distribution and frequencies of investigated polymorphisms were observed according to the presence of CHD ($P > 0,05$). The occurrence of CHD, particularly in female gender of DS individuals is similar as documented in literatures. The disruption of the folate pathway do not contributes to the incidence of CHD among individuals with DS in our study.

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Abstract number: **I6**

lncRNAs

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The recent approaches including whole-genome and RNA-sequencing (RNA-seq) studies have revealed that the transcription landscape in eukaryotes is much more complex than had been previously appreciated. The studies have demonstrated that lncRNAs may represent as alternatively spliced forms of known genes, products of antisense RNAs, double stranded RNAs, retained introns, short open reading frame, RNA polymerase III-derived RNAs and RNA decoys mimicking miRNA targets. Long non-coding RNAs (lncRNAs) play key roles in the regulation of biological processes including response to environmental stresses either transcriptional or post-transcriptional levels. The salinity tolerance of barley may play important role to understand the salinity mechanism. Molecular responses of four barley genotypes *Hordeum vulgare* L. (Hasat, Beyşehir 99, Konevi 98 and Tarm 92) to 150 mM salinity during 3 days germination period were investigated. To reveal effect of lncRNAs, we performed Real-time PCR and fluorescence *in situ* hybridization (FISH) studies. Study demonstrated salinity effected expression levels of four lncRNAs, *CNT0018772*, *CNT0031477*, *AK363461*, and *AK370506* during germination. The study showed there was no statistically difference between barley varieties for expression level of *CNT0018772* ($p>0.05$). However, our study indicated there was statistically significant difference between barley varieties for *CNT0031477* ($p<0.05$). Additionally, there was statistically significant difference between barley varieties for these two barley lncRNAs, *AK363461* and *AK370506*, ($p<0.05$). We were also able to observe the localization of these four lncRNAs on barley chromosomes, in addition, cellular localizations were exhibited under confocal microscope via *in situ* hybridization on barley root preparations. lncRNAs; *CNT0018772* and *CNT0031477* were sequenced and submitted (GenBank MK369941-MK369948). Nowadays, lncRNAs are considered as major regulators. Due to a lack of understanding, the functional characterization of lncRNAs is today challenging that lncRNA function resides predominantly in lncRNA structure and protein interaction repertoire, rather than in the primary sequence context. These results indicate we are in the very early phases of determining the functions of lncRNAs under control and stress conditions.

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Abstract number: **I7**

MODIFICATIONS OF THE COMET ASSAY FOR DETECTING DIFFERENT DNA LESIONS

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The standard comet assay detects single and double DNA strand breaks, and alkali-labile sites, at cell level. However, other DNA lesions are produced by chemical and physical agents, such as oxidized and alkylated bases, bulky adducts and cross-links. To overcome this limitation, the standard comet assay has been modified using several strategies over the past few decades. The first and most validated strategy is the combination of the assay with DNA repair enzymes of several origins for the detection of certain lesions. Several enzymes are used, in particular formamidopyrimidine DNA glycosylase (Fpg), for detecting altered purines, and endonuclease III (EndoIII), for detecting oxidized pyrimidines. It has been demonstrated that the use of Fpg increases the sensitivity of the comet assay in detecting genotoxic compounds. Very recently, a new enzyme has been added to the list of the enzymes used in combination with the comet assay, the human alkyladenine DNA glycosylase (hAAG), which detects alkylated bases. Inhibiting the DNA polymerases involved in the nucleotide excision repair pathway is a strategy that has been used to detect nucleotide lesions, such as DNA adducts. The inhibition of the polymerases leads to the accumulation of (normally transient) DNA breaks during the repair process. To this aim, the DNA polymerase inhibitors aphidicolin and hydroxyurea are used in combination with the comet assay. On the other hand, the 'reverse' comet assay has been designed to detect DNA cross-links. This modification is based on the decrease in DNA migration in the presence of those DNA lesions. These two strategies, while very promising, need to be validated by testing several chemicals and/or physical agents.

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Abstract number: **I8**

PLANT COMET ASSAY IN ENVIRONMENTAL STUDIES: USES, LIMITS AND PERSPECTIVES

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The comet assay is a versatile technique for measuring DNA damage in eukaryotic cells and organisms, and is increasingly used to assess DNA repair. Its main applications are in genotoxicity testing, and in human monitoring, with a so far rather neglected potential in ecogenotoxicology. This is particularly true in plant research. Comet assay was used for the first time on plants in 1993. For a decade, the comet assay remained restricted to some toxicological studies and to few model species including garlic (*Allium cepa*), tobacco (*Nicotiana tabacum*), broad bean (*Vicia faba*), and arabidopsis (*Arabidopsis thaliana*). Since 2010, the technique was exponentially applied to evaluate diverse stressors (organic compounds, radiations, nanoparticles…), and to monitor environmental pollutions in situ. However, despite its increasing applications, the comparison between the number of papers using comet assay on plants (almost 300 during the last 25 years) and on humans (more than 10 000 during the same period), highlights the gap in its uses between genotoxicology and ecogenotoxicology fields. This huge difference can be explained by (i) the difficulty to isolate numerous intact nuclei in plants compared to animal systems, (ii) the lack of a standardize protocol and guideline in plants, (iii) and the lack of a high throughput comet assay scoring method. During the last 5 years, intensive efforts have been done to develop a robust and effective new protocol to extract plant nuclei, as well as an automated high-throughput scoring of plant nuclei. Meanwhile, several authors have used enzyme-modified protocols to detect specific base damage or DNA methylation. This opens new perspectives for the development of this technique in plant studies. During this presentation, we will review the uses of comet assay on plants, and the main bottlenecks of this technique. We will also discuss the current developments and new perspectives.

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ORAL PRESENTATIONS

Abstract number: **O1**

NEXT-GENERATION SEQUENCING AS A TOOL TO STUDY RARE DISEASES

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Majority of rare diseases (RD) are genetic diseases (80%). Therefore, the identification of specific gene defect in each patient is important. Next generation sequencing (NGS) methodology has enabled shortening the diagnostic journey of patients with RD, leading to improved treatment and successful genetic counseling.We have analyzed over 200 RD patients using Clinical-Exome Sequencing TruSight One Gene Panel (Illumina). Variant Studio and various in silico software tools were used for bioinformatics analysis. Filtration and prioritization of variants were performed according to “in-house” designed pipelines and virtual gene panels. NGS studies enabled diagnosis of more than 50 different diseases (haematological, metabolic, endocrinological, pulmonary, immunological, orthopaedic, dermatological, ophthalmological, cardiological, epileptic encephalopathies etc.). It was particularly important for genetically heterogeneous diseases, such as glycogen storage diseases, branched-chain organic acidurias, primary ciliary dyskinesia, MODY or mitochondriopathies. Moreover, different diseases with overlapping clinical manifestations were accurately diagnosed. In our studies mutation detection rate reached 80-100%. Our work enabled the establishment of RD biobank collections containing DNA, RNA, mononuclear cells and tissue samples from over 2000 patients affected with RD. Furthermore, novel variants in DNAI1, MUT, PAH, PCCB, SLC37A4, SPAG16 and SPAG17 genes were functionally characterized in adequate *in vitro* systems such as immortalized patients’ fibroblasts or Crispr/Cas9 edited cell lines. Also, we used TruSeq-Amplicon Cancer Panel to analyse different childhood and adult rare haematological malignancies. Our association studies revealed new diagnostic, prognostic and pharmacogenomic markers, resulting in recommendations for personalized therapeutic modalities in accordance with genomic profile of the patients.

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Abstract number: **O2**

THE GENETIC CAUSES OF THE HEARING IMPAIRMENT IN BOSNIAN POPULATION

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Hearing loss is a very common sensory disorder and can be present at any age of life. Hearing impairment is also the most common congenital sensorineural disorder in children. Approximately 1-3 in 1000 new-borns have a severe hearing impairment. More than 50% of prelingual hearing impairment has a genetic cause. Hearing impairment can be named as syndromic or nonsyndromic. Hereditary sensorineural deafness is caused by monogenic autosomal recessive disorders in over 90% of cases. We used PCR and NGS clinical exome sequencing to find the genetic causes of hearing impairment in a group of 62 children from Bosnia and Herzegovina: 43 with nonsyndromic and 19 with syndromic deafness, including 4 families, with 2 deaf children each. After clinical examination and comprehensive genetic screening for hereditary hearing impairment, a genetic disorder was detected in 32 children (51.6%). We labeled 30 children from 28 families as probands (30/62; 48.4%); six probands (6/30; 20%) from the syndromic group and 24 (24/30; 80%) from the non-syndromic group. Twenty different variants were found in 10 different genes that confirmed the genetic heterogeneity of hereditary hearing impairment. Most of the probands from the non-syndromic group (66.7%) had some of the GJB2 gene variants, the most common being variants c.35delG (60%) and c.313_326del14 (25%), followed by variants in the genes MYO15A, OTOF, TMC1, and LHFPL5. Among the probands from the syndromic group the variants in the USH2A, SF3B4, HDAC8, TSHZ1, and TYR genes were found. Early identification and management of deafness is very important for developing language and social skills. The correct choice of target gene regions, and interpretation of the various variants remain relatively difficult for standard clinical implementation, but over the coming years, the comprehensive NGS method will surely become the gold standard for the etiological diagnosis of all early-onset hearing impairment.

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Abstract number: **O3**

PATHOGENIC MUTATIONS ASSOCIATED WITH COLORECTAL CANCER AND NON-SMALL CELL LUNG CANCER IN BOSNIA AND HERZEGOVINA POPULATION

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Cancer is a group of diseases which involve abnormal cell growth, apoptosis, signal trandsuction, cell cycle regulation, DNA repair, metabolic pathway regulation and contact inhibition. Advances in DNA sequencing technology have enabled more precise and detailed molecular profiling of cancer tissue. Emergence of next generation sequencing enabled two important advancements in cancer genomic analysis: coverage and range. Clinical application of next generation sequencing is detection of the molecular alterations that can have diagnostic, prognostic or therapeutic relevance. The aim of this study is to determine most common pathogenic mutations in colorectal cancer and non-small cell lung cancer in cancer patient samples from Bosnia and Herzegovina. DNA was isolated from formalin fixed paraffin embedded blocks which were previously reviewed by pathologist to determine type and stage of cancer. Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2, which contains primers for amplification of hotspot regions in 22 genes, was used. Obtained mutations were compared to ClinVar database to determine their clinical relevance. For this study 36 colorectal cancer and 14 non-small cell lung cancer samples were sequenced on Ion GeneStudio S5 System with average base coverage of >500X. Pathogenic mutations are found in *ERBB2, KRAS, TP53, PIK3CA, MET, BRAF, NRAS* and *FBXW7* genes in colorectal cancers and *BRAF, TP53, PTEN, KRAS* and *EGFR* in non-small cell lung cancers. In both types of cancer 62 different pathogenic mutations with implications in prognosis or therapy are detected.

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***Abstract number:* O4**

PROTEOMICS-BASED DEVELOPMENT OF NEW TREATMENT FOR PANCREATIC CANCER

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Overall survival for patients with advanced pancreatic ductal adenocarcinoma (PDAC) has not improved over the past 20 years. The aim of this study was to develop a new therapy for PDAC by targeting the nerve-cancer cell crosstalk in combination with standard chemotherapy. 284 mice were implanted orthotopically (in pancreas), subcutaneously, through hemispleen injection (to induce liver metastasis), or tail vein injection (lung metastasis) with cells that were originally derived from PDAC tumor of genetically engineered mouse model with KrasG12D;Pdx1-Cre (Kras mice). The new therapy was botulinum toxin A (BTX) plus ivermectin (IVM) in combination with gemcitabine plus paclitaxel. Results showed that treatment improved median overall survival from 12 days to 28 days in mice with tumor in the pancreas, in which mortality was 100% in 18 days without treatment and 0% in 21 days with treatment. Mortality with lung metastasis was 100% in 17 days without treatment and 0% with treatment until the end of experiment (day 35). BTX and/or IVM alone had little effect on the tumor size and the cell proliferation (Ki67) but enhanced chemotherapy-induced tumor shrinkage rate in mice with tumors in the pancreas or under the skin, liver or lung metastases. In *in vitro* experiments, the cells became resistant to gemcitabine after 20 passages but still responded to IVM. Electron microscopy revealed the formation of vacuoles and lipofuscin bodies in response to BTX plus IVM, suggesting an impaired exocytosis copied with crinophagy. Proteomic analysis using mass spectrometry showed that 95 proteins in the serum and 1463 proteins (e.g. DCLK1) in the tumor tissue were reduced or below detection limit after the treatment. “Proteomic tree” of PDAC revealed possible core signaling pathways in response to the treatment. In conclusion, this new combination therapy might have a great potential in improvement of overall survival for patients with PDAC.

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Abstract number: **O5**

TARGETING NERVE-CANCER METABOLISM IMPROVED OVERALL SURVIVAL IN AGED MICE WITH GASTRIC CANCER

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Gastric cancer (GC) is a disease of aging. Systemically use of cytotoxic drug treatment ("chemo") in elderly patients is usually concerned in regard to quality of life and overall survival (OS). Based on the "nerve-cancer metabolism axis" hypothesis, we wanted to develop a new treatment by combination of Botulinum toxin type A (BtxA, synaptosomal nerve-associated protein 25 inhibitor), RAD001 (mTORC1 inhibitor), and CPI-613 (tumor-specific KDH and PDH inhibitor) using aged transgenic mouse model, i.e. INS-GAS mice that spontaneously develop gastric cancer. 186 INS-GAS mice at 9-15 months of age were randomly assigned to age-and sex-matched groups and followed up until 18-24 months for OS and median survival time (MS). Tumor shrinkage (TS) and the treatment response rate (RR) in terms of percentage of tumor volume reduction and Ki67 proliferation rate were measured. Metabolomics and RNA sequencing analyses were performed. OS and MS were 33 % and 148 days, respectively, in GC mice without any treatment, 40% and 40 days in GC mice that received either chemo or BRC + chemo, 90% and 249 days in GC mice that received BRC. However, TS and RR were 18 % and 33%, respectively, in mice receiving chemo, 32% and 50% in mice receiving BRC, 54% and 75% in mice receiving BRC + chemo. BRC was without affecting the body weight, whereas chemo with or without BRC induced weight loss during and after treatment. RNAseq analysis revealed downregulation of mTOR, nerve growth factor and neuregulin signaling pathways along with Wnt/Yap/Taz-associated genes, whereas glycolysis and gluconeogenesis pathways were upregulated in response to BRC. We concluded that this new treatment regimen (BRC) without cytotoxic drugs increased OS and MS in the aged GC mice by targeting the tumor metabolism rather than proliferation.

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Abstract number: **O6**

A BRIEF STORY OF QIAGEN IN THE FORENSIC FIELD

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QIAGEN is associated with the forensic genetic field for more than 22 years. Along the years, QIAGEN has developed different technologies to improve the work of forensic scientists and experts in the world. The new technologies of reagents and instruments impacted on the outcome of criminal national DNA databases, missing persons identification after natural disasters, identification of kings, airplane crashes, human rights abuses, proving to have high quality even when working with scarce or challenging samples.

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***Abstract number:* O7**

THE VARIABILITY OF DETOXIFYING *GSTP1* GENE POLYMORPHISMS IN THE ROMA POPULATION FROM CROATIA

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The genetic variability of xenobiotic-metabolizing enzymes contributes to individual susceptibility to environmental risk factors (outdoor pollutants, cigarette smoke, diet-related xenobiotics). Glutathione S-transferases (GSTs) are ubiquitous family of multifunctional enzymes which decrease oxidative damage in cells by catalyzing the conjugation of many toxic compounds with glutathione. The *GSTP1* is a polymorphic gene whose certain variants, by altering the gene product protein structure, have been investigated as candidate loci involved in the predisposition to pathologic conditions. This study investigated rs1695 (A313G, I105V) and rs1138272 (C341T, A114V) genotypes, alleles and haplotypes in 440 members of three socio-culturally different and geographically distant Roma (Gypsy) groups. The Baranja and the Međimurje Roma groups belong to Vlax (Bayash) Roma who speak Ljimb`d Bayash, while the Balkan Roma group speaks Romani Chib. Haplotypes were inferred using Phase ver. 2.1. Both investigated loci were in Hardy-Weinberg equilibrium in all the three Roma populations. Minor allele frequency (MAF) of rs1138272 was significantly higher in the Baranja (16.4%) and in the Balkan Roma (14.9%) than in the Međimurje Roma (8.8%) ($p<0.01$ and $p<0.05$, respectively). The MAF of the rs1695 was significantly higher in the Baranja Roma (35.9%) than in the Balkan Roma (25.0%) ($p<0.01$). The most prevalent haplotype in all Roma groups was I105-A114, while the second most frequent in the Baranja and the Međimurje Roma was V105-A114 and in the Balkan Roma group V105-V114 ($p<0.001$). The analyzed *GSTP1* polymorphic loci indicate that Balkan Roma significantly differ from the Baranja and the Međimurje Roma, which is in concordance with their specific histories and distant socio-cultural characteristics.

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Abstract number: **O8**

MONOSOMAL KARYOTYPE IN MYELODISPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA PATIENTS

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Acute myeloid leukemia (AML) is a clinical and biological heterogeneous hematological malignancy. Myelodisplatic syndrome (MDS) is a hematopoetic cell disorder. Cytogenetic is an important prognostic factor in adult AML and MDS, and is an important predictor of survival and disease-free survival. Monosomal karyotype (MK) is defined as 2 or more monosomies, or a single monosomy in the presence of structural abnormalities. Whereas MK is known as a poor prognostic indicator in AML, the prognostic value in MDS is not known. The aim of this retrospective study is to analyse the survival of AML patients with monosomal karyotypes. It also aimed to evaluate monosomal karyotype in MDS patients. A total of 335 MDS and 181 AML patients who diagnosed and followed in the Hacettepe University department of haematology between 2000-2017, were enrolled. Cytogenetic analyses were performed on metaphase cells derived from 24-h unstimulated bone marrow aspirate cultures. A cytogenetic anomaly was detected in 58 of 181 AML patients. 6 of these 58 patients with cytogenetic abnormalities had monosomal karyotype (10,3%). 4 of 6 patients with monosomal karyotypes were died within 15 months. 3 patients with monosomal karyotypes did not response the chemotherapy (idarubicin-ara-c). On the other hand the overall survival of patients with cytogenetic disorders was 85 months. Of 335 patients with MDS, 98 had abnormal karyotypes. 5 of these 98 patients with cytogenetic abnormalities had monosomal karyotype (5,1%). 2 of these 5 patients were male and 3 were female. Transition to acute leukaemia occurred in 3 of 5 MDS patients with monosomal karyotype. Monosomal karyotpe is poor prognostic indicator for AML. In this study, overall survival was decreased in AML patients with monosomal karyotype. The prognostic value in MDS is not known and remains to be elucidated.

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Abstract number: **O9**

ddPCR – NEW GENERATION OF PCR TECHNOLOGY

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ddPCR is third generation of PCR technology. I will explain differences and specificities of this new generation of PCR and explain how all 3 PCR generations can complement each other to get maximum results and to minimise expenses. Because of its high sensitivity and specificity droplet digital PCR (ddPCR) has become one of the most accurate and reliable tools for the examination of genetic alterations in a wide variety applications. ddPCR is currently being applied for rare mutation detection, absolute allele quantification, analysis of copy number variations, gene rearrangements, DNA methylation, and in wide variety of samples. This methodology has proven useful for the evaluation of samples, where poor DNA quality and limited sample availability are major obstacles for standard methods, providing less subjective and more automated quantitative results.

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Abstract number: **O10**

ANGIOGENIN GENE MUTATIONS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS FROM TERTIARY CENTER IN BELGRADE

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Amyotrophic lateral sclerosis (ALS) is neurodegenerative disorder, clinically characterized with progressive weakness of the body, leading to respiratory failure. The disease is caused by damage of motor neurons in brain cortex, brainstem and spinal cord. Between 5 to 10% of patients have familial form of the disease and more than 30 genes have been associated with ALS, so far. Mutations in angiogenin gene (*ANG*) have been associated with ALS in several populations. The aim of this study was to evaluate the contribution of mutations in ANG, a major ALS gene, to the pathogenesis of the disease in Serbian patients, and to investigate possible differences in clinical presentation. The direct sequencing of coding region of ANG gene was performed in 442 ALS patients. The patients with mutations in SOD1, FUS or TDP-43 genes or C9orf72 repeat expansion were not excluded, as there is evidence that ALS is oligogenic. Five different heterozygous mutations (c.3G>A, c.61C>T, c.122A>T, c.136G>T and c.208A>G) were identified in seven patients. Two mutations, M-24I and P-4S, are known mutations that are affecting signal peptide. Variants K17I and I46V are found in 2 and 3 patients, respectively. K17I is described as potentially pathogenic in the literature, but also very common in healthy controls, while I46V is considered as polymorphism. Interestingly, one carrier of K17I mutation had mutation in SOD1 gene and c9orf72 expansion. One novel variant (D22Y) was detected in our study and the carrier of this variant is also a carrier of I46V. The evidence from clinical and *in silico* analyses are supporting the deleterious effect of the novel variant. Our findings are pointing out the relevance of ANG mutations in ALS and confirming the variability among populations of different ethnic origin.

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Abstract number: **O11**

DOES CINNAMIC ACID HAVE BENEFICIAL EFFECTS ON DIABETES INDUCED GENOTOXICITY?

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Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia, which is caused by a failure in insulin secretion and/or action and it triggers various acute and chronic complications in patients. It is one of the major health problems worldwide. The current drugs in diabetes treatment have higher costs, limited efficacy, and tolerability and/or significant side effects. Therefore, patients often have recourse to alternative forms of therapy such as herbal medicines. Cinnamic acid (3-phenyl-2-propenoic acid, CA), a major component of cinnamon, possess a variety of pharmacologic properties such as antioxidant, hepatoprotective, antimalarial and antityrosinase activities. The aim of this study was to investigate the genotoxic and/or antigenotoxic effects of CA on streptozotocin (STZ)-induced diabetes in Wistar albino rats. DNA damage was evaluated in the blood by comet assay and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in the plasma samples were investigated by spectrophotometrically using kits following the manufacturer's procedures. The results of the comet assay showed that there were no statistically significant differences in tail intensity between the sham group and the CA treated groups ($p>0.05$). The DNA damage was found significantly higher in the diabetic group compared to the sham group ($p<0.05$). CA treatment was found to decrease DNA damage significantly in the diabetic group ($p<0.05$). Plasma 8-OHdG levels were significantly higher in the diabetic group compared to the sham group ($p<0.05$). But the levels were found to be significantly lower in the CA treated diabetic group compared to the diabetic group ($p<0.05$). CA alone did not cause significant changes in 8-OHdG levels compared to the sham group ($p>0.05$). In conclusion, CA treatment ameliorated genotoxic effects induced by diabetes and it seems that CA might have a role in the prevention of the complication of diabetes.

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Abstract number: **O12**

THE INFLUENCE OF A THREE-WEEK HYPOCALORIC DIET ON DNA DAMAGE PARAMETERS MEASURED BY ALKALINE COMET ASSAY AND CYTOCHALASIN B-BLOCKED MICRONUCLEUS ASSAY IN OBESE PATIENTS FROM THE SPECIAL HOSPITAL FOR EXTENDED TREATMENT OF DUGA RESA, CROATIA – PRELIMINARY RESULTS

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Obese subjects consistently demonstrate increased DNA damage compared to healthy individuals. Besides excess body weight, obesity is associated with comorbidities such as cardiovascular disease, type 2 diabetes mellitus, hypertension and non-alcoholic fatty liver disease, all of which are connected with oxidative stress. Increased oxidative stress could damage cellular macromolecules including DNA, with genomic instability as one of the driving forces for carcinogenesis. Although conservative weight loss regimens demonstrated beneficiary influence on DNA damage reduction, little is known whether a similar effect can be achieved with a more extensive weight loss using more restrictive calorie reduction diets, so the predictions for human population is mostly based on *in vitro* and animal model studies. The aim of this preliminary study was to examine the impact of a 3-week calorie reduction diet of only 500 kcal in persons with a body mass index of ≥ 35 kg/m^2, without any known comorbidity and therapy, on genomic stability using cytochalasin B-blocked micronucleus assay, alkaline comet assay and modified alkaline comet assay for oxidative DNA damage measurement. Fresh whole blood samples were taken before and after the three weeks hospital treatment with the strict diet regime. The results of this preliminary study on 10 volunteers demonstrated reduced volunteers body weight, reduced levels of DNA damage in alkaline comet assay and in oxidative DNA damage. There was a slight reduction in frequency of micronuclei (parameter of whole chromosome loss or partial chromosome loss), while the frequency of nuclear buds (parameter of gene amplification) and nucleoplasmic bridges (parameter of dicentric chromosomes) remained the same. There was also an increase in the proliferation index and reduction in number of apoptosis. The results indicated a beneficiary effect of this strict calorie reduction diet and should be repeated on a higher number of participants.

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POSTER PRESENTATIONS

Abstract number: **P01**

RELATIONSHIP BETWEEN *H1* AND *H2* HAPLOTYPES OF THE 17q21 INVERSION AND PREGNANCY LOSS IN BOSNIAN POPULATION: A CASE-CONTROL STUDY

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The chromosome inversion 17q21 (known as microtubule associated protein tau) is a common, containing approximately 900 kb, polymorphism. Frequencies of two single nucleotide polymorphisms rs9468 and rs1800547 determine worldwide distribution of *H1* and *H2* haplotypes. The rs1800547A allele and the rs9468T allele are on an *H1* haplotype background, and the G and C alleles are on an *H2* background. Distribution of *H2* haplotype is 20% in European populations, and rare to almost absent in African and Asian populations. Recent studies have demonstrated that *H2* haplotype is ancestral in hominoids, and under positive selection in European populations. The role of non-inverted orientation (*H1* haplotype) and inverted orientation (*H2*) remains unclear, i.a. it is suggested that mothers who are *H1H2* heterozygotes, tend to have more children than *H2H2* homozygotes on average. Thus, we sought to assess the prevalence of haplotypes of the 17q21 inversion in 154 women with pregnancy loss and 154 mothers with at last one live-born child, mean age: 33.0 (±5.4) y/o and 31.4 (±6.7) y/o, respectively. Following DNA extraction from buccal swabs, the genotyping was performed using LightCycler 96 Real-Time PCR System. Statistical analysis was performed using t-test for two independent means. Haplotypes were compared between groups. $P < 0.05$ was considered statistically significant. In women with and without pregnancy loss we identified 115 and 122 *H1H1,* 37 and 27 *H1H2* and 2 and 5 *H2H2* haplotypes, respectively. There was no significant difference in the distribution of haplotypes between women with and without pregnancy loss (p-value = 0.25). Statistically significant difference between the average number of children in women with *H1H2* haplotype ($n_{avg.}$ = 1.54) compared to women with *H2H2* haplotype ($n_{avg.}$ = 1.29), was not found (p-value = 0.773). Haplotypes of the 17q21 were not linked to pregnancy loss and number of live-born children.

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Abstract number: **P02**

DECLINING RATE OF INVASIVE PROCEDURES FOR PRENATAL DIAGNOSIS OBSERVED IN A PRIVATE PERINATAL CLINIC "MEHMEDBAŠIĆ"

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The use of maternal serum marker screening and ultrasound imaging to detect chromosome aneuploidies and other birth defects are a routine part of prenatal care in the first and/or second trimesters. If these tests indicate that a fetus is at increased risk of aneuploidy, invasive methods like chorionic villus sampling or amniocentesis are recommended for diagnosis. The use of non-invasive prenatal testing has grown rapidly, leading to a simultaneous reduction in the application of first-trimester combined tests and invasive diagnostic procedures. Commercially available NIPT has the ability to detect the most frequently observed chromosome aneuploidies. Future advances in NIPT technology promise to expand the range of conditions that can be detected. The goal of this study is to determine the rate of prenatal invasive diagnostic procedures performed over time in the era of non-invasive prenatal testing. This is a retrospective review of the number of invasive prenatal procedures performed between January 2014 and June 2019 among women who were referred to our prenatal clinic for high aneuploidy risk. Starting July 2016, the option of non-invasive prenatal testing became available at our clinic. Total number of invasive diagnostic procedures performed at the clinic within 14,5 yrs. period was 2,154. Total number of amniocentesis performed prior to NIPT was 2,013. There was a significant decline in the number of amniocentesis procedures conducted from July 2016 to June 2019. Starting in July 2016, 81 non-invasive tests were performed at our centre. The number of invasive procedures has declined over time due to the availability of non-invasive prenatal testing. Its high sensitivity and specificity make it an attractive alternative to the serum screens and invasive diagnostics currently in use. It is important is to provide appropriate counselling to the patients and selection of which screening or diagnostic test to use.

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Abstract number: **P03**

FREQUENCY OF FV LEIDEN AND FII G20210A HOMOZYGOTES AND COMBINED CARRIERS IN LARGE COHORT OF PATIENTS FROM SERBIA

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Thrombophilia is a common, multifactorial disease, where environmental and genetic risk factors play a significant role in its pathogenesis. The most frequent genetic risk factors are FV Leiden and FII G20210A mutations. According to our published data, frequencies of FV Leiden and FII G20210A mutation heterozygotes carriers in Serbian patients with thrombosis are 29.3% and 10.9%, respectively. The aim of this study was to establish the prevalence of the FV Leiden and FII G20210A homozygous carriers and combined carriers in thrombophilic Serbian population. The study was carried out in a study group of 2561 patients admitted to The Institute of Molecular Genetics and Genetic Engineering for genetic testing of thrombophilia. Study group consisted of two subgroups: 1476 patients with thrombotic events (subgroup T) suffered from deep vein thrombosis, pulmonary embolism, central vein insult, myocardial infarction, etc. and 1085 females with spontaneous miscarriages (subgroup M). In this study group, 1.17% of combined FV Leiden and FII G20210A heterozygous carriers were detected (1.63% in T subgroup and 0.55% in M subgroup). The 0.98% homozygous carriers of FV Leiden mutation (1.22% in T subgroup and 0.46% in M subgroup) and 0.08% homozygous carriers of FII G20210A mutation (0.14% in T subgroup and 0% in M subgroup) were detected. Although, 0.08% homozygous FV Leiden/heterozygous FII G20210A carriers were identified in study group, no combined homozygous FII G20210A/FV Leiden carrier was detected. This study confirms that homozygosity for both tested mutations, as well as combined heterozygosity, is present with low frequency. According to our results, these genotypes are more common in patients with thrombotic events than in patients with spontaneous miscarriages.

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Abstract number: **P04**

MICRO RNA AS A NOVEL BIOMARKER FOR DIAGNOSIS, PROGNOSIS AND MANAGEMENT FOR PATIENTS WITH *DIABETES MELLITUS*

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Diabetes mellitus is a chronic disorder, not a single disease but a set of metabolic disorders characterized by an increase in blood glucose levels. Chronic hyperglycemia represents a consequence of impaired secretion or action of insulin, a hormone produced by the beta cells of pancreatic islets which plays a key role in controlling blood sugar levels. If *diabetes mellitus* can be detected at an early stage, that implicates better and more developed management for this disorder. Currently used diagnostic criteria and biomarkers have their disadvantages and limitations. In recent years, novel methods for diagnosing *diabetes mellitus* are being analyzed and discovered. One of them is micro RNA (miRNA) and it showed significant potential for diagnosis and even for prognosis and management of *diabetes mellitus*. MicroRNA are small functional molecules of RNA usually consisting of 18-22 nucleotides and were first discovered in nematode *Caenorhabditis elegans*. They regulate genes but are too small to code proteins. Their main role is to bind to a complementary target sequence on mRNA and affect protein synthesis mostly by acting as negative regulators and inhibiting mRNA translation. In this review, we analyzed the benefits and advantages of using several types of miRNA molecules (miRNA 93, 126, 130a, 146a, 155, 197, 223, 335, 375, 802) as potential biomarkers for management and diagnosis *diabetes.* From all types of miRNAs mentioned before, miRNA 223, 146a, 130a and 93 showed more superior benefits and advantages. In summary, this means that these four types of miRNA present the novel biomarker for diagnosis, prognosis, and management of *diabetes mellitus*. This means that these four types of miRNA could have practical usage in the near future.

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Abstract number: **P05**

ASSOCIATION OF *FADS1* GENE POLYMORPHISM WITH DESATURASE ACTIVITY IN TYPE 2 DIABETES

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Long-chain PUFA (LCPUFA) have a key role in biological functions in humans as a main components of the cell membrane, act as signalling molecules, and regulate gene expression. Plasma and tissue LCPUFA levels are influenced not only by diet but also, to a large extent by heritability. Delta-5 (D5D) and delta-6 desaturases (D6D), encoded respectively by *FADS1* and *FADS2* genes, are the rate-limiting enzymes for PUFA conversion and are recognized as main determinants of PUFA levels. Alterations of D5D/D6D activity have been associated with several diseases, from metabolic derangements (metabolic syndrome and type 2 diabetes, T2D) to inflammation and tumorigenesis. Recent genome-wide association studies showed that *FADS1/FADS2* genetic locus was strongly associated with plasma lipids and glucose metabolism. In this study, we investigated the associations of D5D and D6D in T2D risk with rs174550 polymorphism of *FADS1* gene. FA levels in plasma were determined using gas chromatography-mass spectrometry in 390 T2D patients and 252 control subjects, while D5D and D6D activities were estimated from the C20:4n-6/C20:3n-6 and C20:3n-6/ C18:2n-6 ratios, respectively. Our results showed that rs174550 risk T allele was significantly associated with decreased levels of D6D (B=0.072 95%CI 0.014;0.129, p_{add}=0.016) in T2D patients, while in drug-treated T2D patients it was associated with increased levels of D6D (B=-0.77 95% CI -0.149;-0.006, p_{rec}=0.035). Interestingly, the risk T allele was also associated with decreased D5D activity in control subjects (B=77.400 95% CI 36.411;118.409, p_{add}=0.027). Thus, our results suggest that rs174550 polymorphism in the *FADS1* gene affects D6D and D5D in this sample of Bosnia and Herzegovina population.

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Abstract number: **P06**

DAT POLYMORPHISM AND ITS ASSOCIATION WITH IRITTABLE BOWEL SYNDROME

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Irritable bowel syndrome (IBS) is functional disorder of gastrointestinal tract characterized by abdominal pain and discomfort. It is associated with defecation and change in stool form, frequency and abdominal distension, as well as with various psychological conditions. Studies show that serotonin transporter SLC6A4 (SERT) is one of the most promising genes involved in the etiology of irritable bowel syndrome. A very important paralog of SERT is dopamine transporter SLC6A3 (DAT) which has a key role in regulation of dopamine neurotransmission. The polymorphism of variable number of tandem repeat (VNTR) within non-coding region of *DAT* gene is in association with several clinical phenotypes such as deregulation of dopamine transport. The aim of this study was to investigate the association of this polymorphism with clinical phenotypes of patients with IBS. Blood samples were taken from 20 IBS patients whom IBS was diagnosed according to Rome III criteria and 9 healthy volunteers without any gastrointestinal symptoms. Genotyping was performed by convectional PCR followed by sequencing on 3500 Genetic Analyzer. Results showed strong statistically significant association of DAT polymorphism and IBS regarding 434 allele variant (P=0,006), meaning that persons with this allele have a six time higher possibility for IBS than people without this variant. Also, genotypes of 434/434 genotype carriers showed statistically significant association with IBS (P=0,031). Although most of the previous studies have been focused on serotonin, this study has confirmed that the dopamine neurotransmitter system plays a key role in the ethiopathology of IBS.

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Abstract number: **P07**

A RARE MICRODELETION OF CHROMOSOME 19q13.43 SYNDROME – PSYCHOMOTOR DELAYED, INTELECTUAL DISABILLITY, EPILEPSY - CASE REPORT TWO PATIENTS

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Microdeletion syndrome 19q13.43 is a rare syndrome includes facial dysmorphism, intellectual disabilities, difficulty feeding and poor weight gain in infancy. Most patients have impaired EEG and epilepsy. The first patient is 17 years old girl born in the second pregnancy of nonconsanguineous parents birth weight 2,8 kg, Apgar score 8/10. Early psychomotor development was a delay and she has moderate intellectual disability (IQ 45). Seizures began in the second year of life. Dysmorphic features: epichantal folds, synophrys, high arched palate, irregular growth of teeth, maxillary prognathism, low set hair on the neck and head, flexible joints, long, thin fingers, clinodactyly. Magnetic resonance imaging (MRI): ventriculomegaly, hypoplastic corpus callosum. A karyotype is normal with heteromorphism of chromosomes 16. MLPA found microdeletion 19q13.43. The second patient is 12 years old boy born in the second pregnancy of nonconsanguineous parents birth weight 3,4 kg, Apgar score 10 positive psychiatric heredity and exposition to family violence. Seizures developed in the second year of life followed by delayed cognitive development. Dysmorphic features: epichantal folds, hypertelorism, large ears with otapostasis, high arched palate, convergent strabismus, low set hair on the neck, maxillary prognathism, pectus infundibuliform, flexible joints, umbilical hernia, and scoliosis. Also have hyperactivity, attention deficit disorder with autistic behavior. MRI: spreading liquid space on the base of the brain. A karyotype is normal. Using MLPA method we found microdeletion 19q13.43. It is rare microduplication syndrome of the region include several genes such as SCN1B, whose haploinsufficiency is associated with the development of epilepsy. The genes from KRAB-ZNF clusters also found in this region are transcriptional factors in brain differentiation and play a role in the development of cognitive functions. There is no cure for 19q13.43 deletion syndrome, affected individuals need a team of specialized doctors for treating the various problems.

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Abstract number: **P08**

A RARE DE NOVO DUPLICATION 21q22.3 SYNDROME - CASE REPORT

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Here we present a four year old boy with duplication 21q22.3. According to the available literature, region responsible for this duplication syndrome is close to the region for Down syndrome (DS), but these patients have only discreet Down syndrome phenotype. A boy was born from first pregnancy of non-consanguineous parents, birth weight 2.6 kg, Apgar score was 8/10. From early childhood he presented with psychomotor delay, generalized hypotonia, failure to thrive and recurrent respiratory infections. Seizures begun at the age of three and persist despite antiepileptic therapy. He has dysmorphic features including epichantal folds, high arched palate, strabismus, large low set ears and joint hyperlaxity. Magnetic resonance imaging showed asymmetry in the appearance of the skull as well as asymmetry of intracranial structures with wider basal cisterns. Intellectual disability is severe (IQ 36) and he has stereotypical movements. Chromosome analysis was performed on cultivated peripheral blood leukocytes using standard GTG banding technique. Consecutively, we performed multiplex ligation probe amplification (MLPA) screening using SALSA MLPA probemix P036 and SALSA MLPA probemix P070. GTG-banding showed normal male karyotype (46,XY). MLPA analysis indicated duplication in chromosomal region 21q22.3. Data from the literature suggest that the chromosomal region 21q22.3 is related to Down syndrome phenotype. Our patient has nonspecific phenotype: generalized hypotonia and intellectual disability, but no dysmorphic features related to DS. There is further need to evaluate this duplication and correlate genotype with phenotype in our patient.

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Abstract number: **P09**

GENOMIC PROFILING OF GLYCOGEN STORAGE DISEASES

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Glycogen storage diseases or glycogenosis are inherited disorders of glycogen synthesis or degradation, which primarily affect the liver, kidneys and intestinal mucosa. Since the consequences of these disorders are serious and irreversible, molecular-genetic testing is essential for precise diagnosis and optimal medical treatment. We analysed 41 patients with clinical suspicion of glycogenosis and 75 control subjects from Serbia using Sanger and next-generation sequencing. Pathogenicity of novel variants was determined based on expressional, computational analysis and patient's phenotype. The genomic profiling of analysed patients revealed 5 patients with glycogenosis Ia and 31 patients with glycogenosis Ib. Using the next-generation sequencing method we identified patients with glycogenosis III, VI, IXa, cholesteryl ester storage disease and Shwachman-Diamond syndrome. In *SLC37A4* gene of glycogenosis Ib patients we detected 4 novel variants: p.Gly83Glu, p.Gly135Asp, p.Pro191Ser and p.Ser263Glyfs*33. The CRISPR/Cas9 *knocking* method was used to introduce p.Gly83Glu variant in *SLC37A4* gene of HEK293FlpIn cell line in order to establish the new *in vitro* model system for the glycogenosis Ib and to functionally characterize this variant. Computational, expression analysis and clinical presentation in patients undoubtedly confirmed the pathogenic effect of the novel variants (p.Gly83Glu and p.Ser263Glyfs*33) in *SLC37A4* gene. Comprehensive molecular-genetic analysis of patients with clinical suspicion of glycogenosis from Serbia achieved 100% mutation detection rate, allowing early application of appropriate therapy specific to the patient's genotype. The results of this work supported the usefulness of next-generation sequencing for correct diagnostics of glycogenosis and differential diagnosis in patients with overlapping phenotypes. Furthermore, we established a novel human kidney cell model for glycogenosis Ib which lacks *SLC37A4* expression and therefore could be a useful tool to study the pathogenesis of the glycogenosis Ib and to test the latest molecular therapeutics designed to correct metabolic abnormalities related to the glycogenosis Ib.

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Abstract number: **P10**

ddPCR, SIMPLE, ACURATE AND RELIABLE TOOL FOR PRECISION DIAGNOSTICS

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The clinical management of disease is evolving towards more personalized strategies that require a comprehensive knowledge of the complex molecular features involved in tumour development, as well as the development of drug resistance mechanisms leading to disease progression. Because of its high sensitivity and specificity droplet digital PCR (ddPCR) has become one of the most accurate and reliable tools for the examination of genetic alterations in a wide variety of cancers. ddPCR is currently being applied for rare mutation detection, absolute allele quantification, analysis of copy number variations, gene rearrangements, DNA methylation, and in different kinds of clinical samples. This methodology has proven useful for the evaluation of archival tumour tissues, where poor DNA quality and limited sample availability are major obstacles for standard methods, providing less subjective and more automated quantitative results. However, most applications of ddPCR in cancer are focused on liquid biopsies (including cell-free DNA as well as circulating tumour cells) because these represent non-invasive alternatives to tissue biopsies that can more accurately reflect intratumoural heterogeneity and track the dynamic changes in tumour burden that occur in response to treatment at different times during follow-up. A broad spectrum of molecular markers have been interrogated in blood using ddPCR for diagnostic, predictive, and monitoring purposes in various malignancies. Emerging alternative approaches using other body fluids such as cerebrospinal fluid and urine are also currently being developed. This poster aims to give a complete overview of ddPCR applications for molecular screening in oncology and other clinical or scientific uses.

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Abstract number: **P11**

DIFFERENTIAL DIAGNOSIS OF PATIENTS WITH PEDIATRIC LUNG DISEASES AND DISCOVERY OF NOVEL DISEASE-CAUSING GENES AND GENETIC VARIANTS BY USING NEXT GENERATION SEQUENCING TECHNOLOGY

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Primary ciliary dyskinesia (PCD) is a rare disorder that affects lungs, reproductive organs and the internal organs laterality. The disease is inherited in an autosomal recessive or X-linked manner. PCD is clinically and genetically heterogeneous disorder with overlapping symptoms with other paediatric lung diseases. The aim of the study was genomic profiling of suspected PCD patients in order to establish the genetic background of PCD in our patients, to confirm the clinical diagnosis, and to design a strategy for differential diagnosis of PCD patients among patients with similar clinical presentation. Using Clinical-Exome Sequencing Panel, we analysed 93 genes related to PCD and other paediatric lung diseases in a cohort of 22 Serbian patients with clinically suspected PCD. Analysis of obtained results revealed genetic variants in *CCDC39, CCDC40, DNAI1, DNAL1, DNAH5, DNAH11* and *LRRC6* genes, and pointed and confirmed *SPAG16* and *SPAG17* as novel PCD disease causing genes. Twenty variants in these genes were pathogenic, of which fourteen (70 %) were novel. The PCD diagnosis was established in 54.55 % of patients. Analysis of genes related to individual symptoms of PCD, revealed 6 pathogenic variants in *ABCA3, CFTR, MUC2, SCNN1A*, and *SLC26A9* genes, of which 5 (83.33%) were novel. This enabled the diagnosis for additional 28.57% patients. The analysis of the extended list of genes enables mutation detection rate of 95.45% (21/22 patients), while the rate of established diagnosis reached 81.82% (18/22 patients).
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Abstract number: **P12**

THE INFLUENCE OF *UGT1A1* PROMOTER VARIANTS ON THE BILIRUBIN LEVEL IN SERUM OF PATIENTS WITH β-THALASSEMIA MINOR AND CHRONIC HEPATITIS C

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Unconjugated hyperbilirubinaemia due to hemolysis in patients with β-thalassemia minor and liver fibrosis in patients with chronic hepatitis C (HHC) may be significantly affected by the presence of different variants in promotor of the *UGT1A1* gene. *UGT1A1* promoter variant with 6 TA repeats shows the highest expression, while the variant with 7 TA repeats shows a reduced expression of the *UGT1A1* gene. Carriers of *UGT1A1* 7/7 TA genotype develop Gilbert syndrome (GS). The influence of *UGT1A1* promoter variants on bilirubin level was studied in 22 patients with β-thalassemia minor, 24 patients with CHC and 8 healthy individuals. Promoter *UGT1A1* TA variants were genotyped using PCR/acrylamide electrophoresis and Sanger sequencing. Total bilirubin in patients with β-thalassemia was 37.04 μmol/l and was 3.86 times higher than in the control group. The lowest values of bilirubin were measured in β-thalassemia patients carriers of 6/6 TA *UGT1A1* genotype. Heterozygous carriers of 6/7 TA *UGT1A1* genotype had 2.2 times greater bilirubin values than β-thalassemia patients carriers of 6/6 TA *UGT1A1* genotype. Beta-thalassemia patients carriers of *UGT1A1* 7/7 TA genotype had 4.3 times higher bilirubin values than patients carriers of 6/6 TA *UGT1A1* genotype. CHC patients carriers of 6/6 TA and 6/7 TA *UGT1A1* genotypes had mean total bilirubin of 11.19 μmol/l which corresponds to reference values. CHC patients carriers of 7/7 TA *UGT1A1* genotype had a 3-fold higher bilirubin level than carriers of 6/6 TA and 6/7 TA *UGT1A1* genotypes. The *UGT1A1* gene can be treated as a gene modifier in β-thalassemia syndromes. High levels of bilirubin in CHC patients were not the result of liver fibrosis, but due to presence 7/7 TA *UGT1A1* genotype. *UGT1A1* TA promoter variants should be taken into account when treating patients with increased unconjugated bilirubin values.

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Abstract number: **P13**

INFLUENCE OF PRENATAL CYTOGENETIC DIAGNOSTICS IN REDUCTION OF NUMBER OF NEWBORN CHILDREN WITH DOWN SYNDROME AT THE UNIVERSITY CLINICAL CENTER OF THE REPUBLIC OF SRPSKA FROM 2009-2019 YEAR

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Trisomy 21 is one of the most common chromosomal aberrations, which is clinically shown as Down syndrome. The aim of this paper was to present the number of Down syndrome, detected by prenatal cytogenetic diagnostics in the period from 2009 – 2019 at the University Clinical Center of the Republic of Srpska, as well as the number of postnatal detected cases in the same period. It was also a goal to determine extent to which use of prenatal invasive diagnostics influenced on reduction of number of children born with Down syndrome. For prenatal and postnatal karyotyping, samples of amniotic fluid and peripheral blood were processed according to the appropriate protocols for obtaining chromosomes. Karyotype was described according to ISCN 2013. During the mentioned ten-years period, a total of 8 556 prenatal analysis were made. The most common indications for invasive prenatal diagnostics, were serum markers from the blood of pregnant woman, combined with ultrasound findings and age of pregnant women aged 35 and over. The results showed that a total of 134 fetuses with numerical aberrations were detected. Trisomy 21 chromosome as the most common aneuploidy, was detected in 76 cases and made 56,71% of all numerical aberrations with an incidence of 1 in 113 amnion. In relation to 96 850 of live-born children in the Republic of Srpska and 56 cases (0,058%) with Down syndrome, we can conclude that Down syndrome is the most common numerical aberration detected postnatal in our Center. According to data of Statistical Office of the Republic of Srpska there is a significant increased number of pregnant women older than 35, but decreased number of live-born children with Down syndrome which is direct consequence of prenatal diagnostics.

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Abstract number: **P14**

TWO CASES REPORT OF UNBALANCED KARYOTYPE 45,XX,der(4)t(4;22)(p16;q11.2),-22

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In this paper are presented the results of cytogenetic analysis of two cases of unbalanced female 45-chromosomal karyotype, that are the result of translocation between chromosomes 4 and 22, with breakpoints in 4p16 and 22q11.2. Standard cytogenetic analysis using G banding demonstrated a karyotype 45,XX,der(4)t(4;22)(p16;q11.2),-22. In the karyotype there is one normal chromosome 4 and one derivative chromosome 4, as well as one normal chromosome 22. The karyotype is unbalanced due to the partial monosomy of the part p of chromosome 4 (pter → p16), as well as the partial monosomy of the chromosome 22 (pter → q11.2). One case was detected from peripheral blood lymphocytes, in a girl of 16.5 years who has moderate mental retardation, epileptic seizures and poorly developed speech. The second case was detected by prenatal diagnosis from amniotic fluid cells, in a pregnant woman who was referred to amniocentesis due to age and family history. We do not know whether this kind of karyotype has occurred de novo or is the result of translocation t (4; 22) (p16; q11.2) in one of the parents, in either case. Deletion 4p is associated with Wolf-Hirschhorn syndrome (WHS). The 22q11.2 microdeletion is associated with a wide range of overlapping phenotypes including DiGeorge and other similar syndromes. Chromosomal analysis and genetic counseling are typically recommended for parents of an affected child to help confirm or exclude the presence of a balanced translocation or other chromosomal rearrangement in one the parents.

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Abstract number: **P15**

METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM (MTHFR) AS A NON-TRADITIONAL RISK FACTOR OF CARDIOVASCULAR DISEASES IN PATIENTS ON HEMODIALYSIS

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Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in metabolism of folate. Defects in a gene encoding this enzyme can cause a hyperhomocysteinemia, independent risk factor for atherosclerosis. C677T variant of MTHFR polymorphism is considered as a risk factor for blood vessel structure changes and cardiovascular diseases (CVD). Measurement of carotid intima-media thickness (cIMT) is useful for establishing CVD risk. However, there is no sufficient evidence to comprise C677T MTHFR polymorphism as a reliable non-traditional risk factor for CVD. Aim of the study was to test the influence of C677T MTHFR on a cIMT as a marker of CVD in a group of patient on hemodialysis. In addition, we tested association of gene variant and traditional risk factors. Cross-sectional study enrolled 53 patients on hemodialysis treatment. They were divided into two groups - wild-type allele carriers (CC genotype, n=29) and mutated allele carriers (CT+TT genotypes, n=24). The genotype of MTHFR gene C677T variant was determined using polymerase chain reaction and subsequent cleavage with *Hinf*I restriction endonuclease. Carotid intima-media thickness was measured using high-resolution B-mode ultrasonography. Lipid profile parameters were measured on automated analyzers while lipid indexes were calculated. Allele and genotype association with cIMT values, lipid profile parameters and lipid indexes was analysed using SPSS for Windows. For C677T MTHFR, the genotype distribution in all subjects was in Hardy-Weinberg Equilibrium (P=0.234). There was no difference in cIMT values, lipid profile parameters, lipid indexes between two groups of patients. Significantly lower values of non-HDLc was observed in a mutated alleles carrier groups of patients (P=0.023).Results suggest that MTHFR gene C677T variant is not a factor that influence cIMT and can't be considered as reliable non-traditional factor for CVD in a patients on hemodialysis.

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Abstract number: **P16**

Y-CHROMOSOME HAPLOGROUP DIVERSITY OF THE ROMA POPULATION OF NORTH-EASTERN BOSNIA AND HERZEGOVINA

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Previous studies indicated that European Roma share close genetic, linguistic and cultural similarities with Indian populations despite their different geographic location and demographic history. This study is the first report of paternal haplogroup diversity in the Roma population from Bosnia. The main aim of the research was the analysis of the Y-chromosome haplogroup diversity in the examined population and analysis of the influence of paternal gene flow on the Roma gene pool of the population from northeastern Bosnia. Sixty-seven unrelated male individuals of the Roma populations were typed for 23 Y-chromosome short-tandem repeat (STR) loci by the *PowerPlex® Y23 System*. Prediction of Y-chromosome haplogroups from a set of Y-STR loci was made using three different predictors: Whit Athey's Haplogroup Predictor, Jim Cullen World Haplogroup & Haplo-'I' Subclade Predictor i NevGen Y-DNA Haplogroup Predictor. Our results showed that haplogroup H1a1a M82 (64.2%) was the most frequent. In addition to these typical Indian founder lineage, the remaining haplogroups were I1 (16.4%), J2a1 (9.0%), I2a1b3 (7.5%), J2b2a (1.5%), G2a2b2a1b (1.5%), indicating certain amount of genes from the surrounding populations involved during their settlement in the Balkans. The Roman Modal Haplotype (DYS19*15, DYS389I*14, DYS389II*30, DYS390*22, DYS391*10, DYS392*11, DYS393*12) was observed in 26.86% of individuals. Our data confirmed a high level of genetic homogeneity characterized by low levels of haplotype diversity of Roma populations. The data point to the conclusion that the Roma paternal gene pool from northeastern Bosnia could be the result of an early division of the original proto-Roma population and later different demographic history.

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Abstract number: **P17**

PREDICTION OF Y HAPLOGROUP IN ANALYSIS OF HUMAN SKELETAL REMAINS FROM ARCHAEOLOGICAL SITES IN BOSNIA AND HERZEGOVINA

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Archaeological skeletal remains represent valuable source of information in context of determining haplogroups. Y STR ancient DNA (aDNA) analysis is often used for identification, disputable male kinship testing and monitoring migration patterns. The most common Y haplogroup among recent males in Bosnia and Herzegovina is I haplogroup (50%) with I-P3 subhaplogroup. Main goals of this study were successful isolation of aDNA from skeletal remains found at two archaeological sites (Divičani and Kopošići) and prediction of Y haplogroups for obtained Y STR haplotypes. DNA was isolated from teeth and bones samples using an optimized phenol/chloroform DNA extraction procedure, preceding decalcification with EDTA. Quantification of samples was performed with a *Quantifiler Duo quantification kit* for determining the exact concentration of DNA and potential presence of the inhibitors. *PowerPlex® Y23 System* was used for amplification of 23 Y STR loci and analysis was performed on *ABI PRISM 310 Genetic Analyzer*. Analysis of the generated haplotypes and prediction of the haplogroups was done using YHRD database, *Y-DNA Haplogroup Predictor – NEVGEN* and *Athey: Haplogroup predictor – HAPEST*. Partial DNA profiles were obtained for all samples. Comparison of given Y DNA profiles concluded that four persons were in a kinship relations by a paternal line. Subsequent analysis showed that all samples from Kopošići and Divičani sites belong to haplogroup I2a and subhaplogroup I2a1b3 except one case from Kopošići which belongs to haplogroup R1a. Prediction results from both online software's used in this study were in concordance, however predictor NEVGEN calculates subgroups of haplogroup I2a. These preliminary results indicate that genetic structure of male population in medieval B&H is similar to the recent one. Our results also showed that both of these software's can be used for predicting haplogroups, however HAPEST is the recommended one.

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Abstract number: **P18**

DNA ANALYSIS OF SKELETAL REMAINS: CASE OF DISPUTABLE KINSHIP TESTING

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The analysis of DNA from skeletal remains represents one of the biggest challenges in forensics genetics due to number of misinterpretations. First of all, DNA extracted from skeletal remains is found in low copy number, it is frequently degraded and contaminating substances are often presented. Successful isolation of DNA from skeletal remains, generating DNA profiles and testing of disputable kinships were main goals of this study. For this study, DNA was extracted from 12 referent samples of buccal swabs and 11 disputed samples of skeletal remains. These samples were used for paternity (five cases), maternity (four) and kinship testing (three). DNA extraction from buccal swabs was done using *QIAamp DNA Mini Kit* and for skeletal remains organic (phenol-chloroform) extraction was used. In total 24 loci were typed using *PowerPlex® Fusion* system. Electrophoresis of the obtained products was done on *ABI PRISM 310 Genetic Analyzer* (Applied Biosystems). For the obtained DNA profiles of child and potential father Paternity Index (PI), Combined Paternity Index (CPI) and Probability of Paternity (PP) were calculated. As an indicator of the degree of kinship between possible brother and sister Likelihood Ratio (LR) was calculated. In this study 12 complete (referent samples) and 11 partial (disputed) DNA profiles were generated. Given values of PP in five cases regarding paternity testing were above 99,999 % which confirms paternity. Also in all four cases of maternity testing values of PP were higher than 99,999 %, which also confirms maternity assumptions. Regarding analysis of possible brother-sister relations, in all of the three cases LR, was above 1 which leads to conclusion that there is higher probability of kinship relatedness between samples. Kinship analysis can be successfully preformed from skeletal remains, as this study confirmed.

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Abstract number: **P19**

DNA ANALYSIS OF BLOODSTAINS FROM CRIME SCENE: CASEWORKS EXPERIENCE

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Blood is one of the most frequent biological trace found at the crime scene, produced as a result of bleeding victim and/or person who committed the crime. Analysis of bloodstains is often crucial for solving criminal cases and molecular biology methods are routinely used for identification of blood sample donors. In 16 years practice, blood traces were processed in 181 forensic cases at the Laboratory for forensic genetics, Institute for Genetic Engineering and Biotechnology. Blood traces were characterized as traces from clothes and shoes (39%), stains on objects used in crimes (23%), stains on filter papers and swabs (14%), stains on textile (14%) and others (10%). After confirming blood traces by using serological tests, DNA analysis was done using standard methods for DNA isolation, amplification and DNA profile generation. Out of 181 samples, complete DNA profiles were obtained for 117, partial profiles for 44 samples and for 20 samples no profiles were generated. The success of the analysis depends on quantity of trace, proper collection and preservation of blood traces and optimal selection of detection and analysis methods.

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Abstract number: **P20**

COMBINED EXPOSURE OF RADIOFREQUENCY AND UV RADIATION INDUCE ADAPTIVE RESPONSE IN KERATINOCYTE CELLS *IN VITRO*

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The aim of this study was to examine whether radiofrequency (RF) exposure induces genotoxic effects or play a role in the induction of adaptive response after combined exposure in keratinocytes (PromoCell, Germany) *in vitro*. After 24 hours pre-incubation the cells were exposed to 2450 MHz WiFi radiation (SAR: 2 W/kg) for 24 hours and 4 hours later challenged with UV radiation (4 SED) for one hour. The adaptive response condition was compared with one hour UV irradiated cells and sham exposed cells as well. Evaluation of the DNA damage was performed with alkaline comet assay and for each exposure condition 400 cells were examined with Comet Assay IV software (Instem, UK). Data analysis was done by linear mixed-effects models. Our results showed that there was a significant genotoxic effect of RF exposure in keratinocytes. Furthermore, results indicate a significant reduction in DNA strand breaks in RF pre-exposed, subsequently UV exposed cells compared with only UV exposed cells. Consequently the adaptive response was detected. This experiment was done as a part of the project named “Cellular response to co-exposure of radiofrequency (RF) and solar ultraviolet (UV) radiation in human *in vitro* skin model (SKIN-RF)” funded by ANSES. (French Agency for Food, Environmental and Occupational Health & Safety) No. 2015/2 RF /14.

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Abstract number: **P21**

A HIGH-THROUGHPUT COMET ASSAY METHOD FOR THE JOINED PROCESSING OF SAMPLES TAKEN SEPARATELY

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Due to the increasing use of electromagnetic fields in household and telecommunication devices, a large number of studies is being performed on the potential biological effects of non-ionizing radiation. One important aspect of these effects is genotoxicity, the level of damage done to the genetic information. The single cell gel electrophoresis or comet assay is a fast and versatile tool for studying genotoxicity on individual cells. Since its first publication, the method has been modified for several different purposes. Some of these modifications allow an increased throughput, which is essential for working with a large number of samples. However, in some experimental settings the samples are not available at the same time or place, but they still need to be processed together. The comet assay is done on cells embedded in agarose gel. In the traditional form of the assay, cells of monolayer cultures need to be removed from the culture surface to be molded in agarose. However, this removal can cause additional damage, and the time between the treatment and the lysis of molded cells allows DNA repair processes to reduce the damage, thus altering the results. Our aim was to develop a method for the joined processing of small samples taken at intervals or at different locations, with the minimum of additional damage and elapsed time between the treatment and cell lysis. The feasibility of the proposed method has been tested on X-ray irradiated human peripheral blood lymphocytes. In this study we also investigate the biological effects of intermediate frequency (IF) electromagnetic fields on normal human dermal fibroblasts.

The present work was done as a preliminary study under the project “FIGé – Intermediate frequencies and genotoxic stress” funded by ANSES (French Agency for Food, Environmental and Occupational Health & Safety) No. 2017-2 RF-08.

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Abstract number: **P22**

ANALYSIS OF DNA DAMAGE IN PERIPHERAL BLOOD CULTURES TREATED WITH CURCUMIN OR SUNSET YELLOW

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Curcumin, spice used as a natural food colorant, has been long recognized as a potent therapeutic and protective agent and is among the most frequently tested substances in various researches. However, certain genotoxic effects for curcumin were previously reported. Synthetic food colorants, including sunset yellow, are often associated with health risks especially in vulnerable populations. In this study, we aimed to compare curcumin and sunset yellow induced DNA damage using alkaline comet assay in peripheral blood mononuclear cells treated in whole blood cultures. After one hour exposure DNA intensity in both curcumin and sunset yellow treatments was lower than one registered in DMSO (dimethyl sulfoxide) control. Nevertheless, prolonged exposure (24h) has shown significantly lower DNA intensity after treatment with the highest tested concentrations of curcumin and significantly higher DNA intensity in cultures treated with the highest concentration of sunset yellow. Obtained results suggest curcumin's ability to reduce background levels of DNA damage acting as an antigenotoxic agent. Longer exposure to higher concentrations (10 and 20 μM) of sunset yellow significantly increases induced DNA damage, although resulting in lower DNA damage compared to control, even in 24h treatment.

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Abstract number: **P23**

THE IMPORTANCE OF NUCLEAR DIVISION INDEX IN BIOMONITORING HUMAN STUDIES USING THE MICRONUCLEUS ASSAY

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Radiation as a physical agent can cause major alteration to the genetic material, chromosomal instability and cell damage. In this study the micronucleus assay (MN) is being applied for biomonitoring the hospital workers exposure to ionizing radiation or *in vitro* genotoxicity testing of radiation as a predictor of cancer risk, because chromosomal mutation and cell alteration is the main reason for carcinogenesis. The frequency of micronuclei (MNi) and nuclear division index (NDI) are very important factors and usefull indicators for genotoxicity, mitogenic response and proliferative status of the lymphocytes. It was a prospective observational study when MN assay is being applied on control and exposed group (hospital workers with different time of exposure and work activity). The results of this study confirm the high frequency of MNi in hospital workers with long time of exposure and presence of nucleoplasmic bridges (NPBs), nuclear buds (NBUDs) as biomarkers of DNA miss repair complexes. We evaluated when NDI value is lowest than 1.0 all of peripheral lymphocytes are mononucleated, when NDI value is close to 2.0 most of the lymphocytes are binucleated, when NDI value is greater than 2.0 therefore most of the lymphocytes contain more than two nuclei. Our observation confirme, when we apply MN on peripheral lymphocytes the NDI value is better to be close to 2. Also, the NDI value is high in hospital workers with long time of exposure than in control group. The frequency of MNi is high in peripheral lymphocytes in exposed hospital workers than in control group. NDI is useful parameter and could provide better observations for genotoxicity of radiation or other agents in similar studies which biomonitoring the human cells using MN assay.

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Abstract number: **P24**

GENOTOXICITY EVALUATION OF URSOLIC ACID ON MAMMALIAN CELL LINES BY MICRONUCLEUS ASSAY

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Consumption of great amounts of fruits and vegetables rich in phenolic compounds has been associated with the health benefits such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects. Phenolic compounds have been regarded as possible antioxidants, so they have been used in food industry and in the prevention of diseases resulting from oxidative stress. Ursolic acid (3β-hydroxy-12-urs-12-en-28-oic acid) is a well-known pentacylic triterpene which is heavily used in traditional Chinese medicine. *Malus pumila, Ocimum basilicum, Vaccinium* spp*., Vaccinium macrocarpon, Olea europaea, Origanum vulgare, Rosmarinus officinalis, Salvia and Thymus* plants are the main sources of ursolic acid (Ikeda et al., 2008). In recent years, interest in ursolic acid has increased due to its many beneficial effects and low toxicity. Ursolic acid has been using against different diseases including osteoarthritis, rheumatoid arthritis, ulcer, cancer and diabetes In the present study, genotoxic/antigenotoxic effects of ursolic acid were evaluated by micronucleus (MN) assay in human peripheral blood lymphocytes and Chinese hamster lung fibroblast cells (V79). Cells were treated with 5, 10, 25, 50 and 100 µM ursolic acid. Hydrogen peroxide (H_2O_2), 50 µM, was used as positive control and 1% DMSO was used as negative control. In our study, the cells were treated with different concentrations of ursolic acid caused no genotoxic effects alone at all studied concentrations as compared with the negative control. MN frequencies of ursolic acid treated cells were found to be decreased when compared to positive control. It seems that ursolic acid might have a role in the prevention of genotoxic damage.

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Abstract number: **P25**

EVALUATION OF THE GENOTOXICITY POTENTIAL OF WATER FROM THE LAKE HAZNA FROM BOSNIA AND HERZEGOVINA BY MICRONUCLEUS ASSAY IN ERYTHROCYTES OF *CARASSIUS GIBELIO* AND *ALLIUM CEPA* L. ASSAY

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The present study evaluates the genotoxic potential of water from lake Hazna using both micronucleus (MN) assay, in erythrocytes of *Carassius gibelio*, and *Allium cepa* assay, in the root meristem cells. The Lake Hazna near Gradačac (B&H) was built on the Hazna waterfall in 1967. The study included a total of 30 individuals Prussian carp (lat. *Carassius gibelio*, Bloch, 1782) from Lake Hazna. To analyze the number of micronuclei were used microscope slides of differential blood picture of Prussian carp (Pappenheim staining). The Allium test was also used to evaluate the genotoxicity in terms of determining the mitotic index (MI) and monitoring of chromosomal anomalies. For determination the MI 2000 cells were analyzed. The samples of water for MI analyses were taken from 5 different localities of Lake Hazna.
The frequency of micronuclei was in range from 0.0% to 1.7%. Of 30 analysed individuals, in 21 individuals the number of micronuclei ranged from 0.0% to 0.7%, which is considered normal values. In 9 individuals of fish, the number of micronuclei ranged from 0.8% to 1.7%. The incidence of binucleated erythrocytes in the blood of *Carrasius gibelio* in the Lake Hazna ranged from 0.0% to 1.4% per individual. The lowest value of the MI was 10.20% and the highest 12.35%. The results of the micronucleus test and Allium test indicate that water from Lake Hazna has no significant genotoxic potential.

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Abstract number: **P26**

THE INFLUENCE OF HALOGENATED BOROXINE ON THE DEREGULATION OF THE GENES CAUSED BY IMIQUIMOD INDUCED INFLAMMATORY DERMATOSIS IN RATS

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Psoriasis is one of the most common human skin diseases and is considered to have key genetic underpinnings. In this work we present the establishment of rat model of induced skin inflammation as an *in vivo* example of psoriasis. We assessed the gene expression profile in skin biopsies of rats after the application of 5% imiquimod and halogenated boroxine. The goal was to establish viable *in vivo* model for studying effect of psoriasis for testing of therapeutical effects of potential novel drugs. Wistar rats were kept under specific pathogen-free conditions and provided with food and water *ad libitum*. Rats at 9 to 12 wk of age received a daily topical dose of 125 mg of commercially available imiquimod cream on the shaved back for 5 consecutive days. After the 24 hours break following induced skin inflammation, anti-inflammatory treatment with boroxine commenced for four consecutive days of the experiment. Total RNA was extracted from skin tissue of rats which is used as input for the Rat Inflammatory Cytokines & Receptors RT² Profiler PCR Array profiles the expression of 84 key genes mediating the inflammatory response. Comparative analysis of gene expression profiles of skin treated with imiquimod and the boroxine, showed that the profile is characterized by significant deregulation of genes associated with inflammatory processes. It is of note that halogenated boroxine deregulates three genes from the chemokine family (Ccl17, Ccr2, Ccr5) and two genes from the tumor necrosis factor family (Tnfsf10 and Tnfsf13b) that could explain part of anti-inflammatory effect of boroxine on psoriasis like skin changes induced by imiquimod. It can be concluded that induction of psoriasis like inflammatory dermatosis on a rat model was successful and may be used for the investigation strategies of potential drugs with anti-psoriatic effects.

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Abstract number: **P27**

HALOGENATED BOROXINE INFLUENCE ON BASAL LEVEL AUTOPHAGY IN HUMAN MELANOMA GR-M CELL LINE

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Many types of tumors, including melanoma, have an increased basal autophagy, associated with tumor aggression and poor prognosis. Finding new therapeutic that specifically target autophagy and lead to vulnerability of tumor cells to cell death are in the focus of research. Halogenated boroxine ($K_2(B_3O_3F_4OH)$) has potent bioactive properties. In this work we tested influence of halogenated boroxine in concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml on human melanoma GR-M cell line autophagy basal level. The autophagy detection assay that specifically binds to autophagosomes is used to monitor the degree of autophagy. Additionally, expression of two autophagy related genes *BECN1* and *P62* on transcriptional level were analyzed using Real time PCR. Cytotoxic effect on GR-M cells was evaluated with trypan blue exclusion assay. Epifluorescence autophagy analysis of the GR-M cell line negative control (untreated cells), showed a high level of basal autophagy (82.5% of analyzed cells). In cultures with starvation induced autophagy (positive control), the degree of autophagy is reduced, probably as a protective cell mechanism against massive degradation in comparison to basal level. In halogenated boroxine treated cultures, the degree of autophagy is lower in relation to the basal level, except at a concentration of 0.2 mg/ml where the degree of autophagy is higher than the basal level. Under the influence of halogenated boroxine, deregulation of the genes expression *BECN1* and *P62* occurred. Trypan blue exclusion assay showed reduction of cell viability in dose dependent manner (96% - 0.05 mg/ml; 94.31% - 0.1 mg/ml; 93.38% - 0.2 mg/ml; 14.02% - 0.4 mg/ml; 3.33% - 0.8 mg/ml). Despite of halogenated boroxine autophagy related genes expression deregulation and significant cytotoxic effect, significant change in basal autophagy level did not occur indicating that autophagy is not halogenated boroxine cytotoxic molecular target pathway.

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Abstract number: **P28**

CYTOTOXICITY OF 1-SUBSTITUTED 1,2,3,4-TETRAHYDROISOQUINOLINES IN 5637 HUMAN BLADDER CARCINOMA CELL LINE

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A wide range of biological activity on the human body is associated with dopamine, related biogenic amines and phenolic compounds. Due to the potential therapeutic significance of some of the previously reported 1,2,3,4-tetrahydroisoquinolines (THIQs), four 1-substituted THIQs derived *from dopamine and various phenolic aldehydes* were selected for the analysis of the effects on cell growth *in vitro*. Alamar blue colorimetric assay was performed in the 5637 human bladder carcinoma cell line. Vincristine was used as a positive control. The lowest registered growth inhibition of 5637 cell line was at the lowest tested concentration (0.1 uM) of 1-(3-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diole (44.49%) while the maximal registered growth inhibition of 69% was in the highest concentration (100 uM) of 1-(3,4-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diole. Analysis of variance revealed a dose dependent increase in the cytotoxic effect of 1-(3-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diole and 1-(3,4-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diole with a significant increase in the highest concentration ($p<0.01$). The results suggest that our THIQs should be subjected to further studies in other cancer and normal cell lines to investigate diverse cell responses to various THIQs and identify THIQ with the most prominent effects.

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Abstract number: **P29**

CYTOTOXICITY EVALUATION OF HIGHLY OXIDIZED GRAPHENE ON PERIPHERAL BLOOD MONONUCLEAR CELLS

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Due to their unique physical properties, research on possible applications of graphene-based materials in different systems is hot topic in almost every field of science. Their use in biomedicine is based on their very high specific surface area (~ 2600 m^2 g^{-1}) and the possibility for structural modification with different, mostly oxygen and nitrogen, functional groups. However, recent studies indicate that highly functionalized graphenes can have significant cytotoxicity, while ideal, functional-group-free graphenes show no cytotoxic effects. Considering that the latter materials are hydrophobic and generally useless in most biomedical systems, application of graphenes in biomedical systems depends on the possibility for precise modification, in order to achieve the highest possible degree of functionalization without adverse toxic effects. The current study aimed to evaluate effects on cell viability of peripheral blood mononuclear cells (PBMCs) in cultures treated with graphene oxide obtained by modified Hummers method from natural graphite flakes. Three concentrations (0.4 μg/ml, 4 μg/ml, 40 μg/ml) were screened after 48 hours treatment. The cytotoxicity of graphene oxide was investigated by measuring mitochondrial activity in peripheral blood mononuclear cells (PBMCs) using MTT assay (methyltiazolyldiphenyl-tetrazolium bromide). Simple linear regression analysis revealed dose dependent cytotoxicity (p=0.006) of tested graphene oxide. Analysis of variance confirmed that the cytotoxicity was significantly higher (14.4%) in the highest tested concentration compared to 0.3% cytotoxicity in the treatments with the lowest graphene oxide concentration (p=0.03). Results indicate that graphene oxide in concentrations lower than 40 μg/ml does not induce significant cytotoxic effects in PBMCs *in vitro*, probably due to the fact that the lateral size of graphene sheets was comparable to the cell size and should be object of further research.

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Abstract number: **P30**

THE GENETIC STRUCTURE OF POTENTIAL BOSNIAN MOUNTAIN HORSES BASED ON MICROSATELLITE MARKERS

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Autochthonous breeds are an important part of the living world and the richness of each country. Bosnian mountain horse is the only autochthonous horse breed in Bosnia and Herzegovina, well adapted to ecological and geographical conditions of this region. In this study 17 microsatellite markers were employed in order to examine the genetic structure of potential Bosnian mountain horses and to determine the possible sources of undoubtedly autochthonous germplasm. Genomic DNA was extracted from whole blood collected from 61 potential Bosnian mountain horse specimens divided into three groups according to the population of origin: Group 1 = 20 individuals (Bosnian mountain horses from the stud „Borike“); Group 2 = 28 individuals (Bosnian mountain horses from Herzegovina) and Group 3 = 13 individuals (Bosnian mountain horses from the other parts of Bosnia and Herzegovina). Analysis of molecular variance showed that from all genetic variation, 5% is among populations, 14% among individuals and 81% within individuals. Results of the fixation index showed moderate level of genetic differentiation among groups (5,1%). Parwise Fst shows clear genetic differentiation among “Borike’ and other observed groups. Structure harvester predicted two clusters based on 50000 burnin period and 100000 replications and three iterations. MCMC analyse (admixture model) showed that first cluster consists almost all individuals from Borike stud, since other cluster consists individuals from two other observed groups. Factorial Component Analysis (FCA) has confirmed MCMC results. In accordance with the above, only Group 1 (originating from the stud "Borike") has probably the most genetically characteristics of autochthonous Bosnian mountain horses. This stud is carefully guarded and cultivated through generations and that is the basis which should play an important role in the process of revitalizing and preserving the gene pool of our native breed.

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Abstract number: **P31**

DNA BARCODING OF FOUR BUTTERFLY SPECIES OF GENUS *ARGYNNIS* FABRICIUS, 1807 (NYMPHALIDAE: HELICONIINAE) FROM BOSNIA AND HERZEGOVINA

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Bosnia and Herzegovina has a long tradition in morphological and fundamental research of the butterfly fauna dating back to 1844. The problem of studying taxonomic relationships within the order of butterflies, especially between closely related species, currently is being approached quite effectively by molecular-genetic methods. DNA barcoding is a taxonomic method that uses short DNA sequences (DNA barcodes) to enable rapid species identification along with many other different applications. In this study, we obtained seven DNA barcode sequences of four species of the genus *Argynnis* (*A. paphia* Linnaeus, 1758, *A. adippe* (Denis & Schiffermuller, 1775), *A. niobe* Linnaeus, 1758 and *A. aglaja* Linnaeus, 1758). The whole individuals were stored in 96% ethanol. A single leg was used for DNA isolation applying an optimized Taggart extraction protocol. Amplification of the COI region was performed using procedure and primers designed by Folmer. PCR products were sequenced by Macrogen Inc. Europe service. Obtained sequences were stored in the GenBank database. They were sufficiently specific to provide species identification. The difficulty of taxonomical positioning of two closely related species within this genus, *A. adippe* and *A. niobe*, visible when conducting morphological identification are also noticeable at the molecular level. Therefore, it would be useful to analyze additional molecular markers (such as ND5, EF-1 or Cytb) and a larger number of individuals. To estimate taxonomic positions of species within this genus, including species interrelationships, analysis of morphology combined with molecular methods are recommended.

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Abstract number: **P32**

OPTIMIZATION OF NESTED POLYMERASE CHAIN REACTION CONDITIONS FOR MOLECULAR-GENETIC DETECTION OF *BORRELIA BURGDORFERI* SENSU LATO IN *IXODES RICINUS* TICKS

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Borrelia burgdorferi sensu lato is a complex of at least 21 spirochete species widespread dominantly in Northern Hemisphere. Five species have been isolated from humans and described as human pathogens in Europe causing Lyme borreliosis (*Borrelia afzelii*, *Borrelia garinii*, *Borrelia bavariensis*, *Borrelia burgdorferi* sensu stricto, and *Borrelia spielmanii*). They are maintained in enzootic cycles between ticks of genus *Ixodes* and a variety of vertebrate hosts as reservoirs. *Ixodes ricinus* ticks are the main vectors of *Borrelia* species in Europe. Several molecular techniques for the detection and classification of *Borrelia burgdorferi* sensu lato have been described until now. The aim of this study was to optimize the protocol for molecular-genetic detection of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks using polymerase chain reaction targeting the *rrf* (5S)-*rrl* (23S) intergenic region. The commercially available kit was used for total genomic DNA extraction from ticks. Nested polymerase chain reaction for detecting *Borrelia burgdorferi* sensu lato was performed using two primer pairs, SPA1 and SPA2 as the outer primers, while BorRNA F and BorRNA R were inner primers. In both PCR protocols, the primer concentrations and the amount of DNA and PCR templates were shown to have an important role. The crucial points of optimization were annealing temperature and the number of PCR cycles for inner primers. This optimized protocol yielded PCR amplicons between 226bp and 266bp positively correlated with *Borrelia burgdorferi* sensu lato complex.

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Abstract number: **P33**

DIVERSITY OF REPRODUCTIVE PATHWAYS IN *COTONEASTER INTEGERRIMUS* (ROSACEAE) IS DRIVEN BY HETEROPLOID CROSSES AND APOMIXIS

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Interplay of polyploidy and apomixis enriches plant diversity via formation of novel cytotypes and genetic lineagues with predominantly apomictic reproduction. Coexistence of different interacting cytotypes drives exceptional genetic diversity due to heteroploid crosses between cytotypes with different mating systems. We used flow cytometry to assess genome size and ploidy level among 270 *Cotoneaster integerrimus* individuals from 18 populations, with 600 single-seed flow cytometric seed screenings to identify their mating systems. Flow cytometry confirmed the presence of di-, tri-, tetra- and hexaploid cytotype mixtures in heteroploid populations of *C. integerrimus*. Seven populations contained single polyploid cytotype and eleven populations were heteroploid. Generaly, the most abundant cytotype was tetraploid (92.4%), followed by diploid (6.3%), triploid (1%) and hexaploid (0.3%). Relatively high percentage (33%) of tetraploid seeds was sexually produced compared with the rest seeds characterized by pseudogamous apomixis. The highest diversity of reproductive pathways was detected in tetraploids, both sexual and apomictic ones. All triploids were apomictic. As a rule, diploid individuals produced sexually originated seeds having di- or triploid embryos depending on the ploidy level of female gametophyte and fertilising sperm cell. Thus, diploids represent a key factor in enhancement of genetic diversity in the mixed-ploidy and mixed-mating system populations of *C. integerrimus*. Results fulfil the knowledge gap on the reproduction modes of apomictic groups in general and provide critical data for studying the evolution of sexual-agamic complexes.

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Abstract number: **P34**

HETEROCHROMATIN AND rDNA PATTERN REVEALED HETEROMORPHIC SEX CHROMOSOMES IN *DIOSCOREA DUMETORUM*

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The genus *Dioscorea* L. (Dioscoreaceae) include about 600 mostly dioecious and tuberous species, which are mainly widespread in tropical regions. Ten of them are cultivated and of agronomical importance. *Dioscorea rotundata* Poir., commonly call "white yam" and *D. dumetorum* (Kunth) Pax "yellow yam" are the most consumed in Cameroon. For highlighting possible presence of sex chromosomes in *D. dumetorum*, the main goal of this study are 1) to characterize the karyotype of male and female individuals using molecular cytogenetic approaches: fluorochrome banding to detect GC riche DNA regions and Fluorescent *In Situ* Hybridization for physical mapping of rRNA genes, and 2) to determine genome size by flow cytometry for checking an eventual difference in DNA amount between male and female individuals. The mean 2C DNA content obtained for six accessions of *D. dumentorum* was 0.73 pg (714 Mbp) which present small genome size. No difference was found in genome size between male and female individuals. After Feulgen staining the most frequent chromosome number 2*n*= 40 was found in all investigated samples. One chromosome pair of greater length than the other chromosomes in the karyotype was observed. This pair corresponded to sex chromosomes which was additionally confirmed by molecular cytogenetics. Chromomycin banding revealed that the biggest chromosome pair presented the large positive bands. These bands were the similar size on the chromosomes of female individuals while they are of different size in the male individuals. This difference in size of GC-rich regions was also visible in interphase nuclei, which presented one small and one big spots. The *in situ* hybridization experiment confirmed this observation and pointed out that the chromomycin positive regions corresponded to 18S-5.5S-26S rDNA loci. This is the first time that the sex chromosomes have been observed in the genus *Dioscorea*.

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Abstract number: **P35**

HETEROZYGOSIS AS A MEASURE OF THE GENETIC VARIABILITY OF PEDUNCULATE OAK (*QUERCUS ROBUR*, L.) IN THE BOSNIAN-HERZEGOVINIAN PROVENANCE TEST

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Populations of pedunculate oak (*Quercus robur*, L.) in Bosnia and Herzegovina are on the southern edge of the species' distribution range. These populations are significant for the preservation of the diversity of the species in Bosnia and Herzegovina and Europe because of their specific genetic structure. To select the best material for restoration, we established Bosnian-Herzegovinian provenance test in 2009. In this research we analyzed twenty pedunculate oak provenances (Bijeljina, Bosanska Dubica, Bosanska Gradiška, Bosanski Brod, Bosansko Grahovo, Bugojno, Drvar, Hrgovi Srebrenik, Jelah, Kaćuni, Ključ, Kotor Varoš, Miljevina, Mrkonjić Grad, Mutnica Cazin, Olovo, Sokolac, Stojčevac, Žepče, Živinice) in provenance test using isoenzyme markers. We used ten enzymatic systems, with a total of 14 gene locus, and calculated the observed and theoretical (expected) heterozygosity. Provenance Olovo showed the highest value of observed heterozygosity (0.2907), and provenance Bosanska Dubica the lowest (0.1571). Observed heterozygosity was higher than expected for eight provenances (Bosanski Brod, Hrgovi Srebrenik, Jelah, Kaćuni, Kotor Varoš, Olovo, Stojčevac, and Žepče). For nine of 14 gene locus, the average observed heterozygosity for all provenances was higher than expected. Jelah provenance had the highest value of expected heterozygosity (0.2804), and Stojčevac provenance the lowest (0.1867). The existing differences between observed and expected heterozygosity show a deviation of the actual state of starting populations from the equilibrium state. The values of observed heterozygosity that are in the part of the studied population higher than expected show that these populations are in a state of equilibrium and suitable for seed collection. In the other part of the population, such as Bosanska Dubica, the values of the observed heterozygosity are lower than expected, which suggests a deviation from the equilibrium state.

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Abstract number: **P36**

INVESTIGATION OF THE GENETIC DIVERSITY OF POMEGRANATE IN HERZEGOVINA

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Wild pomegranate (*Punica granatum* L.) is indigenous fruit species of Herzegovina. Pomegranate occurs in various types of habitats, and grows individually or in larger and smaller groups. They are characterised by morphological differences especially in colour and size of fruits. Wild populations of pomegranate were surveyed on selected sites in Herzegovina during 2013 and 2014. After evaluations of fruits and shrubs 13 genotypes were selected for DNA isolation and marker analysis. Seven cultivated varieties were included for comparison. Molecular characterization was done with 13 simple sequence repeats (SSR) markers. Number of alleles per locus and polymorphic information content (PIC) were shown for every SSR locus. Three monomorphic loci were excluded from the results. Based on the binary matrix Dice's similarity coefficient was calculated and data were used for UPGMA cluster algorithm. Results were presented in dendrogram. Total number of alleles was 23, or 2.3 alleles per locus in average. PIC value varied from 0.048 to 0.449, with average 0.22. The average observed heterozygosity amounted to 0.19, and expected was 0.25 respectively. Genetic similarity coefficient was high and amounted 0.83 to 0.96 for wild populations and in range from 0.62 to 0.80 when cultivated varieties were included. Similarity among cultivated varieties for given SSRs markers was also high from 0.71 to 0.91. These data indicated a low level of polymorphism for chosen SSRs in particular among wildly grown pomegranate, which indicates narrow genetic diversity.

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Abstract number: **P37**

BACTERIAL GENOME IDENTIFICATION FROM FECAL SAMPLES OF SUBJECTS WITH IRRITABLE BOWEL SYNDROME (IBS)

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Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal tract, characterized by constipation or diarrhoea, without clear organic causes. The prevalence of IBS is about 10-15% in the adult population. There is a scientific evidence that microbiome of intestinal tract is involved in the development of IBS. Intestinal microbiome contains microorganisms that occur naturally in the human gastrointestinal tract, although the composition of the microbiome may vary upon different internal and environmental factors. In our project, DNA identification of the bacterial strains was carried using NGS sequencing panel specific for a number of bacterial strains from DNA isolated from faeces of person diagnosed with IBS and healthy individual. In a faecal sample from IBS-M subject we identified 646 bacterial species from seven families, with Shannon diversity index value of 2.386. The control sample had a smaller diversity with 639 species from seven families and Shannon diversity index value of 2.052. Most of the bacterial species present in the gastrointestinal tract may be found in the faeces. Regarding that fact, knowledge on bacterial composition differences between subjects with IBS diagnosis and healthy volunteers may be beneficial in learning about IBS mechanism, diagnosis and treatment possibilities.

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Abstract number: **P38**

DRUG-INDUCED MODULATORS OF *ESHERICHIA COLI* VIRULENCE GENE EXPRESSION

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Pathogenic microorganisms may be of different strains, which are most prevalent in bacteria and can cause disease due to degradation of the host immune defense. The cause may be a different level of expression of virulent factors, proteins or other molecules synthesized by the enzyme subunit. The activation of these enzymes often occurs as a result of changes in the physiological balance of the host organism, which at the same time leads to a change in the metabolic activity of microorganisms which under such conditions move to opportunistic pathogens and cause disease. One of the most known of these pathogens is *E.coli*, the most common cause of intrahospital infections and gastrointestinal tract infections. Factors that can serve as a trigger in the expression of bacterial genes that encode for different enzymes and lead to the transformation of non-pathogenic bacteria to pathogenic or modulate the level of existing pathogenicity may be different drugs such as antibiotics to which the human organism is exposed. The results of this study showed significant effects of the subinhibitory concentrations of antibiotics on the elicitation of bacterial extracellular proteins as a virulence factors and their influence on the ability to form biofilms, at the same time changing the status of intracellular adhesion gene cluster (ICA) within *E.coli* genome.

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Abstract number: **P39**

PREVALENCE OF *MYCOPLASMA HOMINIS, UREAPLASMA SPP.* AND *CHLAMYDIA TRACHOMATIS* IN ROUTINE GYNECOLOGICAL EXAMINATION IN TEŠANJ AREA

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Mycoplasma hominis, *Ureaplasma spp*. (U*. Urealyticum, U. parvum*) and *Chlamydia trachomatis* can cause serious consequences and are thought to cause various disorders, such as infertility if not treated. The main problem of these infections is that they are mostly asymptomatic and may only be discovered when irreversible damage has already occurred. The aim of this study was to estimate prevalence of *Mycoplasma hominis*, *Ureaplasma spp.* (*U. urealyticum, U. parvum*) and *Chlamydia trachomatis* in sexually active women in Tešanj area. In this study participated 103 women from Tešanj area who came for routine gynecological examination. All women were divided into two groups; women younger than 25 and women older than 25. DNA was extracted from cells in endocervical swab samples and detection was performed using real-time PCR. The overall prevalence was: *M. hominis* 7,77%, *Ureaplasma spp*. (*U. urealyticum, U. parvum*) 37,86% and *C. trachomatis* 0,97%. This study suggests association of infection with women age. Therefore, women younger than 25 seem to be more infected with these bacteria. These findings may be useful to assert importance of routine gynecological examination and in that way avoid negative impacts of *M. hominis*, *Ureaplasma spp.* and *C. trachomatis* infection on the female reproductive tract.

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Abstract number: **P40**

ANTIMICROBIAL SUSCEPTIBILITY OF BIOFILM-PRODUCING AND NON-PRODUCING *PSEUDOMONAS AERUGINOSA* ISOLATES IN THE NON-HOSPITAL ENVIRONMENT

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One of the fundamental attributes of bacteria is their capacity to form biofilms, a multicellular community immersed in extracellular polymer-matrix. Genetic diversity of microorganisms associated with biofilm formation and the variety of conditions in which they occur indicate that biofilms are an ancient form of life. *Pseudomonas aeruginosa* possesses an incredible capability for development and acquisition of new resistance mechanisms to antibiotics. The objective of our study was to investigate antibiotic susceptibility of the biofilm-producing and non-producing *P. aeruginosa* in the non-hospital environment. A total of 98 samples were collected using the wet swab method from different abiotic and biotic surfaces. Biofilm formation was quantitatively evaluated by microtiter plate method (MPA), followed by qualitative tube method (TM). The antimicrobial activity of 11 antibiotics from 4 different classes was tested by the disk diffusion method respecting the National Committee for Clinical Laboratory Standards. Out of the 31 isolates, 26% were non-biofilm producers, and 74% were biofilm producers. Most of the non-producers showed resistance to tetracycline (87.5%), ampicillin (87.5%) and amikacin (75%), while all isolates were susceptible to tazobactam-piperacillin, ciprofloxacin and ceftazidime. Similarly, biofilm-producers were resistant to ampicillin (91.3%), amikacin (69.6%) and aztreonam (56.5%) and were susceptible to tazobactam-piperacillin and ciprofloxacin. Cefotaxime generated the highest number of biofilm producers intermediates (56.5%), while cefepime produced the highest number of biofilm non-producers intermediates (75%). Based on the antibiogram results, we determined the mutual multi-drug resistant (MDR) profile for *P. aeruginosa*. The MDR profile shared by 54.8% of isolates consisted of three different antibiotics, tetracycline, ampicillin and amikacin. Obtained results showed that biofilm production was not associated with antibiotic susceptibility profile for the studied *P. aeruginosa* isolates. We can conclude that biofilms are as widespread in the non-hospital environment as in hospital settings.

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PhD STUDENTS SESSION – ORAL PRESENTATIONS

Abstract number: **OS1**

GENE EXPRESSION OF α-TUBULIN, INVERSIN AND DISHVELLED-1 IN POSTNATAL KIDNEY TISSUE

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Microtubules are essential for ciliary architecture, mitosis, cell differentiation and polarity of the cell. Previously it has been suggested that primary cilium is involved in the nephrogenesis. α-tubulin is the part of the microtubule complex whereas Inversin forms complex with tubulin. This might play important role in kidney maturation. Inversin inhibits the canonical signal Wnt pathway by targeting cytoplasmic Dishvelled-1 (Dvl-1), also it plays the role as the switch between Wnt pathways. The aim of this study was to explore the expression of α-tubulin, Inversin and Dvl-1 in postnatal kidney development as the regular kidney maturation prolongs after birth into the early childhood. The expression of α-tubulin, Inversin and Dvl-1 was studied by double immunofluorescence on the paraffin sections of 1.5- and 7-year old human kidney tissues. The detected positive cells in proximal convoluted tubules (PCT), distal convoluted tubules (DCT) and in glomeruli were obtained by ImageJ software. The retrieved data were then analysed by Kruskall Wallis and Dunn tests. α-tubulin, Inversin and Dvl-1 were expressed in 1.5- and 7-year old kidneys with difference in model staining comparing kidney structures and maturity of the kidneys. α-tubulin had the highest expression in DCT of 7-year old kidneys in comparison with PCT and glomeruli of 1.5- and 7-year old kidney tissue ($P<0.001$). Inversin was highly expressed in glomeruli of 7-year old kidneys in comparison to PCT and DCT ($P<0.0001$). Glomeruli of 7-year old kidney tissue had much higher expression of Dvl-1 than 1.5-year old glomeruli ($P<0.001$). α-tubulin and Inversin expressed higher than Dvl-1 in PCT of 1.5-year old kidney ($P<0.0001$) and in glomeruli of 1.5- and 7-year old kidneys ($P<0.001$). α-tubulin, Inversin and Dvl-1 are expressed and co-localize during postnatal kidney development. Established pattern directs to possible involvement in regular kidney maturation.

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Abstract number: **OS2**

ROLE OF FLOW CYTOMETRY IN DIAGNOSIS OF LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Large granular lymphocyte leukemia (LGLL) is chronic, clonal lymphoproliferative disorder that arises from either memory cytotoxic T-cells or natural killer (NK) cells. Both forms of LGLL commonly manifests with cytopenias (largely neutropenia), splenomegaly, and persistent expansion of circulating LGLs. The diagnostic approach starts with flow cytometric analysis, which as a key first step clarifies T-cell or NK cell lineage. In this abstract, we present a case of T-cell LGLL, and highlight the key role of flow cytometry in adequate diagnostic approach, as well as monitoring of the appropriate clonal disorder. A 55-year-old woman with history of celiac disease was found to have transfusion dependent normocytic anemia with hemoglobin 7.7 g/dL, for which peripheral smear identified concomitant leukopenic neutropenia (white blood cell count 3500/mL, absolute neutrophil count 700-1000, absolute lymphocyte count 2300-2970). Peripheral flow cytometry evaluation showed the following immunophenotype: HLA-DR+, CD3+, CD4+, CD7+ and CD8+. Bone marrow aspirate showed a homogeneous population (30% by cellularity) of lymphocytes, with large cytoplasm and azurophilic granules, with the immunophenotype demonstrating CD3+CD8+CD57+, corresponding to suspected diagnosis of T-cell LGLL. Molecular polymerase chain reaction rearrangement studies on alpha/beta T-cell receptor confirmed clonality, establishing the diagnosis of T-cell LGLL. Patient was initiated on therapy with corticosteroids and cyclophosphamide every 3 weeks, and ultimately showed response with normalization of absolute neutrophil count, loss of transfusion dependency for anemia as well as reduction in population of T-cell LGLs on peripheral and bone marrow aspirates. Due to the lack of a single unique genetic or phenotypic feature and clinic-pathologic overlap between reactive and neoplastic entities, accurate LGL syndrome diagnosis should be based on the combination of morphologic, immunophenotypic, and molecular studies as well as clinical features. For diagnosis and monitoring of LGL proliferations, flow cytometry remains an essential tool.

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Abstract number: **OS3**

EVALUATION OF HEAVY METAL TOLERANCE IN SERPENTINE RHIZOBACTERIA ASSOCIATED WITH *MEDICAGO LUPULINA* L.

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Soils derived from serpentine rocks are characterized by specific and commonly sparse vegetation in terms of both biodiversity and abundance. The lack of essential nutrients, low calcium to magnesium ratio and commonly high concentrations of heavy metals are the main causes for the specificity of serpentine flora. In addition to genetic-physiological adaptation, rhizosphere bacteria with plant growth promoting (PGP) properties through the interaction with plants also contribute to the tolerance of such adverse conditions. *Medicago lupulina* L. is an unremarkable plant that grows at the borders of serpentine formations. It was also found to accumulate Ni at supratoxic concentrations. The samples of rhizosphere associated with the root of *M. lupulina* L. were collected from serpentine outcrops in Central Bosnia (Olovo, Bljuva and Papratnica). Yeast mannitol agar was used for isolation and cultivation of soil bacteria. Assessment of heavy metal tolerance was performed using tryptone yeast agar supplemented with copper, nickel and cobalt. Isolated strains showed strong resistance to copper and slightly weaker resistance to nickel while only few isolates grew in the presence of cobalt. Further research will include identification of tolerant strains using 16S rDNA sequencing and characterization of PGP mechanisms – fixation of atmospheric nitrogen, production of siderophores, phosphate solubilisation, ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity and production of indol acetic acid. Preliminary results of 16S rDNA sequencing of isolates from Olovo and comparison of obtained sequences with NCBI database entries showed highest similarity with genera *Enterobacter*, *Klebsiella*, *Kosakonia*, *Citrobacter* and *Leclercia*. The analysed isolates from Bljuva and Papratnica showed siderophore production and phosphate solubilisation. Strains with expressed PGP traits will be further considered as potential biotechnological tools for inoculation of plants with the aim of improving their heavy metal tolerance, biomass accumulation and use in bioremediation of metal contaminated soils.

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PhD STUDENTS SESSION – POSTER PRESENTATIONS

Abstract number: **PS01**

FINDINGS FROM ACGH IN PATIENT WITH PSYCHOMOTOR DELAY- CASE REPORT

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Unexplained psychomotor delay has an incidence of 1-3% and when associated with congenital anomalies the most common causes of these conditions are chromosomal rearrangements. Initial testing of children with psychomotor delays considers karyotype analysis and metabolic tests. However, introduction of Array Comparative Genomic Hybridization (ACGH) has become the standard method of diagnostics worldwide. ACGH is a highly sensitive method which enables detection of unbalanced chromosomal aberrations and aneuploidies. In this case report, a patient is a sixteen year old girl born to unrelated parents with mild mental retardation and psychomotor delay, hyperacusis, epilepsy, silent nasal speech, clinodactyly of the V finger on left hand, as well as low set ears. Patient had a karyotype interpreted as normal using GTG band analysis. Array CGH was performed using Agilent SurePrint G3 custom CGH+SNP Microarray 8x60K (UCSC, hg19, NCBI Build 37, February, 2009). Results were analyzed by CytoGenomics 3.0 Agilent software. Results of aCGH revealed clinically significant duplication of 17q25.1-q25.3 region with the size of ~7.96Mb. Within the duplicated region 217 genes are present, of which 36 are described as OMIM morbid. Duplications of similar size are described in DECIPHER date base in patients with psychomotor delay, hyperactivity and neoplasm of CNS. Besides duplication, a ~755kb clinically significant deletion was detected in the 17q25.3 region. Deletion involves 18 genes of which 2 are described as OMIM morbid: *TBCD* (MIM604649) and *ZNF750* (MIM610226). Patient with similar deletion was described in DECIPHER date base with notable psychomotor delay. Based on these results FISH analysis is recommended for both parents in order to determine the possible carrier of inversion in the region of 17qter.

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Abstract number: **PS02**

IMP3 EXPRESSION IS DECREASED IN TROPHOBLAST CELLS FROM PREGNANCIES WITH SEVERE AND NON-SEVERE PREECLAMPSIA

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Preeclampsia is the main cause of morbidity and mortality in perinatal period but its etilogy remains unknown. Recent studies suggest that disorders of extravillous trophoblast functions including proliferation, differentiation, invasion and apoptosis, lead to the development of preeclampsia. As cancer and trophoblast cells share similar behavioral patterns, the "cancer-like" mechanisms have been put in the focus of studies. Initial studies on extravillous trophoblast cell lines show important role of oncofetal protein IMP3 (Insulin-like growth factor mRNA binding protein 3) in invasion of extravillous trophoblast. This was the first study on human placentas to determine the expression of IMP3 in trophoblast cells of normal and preeclamptic placentas. The placentas were classified in three groups, 11 placentas with severe, 8 placentas with non-severe preeclampsia and 9 healthy placentas as a control group. Mother-related risk factors, pregnancy and postpartum complications were observed. The expression of IMP3 in extravillous trophoblast was determined by immunohistochemistry and was expressed as a percentage of IMP3 positive cells in the total number of extravillous trophoblast cells. The immunofluorescence staining was used to determine co-expression of IMP3 and proapoptotic factor Caspase-3 in extravillous trophoblast cells. The expression of IMP3 is significantly lower in extravillous trophoblast cells of preeclamptic pregnancies compared to the control group regardless of the stage of the disease, but we didn't find significant difference in IMP3 expression between the groups of severe and non-severe preeclampsia. Imunofluorescence staining revield no co-expression of IMP3 and Caspase-3 in trophoblast cells. There were no differences in the risk factors among these three groups while the frequency of complications was highest in the group of severe preeclampsia. Decreased expression of IMP3 in trophoblast cells of preeclamptic placentas and its negative correlation with Caspase-3 expression indicate the relation of IMP3 with the proper invasion of extravillous trophoblast and its antiapoptotic effect.

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Abstract number: **PS03**

EXPRESSION AND DISTRIBUTION OF FIBROBLAST GROWTH FACTOR RECEPTOR 1, 2 AND OTHER SIGNAL PROTEIN IN HUMAN FETAL LUNG

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The number of patients with developmental lung anomalies increases each year. The data about human fetal lung development are scarce and most studies were done on experimental animal models and cell cultures. Expression pattern of fibroblast growth factor receptor 1 and 2, connective tissue growth factor, zinc finger protein SNAI1, hypoxia-inducible factor 1-alpha, syndecan 1 and special AT-rich sequence-binding protein markers was histologically analysed in normal human fetal lungs from 6th to 16th week of gestation using double immunofluorescence. Following the difference in expression between different stages of development, it was shown that during all stages the expression of receptor and signaling proteins is significantly higher in epithelial then in mesenchymal cells except the connective tissue growth factor that was through all stages of development negative. The expression pattern of fibroblast growth factor receptors 1 and 2 is quite similar. The strongest expression was observed in embryonic stages and expression diminishes through the weeks. Zinc finger protein SNAI1 and hypoxia-inducible factor 1-alpha were negative during the embryonic stage and their expression was observed at the beginning of the fetal stage. Syndecan had a strong expression throughout the observed periods. Special AT-rich sequence-binding protein was also positive throughout the weeks with the strongest expressions in the sixth and seventh gestational weeks. This research provides new insights into the spatial and temporal distribution of signaling proteins at certain stages of normal human lung development, helping to understand the pattern of some developmental lung anomalies and oxygen lung damage, which opens the door for early prevention and treatment.

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Abstract number: **PS04**

CRKL GENE EXPRESSION IN KIDNEYS OF *YOTARI* MICE

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The aim of this study was to explore difference in spatial expression pattern of CrkL protein in the kidney tissue between wild type (*Dab1*$^{+/+}$), heterozygotes (*Dab1*$^{+/-}$) and *yotari* (*Dab1* $^{-/-}$) mice to determine its possible roles in maintenance of kidney function. *Yotari* mouse is constructed by PGK-neo cassette that cause target disruption of the first 47 codons of the gene coding for protein-interacting (PI-PTB) domain. Heterozygotes (*Dab1* $^{+/-}$) were mated and three groups of pups were observed in this study, *Dab1* $^{-/-}$ or *yotari* group, *Dab1* $^{+/-}$ and *Dab1*$^{+/+}$ or control group. Pups were sacrificed four days after birth and samples were embedded in the paraffin. CrkL expression was examined in the kidney sections of control and *yotari* mice by immunofluorescence staining. The percentage of CrkL immunoreactive cells were calculated. For each investigated period we captured at least twenty images per different kidney structure: proximal convoluted tubules (PCT), distal convoluted tubules (DCT) and glomeruli at 40× objective magnification. Any level of cytoplasmic or membrane staining with used marker was regarded as positive. Data were analysed by student (t) test in Past3 statistic program. Statistical significance was considered at $P<0.05$. CrkL was moderately expressed in kidneys of *yotari* mice and occasionally in heterozygotes with specific spatial expression pattern and diverse fluorescence intensity. *Dab1*$^{+/+}$ kidneys contain almost no staining. Expression in DCT of the *yotari* mice significantly increased ($P<0.05$) in comparison to expression in DCT of *Dab1* $^{+/-}$ and *Dab1*$^{+/+}$ mice. There was no statistically significant difference in CrkL expression in PCT and glomeruli between any group of mice. Increased expression of CrkL protein in DCT of *yotari* mice implies its potentially significant role in kidney damage.

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Abstract number: **PS05**

AIFM3 GENE EXPRESSION IN KIDNEYS OF *YOTARI* MICE

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Aim of this study was to describe the spatial distribution differences of the expression pattern of AIFM3 protein between wild type (*Dab1*$^{+/+}$), heterozygotes (*Dab1*$^{+/-}$*)* and *yotari* (*Dab1* $^{-/-}$) mice with the intention to demonstrate the association of its potential roles in maintaining kidney function. *Yotari* mouse is designed with the disruption of protein-interacting (PI-PTB) domain by PGK-neo cassette. By mating *Dab1* $^{+/-}$ we got three groups of pups, *Dab1* $^{-/-}$, *Dab1* $^{+/-}$ and *Dab1*$^{+/+}$. At day four after birth the pups were sacrificed and the samples were embedded in paraffin. In the kidney sections of control and *yotari* mice the expression of AIFM3 protein was examined by immunofluorescence staining. We captured at least twenty images per different kidney structures (proximal convoluted tubules (PCT), distal convoluted tubules (DCT) and glomeruli) of each group of mice at 40× objective magnification. We marked positive any level of cytoplasmic staining. The percentage of AIFM3-immunoreactive cells was calculated and analysed in GraphPad Prism statistic program by 1way ANOVA test with the statistical significance of $P<0.05$. AIFM3 was expressed in all experimental groups with specific spatial expression pattern and distinct fluorescence intensity. Expression of marker in PCT in all groups was present, moderately in *Dab1*$^{+/+}$ and highly in *Dab1* $^{-/-}$ and *Dab1* $^{+/-}$ group. There was no noticeable expression in glomeruli across all groups. Main difference was seen in the expression of marker in DCT. While in control group the expression was detectable in merely 29% of DCT, in the *Dab1* $^{-/-}$ and *Dab1* $^{+/-}$ group it was visible in about 50-53% of DCT ($P<0.05$). The difference in appearance of AIFM3 across the groups in the DCT indicates its potentially important role in the impairment of kidney function of *Dab1* $^{-/-}$ and *Dab1* $^{+/-}$ group of mice.

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Abstract number: **PS06**

CX37, CX40, CX43 AND CX45 GENE EXPRESSION IN DEVELOPING, POSTNATAL AND NEPHROTIC HUMAN KIDNEYS

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Connexins (Cx) are gap junction proteins and are mediators of normal kidney development but also play important role in homeostasis of adult kidney tissue. Cx37 is coded by *GJA4* gene, Cx40 by gene *GJA5*, Cx43 by *GJA1* and Cx45 is coded by *GJC1* gene. Pathological kidney conditions are linked to changes in Cx expression and localisation. The aim of this study was to measure the quantity of Cx37, Cx40, Cx43 and Cx45 in fetal developing stages of the kidney, postnatal kidney tissue and in nephrotic kidney tissue. The expression of Cx37, Cx40, Cx43 and Cx45 was examined by double immunofluorescence on paraffin sections of 8 to 10-, 21 to 22-, 38- week old developing kidney tissue, postnatal 1.5-year old kidney tissue and in nephrotic kidney tissue. Protein expression was quantified in fluorescence intensity units (FIU). FIU represents area under curve of fluorescence intensity histogram, it is a hybrid measure of signal brightness and percentage of area covered by signal on microphotograph. Analyse was performed in ImageJ software and the data were analysed by Welch's ANOVA and unpaired t-test with Welch's correction for post-hoc test. In 8-10 weeks and 38 weeks there were significant differences in fluorescence intensity between connexins ($P<0.0001$). More specifically, in week 8-10 when compared to other connexins Cx43 was least expressed ($P<0.0001$), whereas other connexins had similar levels of expression. In week 38, Cx45 had the highest level of expression among connexins ($P<0.001$), whereas Cx43 was the least expressed ($P<0.001$). In 1,5 year postnatal kidney Cx40 showed higher expression than other connexins ($P<0.01$), and Cx37 and Cx45 were the lowest expressed. There were no significant differences in connexin expression in tissue of patients with nephrotic syndrome ($P=0.927$). Expression of different connexins play an important role in different stages of kidney development.

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Abstract number: **PS07**

PRESENCE OF COMPLEX COMBINED HEMATOLOGIC NEOPLASMS – CASE REPORT

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Concomitant prasence of multiple hematologic malignancies in a single patient spaning several hematologic lineages is a rare phenomenon. Here we present a case report of a patient harboring several hematologic malignancies, with sequential development of neoplasia in myeloid and lymphoid lineages. A fifty-year-old man with no prior medical history first presented with coombs negative hemolytic anemia in 2001. He was treated with corticosteroids with a good response, without further evidence of hematologic dyscrasias. He then presented in April 2019. with diffuse lymphadenopathy (up to 4 cm), splenomegally (up to 27 cm), B symptoms (fevers, nightsweats, weightloss) leukocytosis up to 20,000/µL (mostly lymphocytosis), and anemia (hemoglobin 11 g/dL). PET/CT scan of the whole body revealed absence of bone marrow activity, diffuse FDG-avid lymphadenopathy and community acquired pneumonia. Peripheral blood flow cymetry analysis revealed monoclonal T helper cell immunophenotype which on T-cell α/β rearrangement studies confirmed clonality, corresponding to an underlying T-cell hematologic neoplasm. T-cell prolymphocytic leukemia was suspected based on bone marrow cytomorphologic characteristics of prolymphocytes. Review of medical archives identified that the patient presented earlier in 2012 with signs, symptoms as well as morphologic bone marrow characteristics consistent with indolent systemic mastocytosis, as well as myelodysplastic/myeloproliferative syndrome (<10% blasts, 10% monocytes, no infiltration by T lymphocytes). Presented case demonstrates a rare presence of three separate hematologic malignancies in a single patient. Multidisciplinary evaluation and proper utilization of flow cytometry as well as other molecular tools is critical in initial establishment of accurate diagnosis, and subsequent formulation of adequate therapeutic plan. If a suitable stem cell donor is identified, patient will be planned induction chemotherapy followed by allogeneic stem cell transplantation at a tertiary care centre.

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Abstract number: **PS08**

MULTILOCUS SEQUENCE TYPING ANALYSIS OF *BORRELIA AFZELII* STRAINS ISOLATED FROM *IXODES RICINUS* TICKS FROM SERBIA

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Lyme borreliosis (LB) is caused by bacteria of the *Borrelia burgdorferi* sensu lato complex and is the most common tick-borne disease in Europe. All *B. burgdorferi* sensu lato strains are maintained in natural foci through the transmission cycles of competent tick vectors and a vertebrate reservoir. The human becomes infected after being bitten by infected hard ticks of the genus *Ixodes*. *Ixodes ricinus* ticks are the main vectors of *Borrelia* species in Europe. *Borrelia afzelii* is one of the five *Borrelia* species that cause LB in humans and is the most common species found in *I. ricinus* ticks, reservoirs and isolated from human material in Europe. Skin lesion-erytema migrans, the most frequent manifestation of LB, is caused predominantly by *B. afzelii* (70-90%). Previously published data on the prevalence of *Borrelia* species in *I. ricinus* ticks in Serbia report values from 21.1% to 42.5% depending on the region and detection methods. In Serbia, *B. afzelii* is isolated from ticks and reservoirs, representing second dominating species after *Borrelia lusitaniae*. Available data on the epidemiology of LB are scarce in Serbia. Following previously observed genetic diversity of cultivated borrelia strains, the aim of the current study was further genotypisation of *B. afzelii* based on Multilocus Sequence Typing (MLST) method. In the present study, 13 strains of *B. afzelii* isolated and cultivated from *I. ricinus* ticks were analysed. This typing scheme is based on sequencing 8 chromosomally located housekeeping genes*, clpA, clpX, nifS, pepX, pyrG, recG, rplB,* and *uvrA*. MLST analysis results demonstrate that strains were separated into 8 sequence types from which 3 were not previously represented in the MLST base and revealed 2 new alleles. Obtained results that showed high genetic diversity of *B. afzelii* strains could help to understand the ecology of the pathogen and epidemiology of LB in Serbia.

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Abstract number: **PS09**

ANTIGENOTOXIC EFFECT OF QUERCETIN ON THYROXINE-INDUCED DNA DAMAGE IN HUMAN WHOLE BLOOD CELLS *IN VITRO*

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The binding of thyroid hormones to specific nuclear receptors in target cells induces synthesis of enzymes associated with redox processes, leading to the formation of reactive oxygen species (ROS), which can cause damage of DNA molecule. Quercetin has already been shown to have protective effect against DNA damage, with its most pronounced feature being scavenging of free radicals. The aim of this study was to evaluate antigenotoxic potential of quercetin against thyroxine-induced DNA damage in human whole blood cells by using the comet assay. For that purpose, cells were exposed to 50 μM thyroxine and separately pre-treated or post treated with 500 μM of quercetin. Results showed that DNA damage was significantly reduced in cells pre-treated with this scavenger of free radicals. Obtained results indicate the ability of thyroxine to be a mediator of DNA damage and that quercetin displayed protective effect against thyroxine-induced genotoxicity.

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Abstract number: **PS10**

ASSESSMENT OF DNA DAMAGE IN BLOOD, LIVER AND KIDNEY CELLS IN A HYPERTENSIVE RAT MODEL USING COMET ASSAY

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Hypertension is one of the primary risk factors for heart disease and stroke, the leading causes of death worldwide. Numerous factors have been implicated in the pathophysiology of hypertension: endothelial dysfunction, arterial remodeling and vascular inflammation. Common to all these processes is increased bioavailability of reactive oxygen species in the vessels, heart, brain and kidneys. Oxidative stress and increased reactive oxygen species levels damage all macromolecules, with DNA being particularly susceptible to oxidative damage. The aim of this study was to determine whether there is a difference in the level of DNA damage between normotensive and hypertensive rats using the alkaline comet assay.
Blood samples and cells suspension from liver and kidney from three male spontaneously hypertensive rats were obtained. Three normotensive male Wistar rats were used as a control. Increased level of DNA damage was detected in blood and both of the studied tissues of hypertensive rats compared to the control, where significant difference was present in the liver and kidney cell suspensions. These results indicate that untreated hypertension in rats leads to an increased DNA damage in all of the studied samples, detected by comet assay.

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Abstract number: **PS11**

GENOTOXIC EFFECT OF IRINOTECAN ON HUMAN LIVER AND COLON TUMOR CELLS

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Currently we are faced with an ever-growing number of diagnosed carcinomas with cancers that affect the digestive system having dominance. Irinotecan (CPT-11), a semisynthetic analogue of camptothecin, is one of the most important antineoplastic drugs developed in the last decades primarily for use in the chemotherapy of metastatic colorectal cancer. In this study, the cytotoxicity, genotoxicity and induction of free radicals were examined in hepatocellular carcinoma cell line (HepG2) and the human colon adenocarcinoma cell line (Caco-2) exposed to different concentrations of irinotecan during 2, 4, 24, and 48 hours. The alkaline comet assay was performed for detection of DNA damage in carcinoma cell lines, cell survival was determined by Neutral red test while free radicals were determined with 2',7'-dichlorofluorescein-diacetate (DCFH-DA). The obtained results show that toxicity of irinotecan increased depending on its concentration in the liver cell line, while no dependence on the concentration was demonstrated with colon cells. Also, it has been shown that the cytotoxic effect of irinotecan is associated with the induction of free radicals. With the prolongation of incubation time, the genotoxic effect of irinotecan on both treated cell lines was reached. Primary DNA damage in the investigated carcinoma cell lines was the result of the effect of irinotecan and oxidative stress caused by the exposure of cells to the action of irinotecan.

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Abstract number: **PS12**

$K_2[B_3O_3F_4OH]$ INDUCED APOPTOSIS AND REDUCED CELL VIABILITY IN HUMAN ACUTE MYELOID LEUKEMIA CELL LINE UT-7

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Normal cell cycle control is dysfunctional in tumors, causing over-proliferation of cells and/or decreased capacity of elimination of cells. The leading approaches for strategies in leukemia treatment are based on targeted cell death induction. $K_2[B_3O_3F_4OH]$, also known as halogenated boroxine (HB), shows antitumor potential in various tumor cell lines but their effects on any type of hematological tumors, has not been tested yet. We aimed to analyze the cytotoxic potential and effects on cell viability of HB in human acute myeloid leukemia cell line UT-7 (ACC 137). UT-7 cultures were treated with HB in series of concentrations (0.1, 0.2, 0.4 mg/mL) and incubated for 72 h. TransDetect® AnnexinV-EGFP/PI assay based on epi-fluorescence microscopy was performed for detection of apoptotic and necrotic cells induced by HB. Trypan Blue exclusion assay was used for the evaluation of cell viability. The HB effects were quantitatively evaluated as difference in number of early and late apoptotic and necrotic cells between treated and untreated cultures. Proportion analysis showed that number of early apoptotic cells declined from the 88.9 % to 72.9 % in HB treatments compared to negative control. Late apoptotic cells frequency was relatively increased following HB treatment (from the 11.1 % to 24.6 %). Analysis of variance (ANOVA) followed by Student-Newman-Keuls comparison showed that cell viability percentage of 94.6 % in negative control was decreased up to 24.6 % for the highest HB concentration with statistical significance ($P<0.001$). Increase in number of late apoptotic cells and decrease of UT-7 cells viability were HB dose-dependent. Obtained results indicate the potential of HB to reduce viability of leukemic cells and induce apoptosis which is in accordance to its antitumor properties.

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Abstract number: **PS13**

CELLULAR FACTORS INVOLVED IN RECONSTITUTION OF OXIDATIVE DAMAGED POPULATION OF *USTILAGO MAYDIS*

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Ustilago maydis is a phytopathogenic fungus exhibiting extreme resistance to UV and ionizing radiation. Even regarding this feature that marks *U. maydis* out among eukaryotes, the molecular knowledge that would have a direct bearing on our understanding of *U. maydis*' ecological success is still fragmentary. Our results show that after heavy exposure of a population of *U. maydis* cells to clastogenes a great increase in viability is observed if the treated cells are incubated for prolonged period in distilled water (starvation). This restitution of viability results from cell multiplication of survivors by feeding on the intracellular compounds leaked from damaged cells. This finding suggested that *U. maydis* must possess cellular mechanisms involved not only in recycling of the damaged intracellular compounds but also in protection and maintenance of the *U. maydis* genome integrity. From a screen for mutants defective in the restitution of viability, we identified four genes (*chk1, snf5, hsp* and *tbp1*) that contribute to the process. The mutants in *chk1* and *tbp1* are sensitive to genotoxic agents implying that the gene products are involved in genome protection. The genetic determinants identified by our analysis have already been known to play roles in regulation of cell cycle progression, activation of DNA repair in response to the presence of DNA damage, transcription, chromatin remodelling, which are all crucial for response to stressful conditions. Obtained results will have strong impact on our understanding of the cellular response of *U. maydis* population to oxidative damage.

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Abstract number: **PS14**

SILICON ALLEVIATES COPPER TOXICITY IN CUCUMBER BY INCREASED CU-BINDING CAPACITY AND ENHANCED ANTIOXIDATIVE DEFENSE

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Copper (Cu) is an essential microelement for plant growth acting as a protein cofactor in electron transport (e.g. photosynthesis and respiration), reactive oxygen species (ROS) metabolism and cell wall formation. Excessive Cu could be released into environment due to application of Cu containing fungicides, mining and industrial pollution. Cu present in excess is phytotoxic; it generates ROS via Fenton reaction, thus inducing oxidative stress. Silicon (Si) is the only known mineral element which successfully alleviates various stresses in plants. Beneficial effect of Si in metal toxicity stress is well documented; however, role of Si under Cu toxicity is poorly examined. This study considers the mechanisms of Si-mediated protective effect on cucumber plants under Cu excess. Hydroponically grown cucumber plants were subjected to moderate Cu stress (10µM Cu) and 1.5mM Si supply. Total and cell wall Cu concentration in root and leaf, together with Cu-binding compounds (organic acids and Cu-proteins) and parameters of oxidative stress were analyzed. Beneficial effect of Si on Cu-treated cucumber was evidenced by increased biomass, and lower level of lipid peroxidation indicating reduced level of oxidative stress. Supply of Si decreased total Cu concentration but increased Cu deposition in the root cell wall fraction. Also, Si increased superoxide dismutase (SOD) activity in Cu-treated plants. Concomitantly, protein levels of Cu/Zn SOD isoforms (CSD1 and CSD2) were increased in +Si plants reflecting total SOD activity. The leaf Cu-binding compounds, such as aconitate and plastocyanin (including the plastocyanin gene expression) were higher in the +Si plants. Improved sequestration and complexation of excessive Cu were found to be the main mechanisms of Si ameliorative effect in cucumber plants. Si stimulated antioxidative defense, Cu-binding to the root cell wall and accumulation of Cu-binding compounds: aconitate, SOD and plastocyanin that buffer excess Cu.

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Abstract number: **PS15**

GENERATION OF STABILE *ARABIDOPSIS DSSI* MUTANTS USING CRISPR-CAS9 TECHNOLOGY

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DSS1 gene encodes small, highly conserved protein which belongs to intrinsically disordered protein family. DSS1 protein acts as a multifunctional partner which associates with other complexes and plays vital roles in various cellular processes. For instance, it is known that DSS1 protein is involved in maintenance of genomic integrity and protein homeostasis within 26S proteasome system. Potentially novel role of DSS1 has been proposed recently which implies specific targeting of the oxidized proteins and their further direction to degradation pathway. This mechanism of oxidative stress response is not quite clear. Two highly homologous *AtDSS1(I)* and *AtDSS1(V)* genes have been revealed in *Arabidopsis thaliana* genome, with different chromosomal localization. The function of AtDSS1 isoforms was only examined in interaction with other partners in the homologous recombination. In order to clarify plant DSS1 function, we applied CRISPR/Cas9-mediated targeted mutagenesis in order to obtain individual mutants. Mature *Arabidopsis* plants were transformed with vector containing Cas9 protein and single-guide RNA complementary to the target gene, using *Agrobacterium tumefaciens* mediated floral-dip transformation protocol. We established two stable lines containing mutations in either *AtDSS1(I)* or *AtDSS1(V)* gene. High resolution melting method followed by Sanger sequencing enabled us to select plants with desired mutations in *DSS1* genes. The mutants with 2nt substitution in *AtDSS1(V)* and 1nt gene insertion in *AtDSS1(I)* gene were chosen for further study. The former mutation led to altered ORFs and the latter caused premature stop codon. Morphological analysis of the single *Atdss1* mutant plants reveled differences in rosette shape, stem length and branching pattern. In addition, the *dss1(V)-/dss1(V)-* lines showed increased sensitivity to oxidative stress as compared to the wild-type plants. In conclusion, as an important method for mutagenesis, CRISPR technology proved the ability to generate stable heritable mutants and the opportunity to characterize duplicated genes which share highly similar DNA sequences in plants.

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Abstract number: **PS16**

GENETIC CHARACTERISATION OF ALMOND (*PRUNUS AMYGDALUS L.)* USING MICROSATELLITE MARKERS

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The simple sequence repeat analysis (SSR) has been successfully used to examine the origin, geographic divergence and distribution of almond germplasm in the Mediterranean area. Mediterranean germplasm is characterized by a rapidly developed wide diversity determining a massive genetic pool of cultivars in several growing areas. As the whole, regions of Bosnia and Herzegovina, Croatia and Montenegro were considered as one of the trade routes along which almond was spread throughout the shores of the Mediterranean Sea. Microsatellite or simple-sequence repeat (SSR) markers seem to be the appropriate marker system for cultivar identification, given their high polymorphism, codominant inheritance and the simplicity of the methods required for their detection. In this study, 10 SSR markers have been used to analyze 120 almond genotypes from their natural environment, including the most of almond genotypes spread in Herzegovina and foreign genotypes from Mediterranean areas in order to determine the level of genetic diversity and elucidate phylogenetic and possible parentage relationships between accessions and foreign germplasm. The genetic diversity between Bosnia and Herzegovina, Croatia and Montenegro germplasm and the commercial cultivars have been studied, through the same set of ten above mentioned SSR markers. The main aim of the study was to contribute to the identification of the most suitable local genotypes, as they can be still important to sustainable agriculture (that leans on biodiversity safeguard, local products for agrifood industry) and for landscape preservation. These data present the first overview of molecular genetic diversity of almond cultivars from the Mediterranean area which represents a valuable source of variability to be used for breeding.

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GRADUATE STUDENTS SESSION – POSTER PRESENTATIONS

Abstract number: **PS17**

MODULATORY EFFECTS OF DELPHINIDIN AND HALOGENATED BOROXINE ON *CAT* GENE EXPRESSION IN CULTURED LYMPHOCYTES

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Bioflavonoids present natural, polyphenolic compounds with numerous protective properties on human organism, such as antioxidant, antimutagenic, anticarcinogenic. There are several studies which have examined these properties of selected bioflavonoid, delphinidin. Halogenated boroxine (HB; dipotassium-trioxohydroxytetrafluorotriborate) has been proven to have antiproliferative role in various cell types *in vitro* and *in vivo*, and genotoxic effect in higher concentrations. It also inhibits catalase activity. When HB treatment was combined with bioflavonoids (delphinidin and luteolin), genotoxic effects of HB were decreased. The aim of this study was to test for protective, antioxidative effect of delphinidin by determination of a relative expression of *CAT* (*catalase*) gene and comparison between different treatments. Blood samples were collected from five healthy volunteers and cultured at 37 °C with a cultivation period of 72 h. Each lymphocyte culture was treated with delphinidin and HB individually, and simultaneously. Total RNA was isolated from harvested cell cultures using Quick-RNA™ Mini Prep Plus kit and then was reverse-transcribed using Proto Script First-Strand cDNA Synthesis kit. SYBR based Real-Time PCR amplification method for analysis of relative gene expression level in treated cultures was used. Analysis of results included normalization of ratio of target (*CAT*) and housekeeping (*GADPH*) gene and statistical analysis (REST®). Significant up-regulation of *CAT* expression was found in delphinidin-treated cultures and simultaneous treated cultures when they were compared to HB-treated cultures. Significant difference between delphinidin-treated cultures and cultures treated simultaneously with HB and delphinidin was not found. Also, none of tested treatments was significantly different from control treatment (DMSO). Variation in relative *CAT* expression between different treatments may indicate protective role of delphinidin against genotoxic/oxidative effects of HB through transcriptional regulation of anti-oxidative gene - *CAT*. Considering statistically non-significant results in comparison to control and for precise determination of protective, antioxidative effects of delphinidin more treatments and genes should be explored.

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Abstract number: **PS18**

BIHOR DNA PROJECT - RESULTS OF RESEARCH

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DNA genealogical research has become much more popular in the recent years. Today are many commercially genetic tests available on the market. For genealogists it is especially interesting Y chromosome test, which test the diversity of selected molecular genetic markers located on the Y chromosome exists only in males. Given the difficult-to-reach mountain area characteristic for the Montenegro, the assumptions of earlier researchers are that no more than six to eight Y haplogroups are expected among the mountain population. The results of the previous genetic research male population in Montenegro have present that the leading haplogroup is I2, while in the second place is the haplogroup Ev. However, those researches did not include the region of Northeast Montenegro, better known as the Bihor region, which includes three municipalities: Berane, Petnjica and Bijelo Polje. This paper presents the results of data were obtained by testing 44 samples of DNA materials taken from the male inhabitants from the area of upper Bihor as well as an analysis of the obtained data. The aim of the research is to present the genetic composition of the male population of upper Bihor. The results of the research point out that there are 7 Y haplogroups among the male population of upper Bihor, of which the most common haplogroup is R-M268 identified in 50% of samples, at the second place is I2a identified in 18,18%, while in the third place is haplogroup G2-M201 identified in 11.36% of samples. The other four haplogroups are present in a lower percent.

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Abstract number: **PS19**

DNA ANALYSIS SUGGEST POTENTIAL KIN RELATIONSHIP BETWEEN TWO PERSONS FROM DISTINCT MEDIEVAL ARCHAEOLOGICAL SITES

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Bosnia and Herzegovina is rich in a numerous historical monuments that remained from Middle Ages. Archaeological sites are source of numerous artefacts and skeletal remains that can answer to many questions about identity, lifestyle and relationships between medieval people, among which are also historically important persons. In our study, skeletal remains from two archeological sites (Mravinjac-Čelebići and Biskup, Glavatičevo) were subjected to DNA analysis. No precise archaeological or historical data about identity of buried persons were available for the site of Mravinjac- Čelebići. The other site - Biskup, Glavatičevo - is known as a burial site of members of well-known medieval Sankovic family who ruled Mravinjac area in the late medieval period. Skeletal remains from both sites were subjected to DNA analysis. For DNA isolation from 12 teeth (of 6 different skeletons), modified phenol-chlorophorm method was used following RealTime PCR quantification. Amplification of 23 STR loci was performed with PowerPlex® Fusion multiplex system. Among 6 generated and analyzed partial archaeological DNA profiles, for two persons calculated likelihood ratio (LR) was above 2 indicating high probability of kinship. It stays unrevealed if members of Sankovic family are buried at both sites or they left descendants at Mravinjac locality. Our research is another example how much is important to develop multidisciplinary approach for investigation of archaeological skeletal remains and how DNA profiling can help when there are no precise archaeological data.

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Abstract number: **PS20**

EVALUATION OF THIOMERSAL AND PARACETAMOL GENOTOXICITY IN HUMAN LYMPHOCYTE CULTURE

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Although its use has been reduced, thiomersal is still present as conservans in multidose containers of some vaccines or as a trace from pathogen inactivation process in production of vaccines. Paracetamol is most often applied analgoantipyretic for treatment of postimmunisation pirexy. Prophylactic use of paracetamol upon vaccination is still present, despite its proven effect on reducing immune response to some vaccines. Because interaction possibility of these two substances in vivo, it was found interesting to made *in vitro* examination of their genotoxicity. Genotoxic effects of thiomersal and paracetamol and influence of their common application were examined on human lymphocytes cultures. Blood samples of three healthy donors were included in the study. Tested substances were added 24 hours after culture were started. Following concentrations were examined: thiomersal 1 μg/ml, thiomersal 0.5 μg/ml, paracetamol 20 μg/ml, thiomersal 0.5 μg/ml with paracetamol 20 μg/ml and thiomersal 1.0 μg/ml with paracetamol 20 μg/ml. Cultivations were lasted for 72 hours, then analysis of structural chromosomes aberrations was performed. Results shown that frequency of structural chromosome aberrations was significantly increased in all treated cultures in comparison with negative control. Number of structural chromosome aberrations was highest in culture treated with 1 μg/ml of thiomersal. In cells treated with thiomersal 1 μg/ml with presence of 20 μg/ml of paracetamol, the number of aberrations was significantly decreased, while paracetamol suppressing effect on number of structural chromosome aberrations related with 0.5 μg/ml of thiomersal is not found as significant. Induction of structural chromosome aberrations is shown as a sign of genotoxicity for examined concentration of thiomersal and paracetamol and their common treatment of lymphocytes cultures. Suppressing effect of paracetamol on thiomersal genotoxicity in lymphocytes culture treated with 1 μg/ml of thiomersal was shown as indicative for furter examination of paracetamol use in prevention of genotoxicity.

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Abstract number: **PS21**

CYTOGENOTOXIC POTENTIAL OF THREE DIFFERENT PARABENS *IN VITRO*

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Parabens (PBs) are alkyl esters of p-hydroxybenzoic acid. They are used as preservatives in consumer products, including pharmaceuticals, foods and cosmetics. Also, they presence has been confirmed in human breast cancer. The objective of this study was to determine the possible cytogenotoxic effects of the three different parabens, methylparaben ($C_8H_8O_3$), ethylparaben ($C_9H_{10}O_3$) and butylparaben ($C_{11}H_{14}O_3$), in plant cells (*Allium cepa*) and human lymphocytes. Our results for all three PBs showed that mitotic index in *Allium* test decreased with increasing concentration (100 μg/ml, 250 μg/ml and 500 μg/ml). None of the tested PBs showed inducing effect on root growth, on the contrary, there was stagnation in growth at higher concentration. All three tested concentration of buthylparaben showed slight decrease in length of the root. Increased frequency of chromosome aberrations (CA) in comparison with controls was observed in *Allium* test. Ethylparaben increased frequency of apoptosis (500 μg/ml), while methylparaben increased frequency of necrosis (100; 500 μg/ml). In human lymphocytes differences between PBs and control was observed for accentric fragment (250 μg/ml methylparaben), chromatid break (100 μg/ml ethylparaben), aneuploidy (100 μg/ml ethylparaben) and polyploidy (100 μg/ml methylparaben, and 250 μg/ml butylparaben). Increased frequency of apoptosis was induced by methyl- and ethylparaben (250; 500 μg/ml for both PBs). Results of mitotic index in human lymphocytes matches with results of plant model. Our results demonstrated that: PBs may have a genotoxic effect and have cytotoxic potential in human peripheral lymphocytes as well as in *Allium cepa*; that the long-term and intensive use of the tested substance may pose a risk to humans. Further studies are needed to strengthen these findings.

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Abstract number: **PS22**

COMPARATIVE CYTOTOXICITY ANALYSIS OF EXTRACTS OF *THYMUS BRACTEOSUS* VIS EX BENTHAM AND *ACINOS ORONTIUS* (K.MALY) ŠILIĆ

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Endemic species *Thymus bracteosus* Vis ex Bentham and *Acinos orontius* (K.Maly) Šilić belong to the family Lamiaceae that is frequently used in folk medicine due to its well-known therapeutic effects. Safety assessment of a traditionally used extracts of these two plant species has been poorly investigated so far, thus cytotoxicity testing of their extracts on the cells viability *in vitro* is needed to predict potential toxic effects in humans. For more rational utilization of these Bosnian and Herzegovinian endemic species in folk medicine, and assessment of their potential clinical relevance, this study aimed to analyze and compare cytotoxic effects of aqueous and dimethyl sulfoxide extracts of *Thymus bracteosus* and *Acinos orontius*. Various concentrations of individual plant extract samples (0.01, 0.05, 0.1, and 0.2 mg/ml) were screened after 48 hours treatment to assess effects on cell viability of peripheral blood mononuclear cells (PBMCs) in culture, using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. Results have shown dose-dependent effects on cell viability for all tested extracts with a significant increase in cell viability in treated cultures compared to controls ($p<0.001$). Moreover, dimethyl sulfoxide extracts at higher concentrations (0.1- 0.2 mg/ml), and all aqueous extracts of *Thymus bracteosus* had a more profound effect on PBMCs viability compared to extracts of *Acinos orontius.* Cytotoxicity of tested extracts has not been confirmed in applied concentrations. However, increase in culture proliferation indicates further studies potential of these extracts to modulate molecular mechanisms of cell cycle both in normal and cancer cell lines.

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UNDERGRADUATE STUDENTS SESSION – POSTER PRESENTATIONS

Abstract number: **PS23**

TURNER SYNDROME WITH HAPLOINSUFFICIENCY IN XP22.3 AND XQ28 MICRODELETION

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Turner syndrome is a chromosomal disorder in which females have only one X chromosome while other is missing or structurally altered. Characteristic features such as short stature, webbed neck, and early loss of ovarian function are usually going with normal intelligence. Patient is a female child in the age of 7,5 years old, sent to the genetic specialist under diagnose of severe psychomotor development delay and reduced growth. During the inspection, patient was presented with reduced growth (112 cm), webbed neck, low hairline at the back of the neck, widely spaced nipples and clinodactyly. The patient seemed severely hyperactive, with occasional stereotypic movements, a narrow vocabulary and without the capability of making sentences. Karyotipization showed karyotype 46, X0 (Turner syndrome). Throughout the years patient developed severe aggression toward herself and mother, continued to express hyperactivity with extremely poor development of higher cognitive functions. Since this difficult and global psychomotor and somatic development delay is not characteristic for Turner syndrome, a genetic specialist recommended a subtelomeric screening. MLPA showed haploinsufficiency in Xp22.3 which according to literature is responsible for neurocognitive deficits in this patient. Screening also showed Xq28 microdeletion which affects the expression of MeCP2 gene. This mutation clinically correlates with Rett like phenotype which is characterized by significant intellectual impairments together with an autistic spectrum disorder. This case emphasizes the importance of clinical monitoring of such complex patients in order to detect deviations of the classical presentation of syndromes. It also encourages us to rethink about diagnose, if we notice abnormal clinical aspects, even after it was confirmed so that we continue to observe in the purpose of reaching the ultimate cause that can explain the overall clinical picture.

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Abstract number: **PS24**

CONGENITAL HYPOTONIA AND EPILEPSY AS MAIN CLINICAL SIGNS OF WOLF-HIRSCHHORN SYNDROME

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Wolf-Hirschhorn syndrome (WHS) is a rare genetic disorder caused by a deletion of the short arm of chromosome 4. Main characteristics are distinctive facial feature consisting of "Greek warrior helmet" appearance, delayed growth and development, hypotonia, variable midline defects, and seizures. This case represents two children with WHS. First, female child of healthy nonconsanguineous parents, born in the fourth pregnancy with intrauterine growth failure. Postnatal presenting with hypotonia and dysmorphic features. Karyotyping confirmed WHS (karyotype: 46, XX, del (4) (p15.32)dn) with characteristics: typical high-tone crying, microcephaly, hypertelorism, epicanthal folds, blepharophimosis, micrognathia, gothic palate, clinodactyly, camptodactyly, growth failure, respiratory infections, and epilepsy. The girl now has 8.5 years, BW 14 kg, epilepsy, immunodeficiency and severe global psychomotor delayed. The second case is a male child of nonconsanguineous parents born in a third pregnancy which was poorly controlled. The mother has epilepsy from the infant age, high myopia, and border cognitive functioning. Karyotyping confirmed WHS. As in the first case, phenotypic features, respiratory infections, and epilepsy are present. Differences are a cleft palate, hypospadias with cryptorchism and brain developmental anomaly. At the age of 3.5 years, he acquired severe pneumonia and respiratory insufficiency with multiorgan failure. By the end of life, he remains in palliative care and dies at the age of 4 years and 9 months. It is a rare genetic syndrome characterized by typical phenotype, hypotonia, growth failure, and refractory epilepsy. Most patients die within the first year of life, but this case represents a prolonged survival. For individuals affected by WHS there is no specific treatment, they need multidisciplinary monitoring in the direction of improving the quality of life. The therapeutic measures should be on supporting the parents and also suggest genetic counseling for the next planned pregnancies.

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Abstract number: **PS25**

URINE qRT-PCR ASSAY AS A SCREENING TOOL FOR THE DETECTION OF CONGENITAL HUMAN CYTOMEGALOVIRUS INFECTION OF INFANTS IN SARAJEVO CANTON

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In the past 10 years, CMV infection in infants has become one of the most extensively studied vertically transmitted infections. Despite of being massively investigated by various biomedical research institutions, tremendous adverse effects of vertically transmitted CMV infection are still not well introduced to the general public in Sarajevo Canton, which results in low awareness of potential expectant mothers. As a consequence of low awareness about CMV, incidence of CMV infected infants is increasing each year. Stressing this out, the purpose of this study is to elucidate sensitivity of urine samples for CMV detection in infants as well as to reflect importance of real-time PCR as additional technique for diagnostics of CMV infection in infants. Quantitative real-time PCR is used in this study as the primary technique for evaluation of CMV infection incidence of infants in Sarajevo Canton in the period between October 2017 and April 2019. Real-time PCR results of patients included in this study positively evaluate gold medical standards for CMV detection, emphasizing that urine samples are sufficiently sensitive for detection of CMV DNA in infants. Furthermore, results of several infants have shown higher number of CMV DNA copies detected in urine compared to blood samples, thus demonstrating the importance of urine sample in CMV diagnostics. In conclusion, urine samples are shown to be reliable source for diagnostics of CMV infection in infants. Likewise, simultaneous analysis of urine and blood samples could notably contribute to establishment of more specific therapies for infected infants. Findings of this study may contribute to the classification of CMV test in one of the first trimester pregnancy screening tests in Sarajevo Canton, which could potentially result in decreased incidence of CMV infection in infants.

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Abstract number: **PS26**

DNA ANALYSIS OF ABORTED FETAL TISSUES IN FORENSIC CASES

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Prenatal samples are most commonly analyzed in rape cases, when assailants are identified through the paternity testing. Analysis can be very challenging since analyzed material frequently contains both mother and fetal DNA. Final results must be unambiguous so it is essential to use markers with high discriminatory power. Here we present seven cases of rape including incest, human trafficking and rape of minor. DNA analysis of aborted material was used for paternity testing to provide evidence of guilty of suspected persons. For DNA extraction from biological samples QIAamp DNA Mini Kit was used. Sixteen STR markers were amplified applying PowerPlex 16 amplification kit and DNA profiles were generated on 310 ABI PRISM Genetic analyzer using GeneMapper® ID v3.2 Software. In one case mixed DNA profile was created from fetal tissue. Calculated paternity indexes proved that all suspects committed crimes for which they have been accused. Results of this study once again emphasize importance of STR-DNA analysis in resolving cases of rape and sexual violence.

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Abstract number: **PS27**

CRITICAL POINTS IN PERFORMING ALKALINE COMET ASSAY ON ORAL LEUKOCYTES

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Comet assay is a simple and rapid technique used to access single-cell DNA damage. This single-cell gel electrophoresis technique is used for assessment of genotoxic effects of various agents by the visual or software-based analysis of comets stained with fluorescent dyes. Oral leukocytes are suitable sample for human biomonitoring studies as their collection is non-invasive and thus convenient for vulnerable populations, such as patients or children. However, there are numerous variables that relate to isolation and performance of comet assay in oral leukocytes (e.g. seasonal temperature variations, inter-individual variations in yield etc). Hence, this justifies the aim of this study to optimize protocols in order to attain reproducible and equally continuous results. Oral leukocytes were isolated from healthy individuals, smokers and nonsmokers by using commercially available or laboratory made physiological saline solution and alkaline (pH=10) comet assay was performed. Effects of centrifuge conditions, room temperature, buffer storage conditions and sample concentration were evaluated. The optimal results were obtained within 20-25°C laboratory temperature range, with commercially available normal saline solution and freshly made buffers and solutions. We recommend frequent pH checkup of chemicals in order to maintain alkaline conditions, and optimization of centrifugation conditions for each manufacturer and laboratory. Among the critical factors that also should be considered is air-conditioning during the summer season.

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Abstract number: **PS28**

TWO-COMPONENT VAULT NANOPARTICLE SYSTEM FOR EFFICIENT TREATMENT OF LATENT AND ACTIVE TUBERCULOSIS

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Mycobacterium tuberculosis has the ability to obstruct the process of phagosome-lysosome fusion, which is the first step in the process of establishing a latent tuberculosis infection inside of the lungs. However, M. *tuberculosis* strains are faced with yet another obstacle presented by the host's immune system - the noxious environment of the inactivated phagosome (macrophage). Cytokine-mediated nitric oxide synthase produces low levels of nitric oxide within human macrophages during infections, whose bacteriostatic and bactericidal properties have the ability to control or eradicate pulmonary infections. Inactivated macrophages harbouring *M. tuberculosis* have poor oxygen permeability, which creates noxious conditions facilitated by hypoxia and nitric oxide. Such conditions prevent aerobic respiration and replication, thus triggering the activation of a genetic program designated as the *DosRST* regulon. This regulon plays a crucial role in maintaining metabolic homeostasis and non-replicating persistence; it enables a latent, chronic infection that does not respond to currently available therapeutic regiments. Latent tuberculosis is established either asymptomatically - without the patient's awareness due to absence of symptoms - or after treatment of a symptomatic tuberculosis infection. Eradicating viable tubercule bacilli from the body is currently impossible due to the protection provided by the *DosRST* regulon, which also assists in re-activation of the bacilli when conditions are favourable. The aim of this work is to explore and present a novel two-component therapeutic method for treatment of pulmonary tuberculosis using a vault nanoparticle-based drug delivery system. Such a system would primarily carry *DosRST* inhibitors inside pulmonary macrophages using *M. tuberculosis-specific* macrophage receptors. Benefits, difficulties, potential risks and the selection of appropriate vault nanoparticle carriers shall also be explored. The second component of this system will be used to prevent latent tuberculosis from being established during treatment of active infection by targeting Mannose-capped lipoarabinomannan present within the mycobacterial cell wall.

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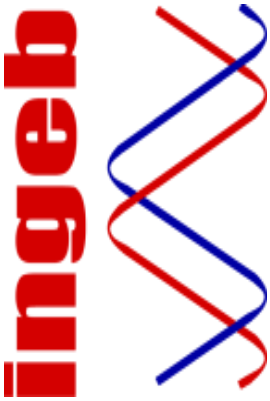
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