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# Journal of Bioanthropology

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Journal of Bioanthropology

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Journal of Bioanthropology is a multi and interdisciplinary scientific journal that focuses on scientific research in the field of biological anthropology, bioarchaeology, biomechanics, biomedicine, ergonomics, forensics, genetics, human evolution, molecular anthropology, public health and related subjects. Official language of the Journal is English.
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Journal of Bioanthropology operates a double-blind peer-review system, where the reviewers do not know the names or affiliations of the authors and the reviewer reports provided to the authors are anonymous.
Submitted manuscripts will generally be reviewed by two to three experts who will be asked to evaluate whether the manuscript is scientifically sound and coherent, whether it duplicates already published work, and whether or not the manuscript is sufficiently clear for publication. Reviewers will also be asked to indicate how interesting and significant the research is. The Editors will reach a decision based on these reports and, where necessary, they will consult with members of the Editorial Board.
 Journal of Bioanthropology is official journal of The International Society of Applied Biological Sciences (ISABS).

For issue vol.2, no.1 in 2022, we were privileged for the opportunity to announce excellent scientific lectures to be presented at the Twelfth ISABS Conference on Forensic and Anthropological Genetics and Mayo Clinic Lectures in Individualized Medicine.

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Journal of Bioanthropology



# PROGRAM AND ABSTRACTS
# The Twelfth ISABS Conference on Forensic and Anthropological Genetics and Mayo Clinic Lectures in Individualized Medicine

## June 22-27, 2022, Dubrovnik, Croatia

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Ljudevita Gaja 32, Zagreb, Croatia

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*Guest Editors:*

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*Prepress:*

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# PROGRAM AND ABSTRACTS

THE TWELFTH ISABS CONFERENCE
ON FORENSIC AND ANTHROPOLOGICAL GENETICS
AND MAYO CLINIC LECTURES IN INDIVIDUALIZED MEDICINE

JUNE 22-27, 2022
Hotel Dubrovnik Palace
Dubrovnik
CROATIA

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# DUBROVNIK PALACE HOTEL – LAYOUT

ISABS CONFERENCE
on Forensic and Anthropological Genetics and Mayo Clinic Lectures in Individualized Medicine
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## 9th Floor


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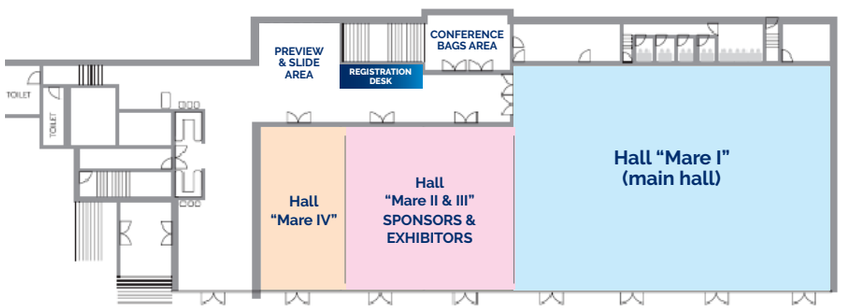




ISABS CONFERENCE
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| HALL/DAY | TUESDAY, JUNE 21 | WEDNESDAY, JUNE 22 | THURSDAY, JUNE 23 | FRIDAY, JUNE 24 | SATURDAY, JUNE 25 | SUNDAY, JUNE 26 | MONDAY, JUNE 27 |
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| MARE I (10th Floor) | | | 09:00 a.m. - 10:30 a.m. Opening Ceremony | 08:30 a.m. - 11:35 a.m. Oral Session 2 & Selected Abstracts | 08:30 a.m.- 11:35 a.m. Oral Session 4 & Selected Abstracts | 08:30 a.m. - 11:35 a.m. Oral Session 6 & Selected Abstracts | 08:30 a.m. - 11:35 a.m. Oral Session 8 & Selected Abstracts |
| | | | 10:30 a.m. - 12:15 a.m. Opening Session | 12:30 p.m. - 02:45 p.m. Nobel Laureate Session | 01:30 p.m. - 05:00 p.m. Oral Session 5 & Selected Abstracts | 01:30 p.m. - 04:35 p.m. Oral Session 7 & Selected Abstracts | 01:30 p.m. - 02:05 p.m. Special Plenary Session |
| | | | 02:00 p.m. - 05:05 p.m. Oral Session 1 & Selected Abstracts | 03:00 p.m. - 04:15 p.m. Oral Session 3 | 08:00 p.m. - 09:00 p.m. Evening Lecture 1 | 08:00 p.m. - 09:00 p.m. Evening Lecture 2 | 02:05 p.m. - 04:15 p.m. Oral Session 9 & selected Abstracts |
| | | | | 06:00 p.m. - 07:15 p.m. Nobel Spirit Broadcasting | | | 04:15 p.m. - 04:25 p.m. Closing Remarks |
| MARE II & III (10th Floor) | | | SPONSORS & EXHIBITORS | SPONSORS & EXHIBITORS | SPONSORS & EXHIBITORS | SPONSORS & EXHIBITORS | SPONSORS & EXHIBITORS |
| MARE IV (10th Floor) | | 12:30 p.m. - 02:50 p.m. Interdisciplinary Session AAFS & ISABS | 12:15 p.m. - 02:00 p.m. Joint session: ISABS; St. Catherine, Regiomed Klinken, University of Split (by invitation) | 08:30 a.m. - 12:05 p.m. Bioanthropology and global health in the times of crisis | 11:35 a.m. - 01:30 p.m. IRI 2 Workshop: Osteoarthritis (IRI 2 partners only) | | |
| | | | 03:25 p.m. - 05:10 p.m. Bioanthropology and global health in the times of crisis | | | | |
| DUBRAVA (9th Floor) | 04:00 p.m. - 07:00 p.m. Satellite Joint Event CSNG&CSPPM (8th Croatian Human Genetics Conference &1st Croatian Personalized and Precision Medicine Conference) | 08:30 a.m. - 03:30 p.m. Mayo Clinic Short Course on Epigenomics | | | | | |
| | | 03:30 p.m. - 03:50 p.m. Sponsor Lecture 10x Genomics | | | | | |
| SUNSET LOUNGE (HOTEL LOBBY) (9th Floor) | | | | | 11:35 a.m. - 01:30 p.m. Poster Session I: Anthropological Genetics & Future Scientist Award | 11:35 a.m. - 01:30 p.m. Poster Session II: Forensic Genetics | 11:35 a.m. - 01:30 p.m. Poster Session III: Individualized Medicine |

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# WELCOME TO THE TWELFTH ISABS CONFERENCE ON FORENSIC AND ANTHROPOLOGICAL GENETICS AND MAYO CLINIC LECTURES IN INDIVIDUALIZED MEDICINE!

Dear Colleague,

We invite you to join us at the 12th ISABS Conference on Forensic, Anthropological and Medical Genetics, Dubrovnik, Croatia, June 22 – 27, 2022. The conference is the next in the series of biennial events organized by the International Society for Applied Biological Sciences (ISABS), a society dedicated to the promotion of applied molecular biology (www.isabs.net).

*Since the initiation of the series in 1997, we have strived both to focus and broaden the scope of the conferences. The focus has been on the application of the cutting-edge analytical methodology in forensic science. In 2003 programs in forensic medicine and in cellular and molecular medicine ran in parallel with the introductory and closing sessions held jointly. The Cellular and Molecular Medicine program was co-organized with the Mayo Clinic, Rochester, Minnesota, USA. Since 2007 we have broadened the area of interest by the introduction of molecular anthropology that, in large part, shares the methodology with forensic genetics. In 2009, we introduced selected topics from individualized medicine, another applied discipline based on the advances in mapping the human genome. In 2011 we included the most recent and most interesting topics in molecular medicine. In 2013 we included genetics applied to the crossing of forensic science, anthropology, and translational medicine. Mayo Clinic again, same as in the past ten years joined our efforts. It provided a critical link to the cutting-edge clinical applications of genetics. The overall effort culminated in the incorporation of individualized medicine as the third cornerstone, together with forensic and anthropological genetics. We feel this integration of the three areas, united by technology and applicative intent, provides an unprecedented opportunity for further progress in every respect. In the clinical part of the conference, topics covered the latest achievements in regenerative medicine, gene and cell therapy, individualized medicine, new molecular procedures and methodology for early detection of cancer, the clinical importance of circulating tumor cells, and immune therapy in cancer treatments. In 2015 we introduced up-to-date results in genomics of individualized medicine, a program in anthropology genetics concerning ancient and modern human genome history, and human genetic history of the continents, and forensic genetics program with special emphasis on new knowledge in Next Generation Sequencing (NGS) in forensics, DNA investigative intelligence, and advancements in forensic DNA routine. At the jubilee 10th ISABS conference in Dubrovnik in 2017, the Nobel Spirit session took place for the first time. This jubilee conference marked a breakthrough towards personalized and regenerative medicine and microbiome analysis in forensics, anthropology, and medicine, and in 2019 we continued with the same "Nobel Spirit" achievements.*

*We are pleased that the program in 2022 will be more interesting than ever and it will include the following topics: Mathematical Modeling in Cancer Therapy, Gene Therapy, Archaeological Genetics, New perspectives in Human Forensic Molecular Biology, Genomics in Medicine, Pharmacogenomics and Drug Development, Stem Cells in Medicine, Regenerative Medicine, Ribosomes in Medicine, Epigenomics, Crime Scene Investigation, Forensic Genetics, and Mass Catastrophes Managements. This year, the third "Nobel Spirit" will provide a forum to the three Nobel laureates to stimulate public discussion on the role of science in solving global health issues, acute regional problems such as brain drain, demographic decline, as well as cultural and social change. In addition, we are organizing*

*a very stimulating Session on Bioanthropology and global health in the times of crisis, as well as Joint Event ISABS and Ministry of the Interior - Crime Scene Investigation Training Course: Mystery on the ship —Investigation of the water-related crime scene.*

Together with several conference regulars, this 2022 year will bring many new and exciting names including Nobel Prize laureates Aaron Ciechanover, Sir Richard John Roberts, and Thomas Südhof. Special Plenary Lectures will be held by Garry Kasparov (New York, USA) and Greger Larson (University of Oxford, Oxford, United Kingdom).

This year again, program directors Tamas Ordog and Manfred Kayser invested both their creative energy, effort, and time to compile a stellar program. Kudos should be addressed to them; shortcomings and complaints should be addressed to us.

As before, the conference is structured to allow close interaction of the international faculty and attendees. We continue to have traditional Young Investigator Awards and High School Student Future Scientist Awards. Together with formal presentations, there will be meet-the-professor sessions, a gala dinner, and other social occasions meant to enhance opportunities for scientific intercourse, but also to introduce the participants to the town of Dubrovnik, one of the most prominent tourist destinations in the Mediterranean Sea; it carries the appellation of the pearl of the Adriatic. With its remarkable history, Dubrovnik is a city that leaves nobody unmoved, so delighted by its beauty, George Bernard Shaw said "Those who seek paradise on Earth should come to Dubrovnik".

We look forward to seeing you in Dubrovnik!

Dragan Primorac and Stanimir Vuk-Pavlović

Conference Founders





# ABOUT DUBROVNIK

Sitting in the southernmost part of Croatia, harboring centuries of heritage created by the noble skills of the finest builders and artists, Dubrovnik is one of the most prominent tourist destinations in the Mediterranean Sea; it carries the moniker of "The Pearl of the Adriatic".

The prosperity of the city was historically based on maritime trade; as the capital of the maritime Republic of Ragusa, it achieved a high level of development, particularly during the 15th and 16th centuries, as it became notable for its wealth and skilled diplomacy. Dubrovnik used to be an independent, merchant republic for 700 years (abolished by Napoleon in 1806).

The old town was completed in the 13th century and remains virtually unchanged to the present day. Although severely damaged by an earthquake in 1667 and again in the 1990s by armed conflict, Dubrovnik managed to preserve its beautiful Gothic, Renaissance and Baroque churches, monasteries, palaces, and fountains. Among the variety of archaeological, historical, and cultural monuments are 1,940 m long defensive walls (from 1979 inscribed into the UNESCO World Heritage List) that surround the city.

There are only two entrances to the old town which lead to Stradun, the city's promenade. From the Onofrio Fountain to the City bell tower, the filigree-like Gothic and Renaissance facades of the Sponza palace and Ducal palace, the Baroque church of St. Blasius (St. Blaise, or Sveti Vlaho as the locals call him, is the city patron), the Cathedral of the Assumption of Our Lady, or St. Ignatius and the Jesuit College, every step in this town will be an experience par excellence. The city's glorious walls, fortresses, and bastions offer a view of the magical Elaphite islands- Šipan, Lopud, and Koločep, scattered like pearls in the azure of the sea.

With its remarkable history, Dubrovnik is a city that leaves nobody unmoved, so delighted by its beauty, George Bernard Shaw said, "Those who seek paradise on Earth should come to Dubrovnik".

# 12TH ISABS CONFERENCE,

# JUNE 22-27, 2022, DUBROVNIK, CROATIA

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**Alen Juginović** (Harvard University, Boston, USA and St. Catherine Specialty Hospital, Zagreb, Croatia), Vice-Chair

- ***IT support***

**Stipe Pavela**

- ***Administrative support***

**Severina Ević** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Vlatka Habjanec** (St. Catherine Specialty Hospital, Zagreb, Croatia)

- ***Ph.D. Students Committee***

**Vid Matišić** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Vilim Molnar** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Martina Smolić** (Faculty of Dental Medicine and Health and Faculty of Medicine, University of Osijek, Osijek, Croatia)

- ***Students Committee***

**Petar Brlek** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Martin Čemerin** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Zvonimir Mlinarić** (Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia)
**Kristijan Vrdoljak** (St. Catherine Specialty Hospital, Zagreb, Croatia)

- ***High School Students Committee***

**Ivana Erceg Ivkošić** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Josip Crnjac** (Department of Forensic Sciences, University of Split, Split, Croatia)
**Ivan Dolanc** (Institute for Anthropological Research, Zagreb, Croatia)





# Young Investigator Award

## Recipients of the 2022 Young Investigator Award

- Elena Essel, Germany (Antropological Genetics)
- Vid Matišić, Croatia (Pharmacogenomics)
- Karlo Miškec, Croatia (Epigenetics)
- Amira Nabil, Egypt (Molecular diagnostics)
- Rachelle Turiello, USA (Forensic Genetics)

## Recipients of the 2019 Young Investigator Award

- Viktoria Dotz, The Netherlands (Personalized Medicine)
- Benjamin Planterose Jiménez, The Netherlands (Forensic Genetics)
- Elena Zavala, Germany (Anthropological Genetics)
- Recipients of the 2017 Young Investigator Award
- Mateja Hajdinjak, Germany (Anthropological Genetics)
- Goran Josipović and Vladimir Zanki, Croatia (Personalized Medicine)
- Sabriya Syed, USA (Personalized Medicine)
- Atina Vidaki, The Netherlands (Forensic Genetics)
- Recipients of the 2015 Young Investigator Award
- Dora Polšek, Croatia (Molecular Therapy)
- Niraj Rai, India (Molecular Anthropology)
- Barbara Zajac, Germany (Genetic Analysis of Forensic Non-human Material)

## Recipients of the 2013 Young Investigator Award

- Matko Čančer, Sweden (Gene Therapy)
- Dora Markulin and Branka Gršković, Croatia (Genome-based Applications in Forensic Science)
- Slave Petrovski, USA (Personalized Genomics)
- Antoinette Westen, The Netherlands (Genome-based Applications in Forensic Science)

## Recipients of the 2011 Young Investigator Award

- Mark Barash, Australia (Forensic DNA Phenotyping)
- Rebecca S. Just, USA (Genome-based Applications in Forensic Science)
- Renato Polimanti, Italy (Molecular Anthropology)
- Martina Smolić, Croatia (Molecular Therapy)

## Recipients of the 2009 Young Investigator Award

- Chiara Barbieri, Germany (Molecular Anthropology)
- Fernanda Toledo Gonçalves, Brasil (Individualized Medicine)
- Pavlo Feliksovich Tatarskyy, Ukraine (Individualized Medicine)
- Antoinette Westen, Netherlands (Forensic Genetics)

## Recipients of the 2007 Young Investigator Award

- Kaye Ballantyne, Australia (Molecular Anthropology)
- Tomislav Domazet-Lošo, Croatia (Molecular Anthropology)
- Coralie Frassati, Switzerland (Molecular Anthropology)
- Grzegorz Kaczmarczyk, Poland (Forensic Genetics)
- Agnieszka Krzyżańska, Poland (Forensic Genetics)
- Taeko Kashima, Japan (Molecular Anthropology)

### Recipients of the 2005 Young Investigator Award

- Mirela Baus Lončar, Germany (Molecular and Cellular Medicine)
- Tracy Johnson, USA (Forensic Genetics)
- Vedrana Montana, USA (Molecular and Cellular Medicine)
- Caroline Round, United Kingdom (Forensic Genetics)

### Recipients of the 2003 Young Investigator Award

- Chiara Magri, Italy (Molecular and Cellular Medicine)
- Robert J. Shelton, CO, USA (Forensic Genetics)

### Recipients of the 2001 Young Investigator Award

### Forensic Identity Testing: Frontiers in Molecular and Cellular Medicine:

- Rima Dada, India
- Katja Drobnič, Slovenia
- Anna Gareeva, Russia
- Nguyen Hoai Giang, Vietnam
- Tomasz Kupiec, Poland
- Lucia Cifuentes Ovalle, Chile

### High School Student Future Scientist Award

- Recipients of the 2022 High School Student Future Scientist Award
- Filip Bulat, VII. Gymnasium, Zagreb, Croatia
- Ante Čolak, II. Gymnasium, Zagreb, Croatia
- Mei Đulabić Chalfe, V. Gymnasium, Zagreb, Croatia
- Robertina Filković, V. Gymnasium, Zagreb, Croatia
- Lucija Glavičić Marović, XV. Gymnasium, Zagreb, Croatia
- Đurđica Kovačić, III. Gymnasium, Split, Croatia
- Nika Adriana Marijanović, Classical Gymnasium, Zagreb, Croatia
- Frane Marušić, Jure Kaštelan High School, Omiš, Croatia
- Nika Miličević, V. Gymnasium, Zagreb, Croatia
- Goran Narančić, XV. Gymnasium, Zagreb, Croatia
- Rea Pešušić, Vladimir Prelog Science School, Zagreb, Croatia
- Recipients of the 2019 High School Student Future Scientist Award
- Božo Bradarić Lisić, Vladimir Prelog Science School, Zagreb, Croatia
- Matea Bürger, VII. Gymnasium, Zagreb, Croatia
- Sara Caktaš, XV. Gymnasium, Zagreb, Croatia
- Angela Kalpić, Medical School Split, Split, Croatia
- Rea Pešušić, Vladimir Prelog Science School, Zagreb, Croatia
- Petar Škrobo, M.A. Reljković Gymnasium, Vinkovci, Croatia
- Recipients of the 2017 High School Student Future Scientist Award
- Filip Bognar, XV. Gymnasium, Zagreb, Croatia
- Lovro Jančić, Karlovac Gymnasium, Karlovac, Croatia
- Rej Kovačević, VII. Gymnasium, Zagreb, Croatia
- Lara Primorac, IB World School – XV. Gymnasium, Zagreb, Croatia
- Magda Topić, XV. Gymnasium, Zagreb, Croatia
- Borna Branimir Vuković, V. Gymnasium, Zagreb, Croatia





# Scientific Program Information

## Badges

Badges will be provided to participants, accompanying persons, exhibitors and press at registration and will be required for admission to all conference facilities and scientific and social events during the Meeting. The badges will be checked by security guards at the conference venue. Any individual who is not wearing an official meeting badge will be directed to the registration desk to register or, if already registered, to purchase a replacement badge. Handling fee for replacement badges is €10.

## Certificate of Attendance

Confirmations of attendance will be issued at the registration desk.

## Credits

The 12th ISABS Conference on Forensic Genetics and Molecular Medicine has been approved for 10 (participants) or 15 (lecturers) points by the Croatian Medical Chamber. Credits are intended for medical doctors, members of Croatian medical Chamber, in order to extend their medical doctor's license. If you are the member of Croatian Medical Chamber and you qualify for the credits approved for the participation in 12th ISABS, please enter your VAT number during online registration.

## Young Investigator Award

Knowing the importance of including young researchers as active participants of the conference, ISABS encourages PhD and graduate students working toward a degree in the fields related to the conference program themes as well as postdoctoral researchers to join the conference and apply for Young Investigator Award (YIA). Therefore, for each ISABS conference the YIA is presented for outstanding research presented by investigators who are less than thirty five years old. Scientific Advisory Committee of the conference selects finalists from nominees who submit abstracts. Besides the attractive prize, each award recipient is given the opportunity for a podium presentation. Author(s) of each selected abstract receive the Young Investigator Award Certificate as well.

## High School Student Future Scientist Award

High School Student Future Scientist Award is a joint project of the International Society of Applied Biological Sciences (ISABS), the Ministry of Science and Education of the Republic of Croatia and the Croatian Education and Teacher Training Agency. The Scientific Committee of the International Society for Applied Biological Sciences (ISABS) in collaboration with the Croatian Education and Teacher Training Agency and the Croatian Ministry of Science and Education awards the best Croatian high school students' essays with The ISABS Future Scientist Award. The essays must be in the field of human biology, genetics, chemistry, and infectious disease (such as COVID-19). ISABS will also reward all teachers-mentors of the winning students. The awarded students will receive The ISABS Future Scientist Award, certificate, cash prize of 1,000 kuna, and free registration for the scientific program of the conference, including lectures of the Nobel Laureates. They will also be entitled to attend

the opening ceremony of the ISABS Conference and the Conference banquet. In addition, they will be provided with conference materials (a book of abstracts, a special volume of the Croatian Medical Journal, etc.). Furthermore, their mentors will be awarded with free conference participation and they will receive a certificate as well.

## Registration Desk Hours

| | |
|---|---|
| Tuesday, June 21, 2022 | 14:00 – 20:00 |
| Wednesday, June 22, 2022 | 8:00 – 16:00 |
| Thursday, June 23, 2022 | 8:00 – 17:00 |
| Friday, June 24, 2022 | 7:30 – 17:00 |
| Saturday, June 25, 2022 | 7:30 – 17:30 |
| Sunday, June 26, 2022 | 7:30 – 17:00 |
| Monday, June 27, 2022 | 7:30 – 17:00 |

## Sponsor Exhibition

Setup: June 22, 2022
Dismantling: June 27, 2022 by 17:00

## Exhibit Hall Hours

| | |
|---|---|
| Wednesday, June 22, 2022 | 8:30 – 17:00 |
| Thursday, June 23, 2022 | 8:30 – 17:00 |
| Friday, June 24, 2022 | 8:30 – 17:00 |
| Saturday, June 25, 2022 | 8:30 – 17:00 |
| Sunday, June 26, 2022 | 8:30 – 17:00 |
| Monday, June 27, 2022 | 8:30 – 17:00 |

## Poster Setup and Removal

Posters will be displayed during the entire conference. Poster board numbers can be found in the conference book of abstracts. Conference staff at the registration & info desk will help you locate the board. Adhesive material will be made available at registration & info desk.

Posters mounting: **Wednesday, June 22, 2022, 8 a.m. – 4 p.m.**
Posters removal: **Monday, June 27, 2022, 5 p.m. – 6 p.m.**

ISABS is not responsible for material left after the Conference is over. Posters will not be stored or sent to the authors after the Conference.

### Poster Sessions

| | |
|---|---|
| Saturday, June 25, 2022 (Session 1, Anthropological Genetics) and FSA | 11:35 to 13:30 |
| Sunday, June 26, 2022 (Session 2, Forensic Genetics) | 11:35 to 13:30 |
| Monday, June 27, 2022 (Session 3, Individualized Medicine) | 11:35 to 13:30 |

Presenters are required to be present by their poster for the discussion and to answer questions.

## Program Changes





Organizers assume no liability for any changes in the program due to external or unforeseen circumstances. For any program changes please check the updated Conference Program (https://isabs.hr/doc/12_ISABS_Conference_Program.pdf) or ask at the Registration desk.

**Official language** of the conference is English. No translation will be provided.

**Slide and PowerPoint Preview Room** will be available to all presenters.

## Info Center

Info Center will be available at the registration desk. Service Center provides photocopying, typing, and computer printouts at cost.

## Smoking Policy

The 12th ISABS Conference on Forensic Genetics and Molecular Medicine is officially declared as a "Non-smoking-Conference".

## Special requirements

Registrants with special requirements for physical communication and dietary requirements should contact technical organizer in advance: isabs@spektar-holidays.hr

## Conference staff

If you should have any questions, the conference staff will be pleased to help you. It will be easy to recognize them by the special name badge they will be wearing.

# General Information

## COVID-19 RELATED INFORMATION

ISABS will be following and monitoring official requirements and providing guidance and updates as they become available. We are committed to a safe and enjoyable event for all our attendees.
For current information please check the official website of the Ministry of the Interior of the Republic of Croatia.
It is your responsibility to be timely informed about information regarding the conditions of entry into the Republic of Croatia considering current COVID-19 pandemic.

## GSM OPERATORS

Currently, there are five GSM operators offering the GSM service in Croatia.
T-Mobile operating under +385 98 xxxxxx and +385 99 xxxxxx.
A1 operating under +385 91 xxxxxx.
TELE2 operating under +385 95 xxxxxx.
Tomato operating under +385 92 xxxxxx.
Bonbon operating under +385 97 xxxxxx.

Please contact your local GSM operator to check the availability and the costs of roaming services.

## USEFUL TELEPHONE NUMBERS

Country code: (+/00) 385
Area code (Dubrovnik): (0)20
Police: 192
Fire-fighting center: 193
Emergency: 194
Time check: 18095
Information local calls: 18981
General information service: 18981
Information national and international calls: 11802
Wake-up calls: 18100
Roadside vehicle assistance: 1987
Weather forecast: 18166

## OPENING HOURS

**Bank and Post Office Hours** are usually opened from 8:00 to 19:00, Monday through Friday and from 8:00 - 12:00 on Saturdays.

**Non-Governmental offices** work from 8:30 to 17:00, Monday to Friday.

Most **shops, grocery and department stores** are open non-stop, from 6:00 or 7:30 to 19.30 or 20:00.





**Restaurants:** Most restaurants in Dubrovnik are open from 8:00 – 23:00. Service charges are included in the price, unless explicitly mentioned otherwise, but an additional tip of 5 to 10 percent is expected. Some restaurants may have a cover charge.

## CURRENCY & EXCHANGE

The basic Croatian currency unit is the Kuna (HRK), made up of 100 Lipa. Foreign currency can be exchanged for local money at banks, post offices, and exchange offices, according to the valid rates of exchange. Visit the exchange office on the Web for valid exchange rates.

**Cash Machines:** ATMs accepting all major bank cards and credit cards are located at numerous sites in Dubrovnik.

## CREDIT CARDS

All major credit cards are normally accepted throughout Croatia, as advertised at points of sale, such as: American Express, Diners Club, Euro card/Master card, Visa, JCB, and Eurocheques. Traveler's Cheques are also accepted.

## TAX FREE

Foreigners can claim a sales tax refund within one year for purchased goods. Don't forget to ask the salesman to fill out the tax refund form when purchasing goods.

## MISCELLANEOUS

**Electricity Supply:** 220-240 V, 50 Hz.

**Water:** Tap water is drinkable in all parts of Croatia.

**Travel and Health Insurance:** Participants are advised to make their own arrangements pertinent to health and travel. By registering for the 12th ISABS Conference on Forensic and Anthropological Genetics and Mayo Clinic Lectures in Individualized Medicine, participants agree that neither the organizers and its agents nor the sponsors and exhibitors nor the Hotel Dubrovnik Palace assume any liability whatsoever.

**Taxi:** Numerous taxi stands are located throughout Dubrovnik city center and in front of hotels. Hotel staff will be glad to help you.

**Hotel Information:** Hotel Dubrovnik Palace nestles on the scenic seafront between a pine forest and the turquoise coastal waters of the lush Lapad peninsula. Hotel address: Masarykov put 20, 20000 Dubrovnik.

# INVITED SPEAKERS

## *Nobel Laureate Lectures:*

**Aaron Ciechanover** (Nobel Prize in Chemistry 2004; Technion – Israel Institute of Technology, Haifa, Israel): The road for cure and prevention of a pandemic is strewn with bioethical issues: lessons from the COVID-19 pandemic

**Sir Richard John Roberts** (Nobel Prize in medicine in Physiology and Medicine 1993; Northeastern University, Boston, MA, USA & New England Biolabs, Ipswich, MA, USA): Combating hunger and climate change with biotechnology

**Thomas Südhof** (Nobel Prize in Physiology and Medicine 2013; University of Stanford, Palo Alto, CA, USA): Towards an understanding of Alzheimer's disease as a synaptic disorder

## *Mayo Clinic Lectures in Individualized Medicine and Short Course on Epigenomics Program:*

**Julie G. Allickson** (Mayo Clinic, Rochester, MN, USA): Building the ecosystem of regenerative medicine in the Mayo Clinic enterprise

**Veronique Belzil** (Mayo Clinic, Jacksonville, FL, USA): Epigenomics in medicine: Epigenetic basis of human disease—neurodegenerative diseases; Single-cell profiling of the human M1 and DLPFC in ALS and FTLD

**Moritz Binder** (Mayo Clinic, Rochester, MN, USA): Epigenetics in the lab: Bioinformatic analysis of epigenomic data

**John "Al" Copland** (Mayo Clinic, Jacksonville, FL, USA): Therapeutic targeting of the FOXO3 cis-regulome in anaplastic thyroid cancer

**Julie M. Cunningham** (Mayo Clinic, Rochester, MN, USA): Molecular basis of epigenetic control: Epigenetic regulation by post-synthetic DNA and RNA modifications

**Henry A. Erlich (**Children's Hospital Oakland Research Institute, Oakland, CA, USA): Non-invasive prenatal testing for the beta-hemoglobinopathies using next-generation sequencing and probe capture

**William A. Faubion** (Mayo Clinic, Rochester, MN, USA): Epigenomics of inflammatory bowel disease

**Mark A. Frye** (Mayo Clinic, Rochester, MN, USA): Bipolar disorder from a multiomic perspective

**Alexandre Gaspar Maia** (Mayo Clinic, Rochester, MN, USA): Epigenetic states and inheritance: Cellular reprogramming; Single-cell multiomic analysis to identify cell-specific transcriptional dependencies in cancer and COVID-19 inflammatory response

**Struan F.A. Grant** (Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA): 3D genomic strategies to understand complex trait genetic architecture

**Feda Hamdan** (Mayo Clinic, Rochester, MN, USA): Molecular basis of epigenetic control: Chromatin architecture and nuclear organization

**Haojie Huang** (Mayo Clinic, Rochester, MN, USA): Molecular basis of epigenetic control: Epigenetic regulation by the RNA world; Massive epigenetic reprogramming triggered by a single genetic alteration

**Tae Hyun Hwang (**Mayo Clinic, Jacksonville, Florida) Machine learning- and AI-driven approaches to dissect tumor immune microenvironment using digital pathology and spatial transcriptome for novel immune and cellular therapy development in gastrointestinal cancer

**Steven A. Johnsen** (Robert Bosch Center for Tumor Diseases, Stuttgart, Germany): Dynamic enhancer-promoter interactions mediate chemoresistance in PDAC

**Purna Kashyap** (Mayo Clinic, Rochester, MN, USA): Multi-omics to mechanisms: The road to microbiome-driven precision medicine

**Adrijana Kekić** (Pharmacy Clinical Practice, Mayo Clinic Arizona, Phoenix, AZ, USA): Pharmacogenomics in clinical practice: Lessons from the Individualized Medicine Clinic

**Manolis Kellis** (Massachusetts Institute of Technology, The Broad Institute, Cambridge, MA, USA): From genomics to therapeutics: Single-cell dissection and manipulation of disease circuitry

**Gordan Lauc** (University of Zagreb and Genos, Ltd., Zagreb, Croatia): Glycans as biomarkers and functional effectors in cardiometabolic diseases

**Konstantinos N. Lazaridis** (Mayo Clinic, Center for Individualized Medicine, Rochester, MN, USA): Transforming clinical practice through individualized medicine today and tomorrow

**Zhenkun Lou** (Mayo Clinic, Rochester, MN, USA): Sensitizing cancer cells to DNA damage-inducing agents and anti-tumor immunity

**Weibo Luo** (University of Texas Southwestern Medical Center, Dallas, TX, USA): Crosstalk between hypoxia and epigenetics in breast cancer

**Ulrika Marklund** (Karolinska Institute, Solna, Sweden): Development of neuronal cell diversity in the gut revealed by single-cell transcriptomics

**Aleksey Matveyenko** (Mayo Clinic, Rochester, MN, USA): Circadian etiology of Type 2 diabetes

**Ian Maze** (Icahn School of Medicine at Mount Sinai, New York, NY, USA): Protein monoaminylation in brain: novel mechanisms of neural development, plasticity and disease

**Eva Morava-Kozicz** (Mayo Clinic, Rochester, MN, USA): Epigenomics in medicine: Epigenetic syndromes; Metabolic repair: A new approach to the therapy of abnormal glycosylation

**Tamas Ordog** (Mayo Clinic, Rochester, MN, USA): Epigenetic states and inheritance: Epigenetic inheritance and environmental epigenetics; Epigenetics in the lab: Epigenomic methodologies; Impact of disordered metabolism on 3D gene regulation in diabetes

**Mrinal Patnaik** (Mayo Clinic, Rochester, MN, USA): Epigenomics in medicine: Epigenetic basis of human disease - Cancer

**Dragan Primorac** (ISABS & St. Catherine Specialty Hospital, Zagreb, Croatia; Universities of Split, Osijek and Rijeka, Croatia; Eberly College of Science, The Pennsylvania State University, University Park, State College, PA, USA; The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Regiomed Kliniken, Coburg, Germany): Osteoarthritis: From molecular pathways to therapeutic advances

**Elias Puchner** (University of Minnesota, Minneapolis, MN, USA): Characterizing locus-specific chromatin structure and dynamics with correlative conventional and super-resolution imaging in living cells

**Keith D. Robertson** (Mayo Clinic, Rochester, MN, USA): Fundamental mechanisms underlying the regulation of gene transcription; Targeting epigenetic regulator mutations in kidney cancer

**Vijay Shah** (Mayo Clinic, Rochester, MN, USA): Multiomics reveals new pathobiologic targets for alcohol-associated hepatitis

**Natalia Tretyakova** (University of Minnesota, Minneapolis, MN, USA): Regulation and deregulation of DNA epigenetic marks

**Liewei Wang** (Mayo Clinic, Rochester, MN, USA): Cancer pharmacogenomics: discovery and translation

**Zong Wei** (Mayo Clinic, Scottsdale, AZ, USA): Molecular basis of epigenetic control:



Invited speakers

12th ISABS Conference on Forensic and Anthropological Genetics
and Mayo Clinic Lectures in Individualized Medicine
June 22-27, 2022, Dubrovnik, Croatia

Epigenetic regulation through histone proteins and chromatin-modifying complexes; Signal-dependent chromatin remodeling in metabolism and inflammation

**Yi Xing** (Children's Hospital of Philadelphia, Philadelphia, PA, USA): Long-read strategies to study the human transcriptome

### *Forensic Genetics and Anthropological Genetics Program:*

**Jack Ballantyne** (University of Central Florida and National Center of Forensic Science, Orlando, FL, USA): RNA sequencing applications in forensic genetics

**Joachim Burger** (University of Mainz, Mainz, Germany): News from the Neolithic

**Angel Carracedo** (University of Santiago de Compostela, Santiago de Compostela, Spain): Impact of omics on forensics

**Wolfgang Haak** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): Population genetics, kinship practices and social organization in prehistoric societies of Europe

**Mateja Hajdinjak (**The Francis Crick Institute, London, UK): Stone Age encounters: Neanderthal ancestry of early humans in Eurasia

**Mitchell Holland** (Pennsylvania State University, State College, PA, USA): A forensic genomics approach for the identification of Sister Marija Krucifiksa Kozulić of Rijeka

**Jodi Irwin** (Federal Bureau of Investigation - FBI, Quantico, VA, USA): Recovery of nuclear DNA from hair shafts associated with the Romanov family

**Mattias Jakobsson** (University of Uppsala, Uppsala, Sweden): Archaic and modern humans in island Southeast Asia

**Janet Kelso** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): The functional impacts of gene flow between archaic and modern humans

**Johannes Krause** (Max Planck Institute for the Science of Human History, Jena, Germany): The genetic history and origin of the Black Death

**Charla Marshall** (Armed Forces DNA Identification Laboratory, Dover, DE, USA): Capture and MPS for human identification

**Tomislav Maričić** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): Genome editing, stem cells and Neanderthals

**Mait Metspalu** (University of Tartu and Estonian Biocenter, Tartu, Estonia): The genesis of the genetic landscape of northeast Europeans

**Matthias Meyer** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): In the absence of skeletal remains: studying ancient human DNA preservation in sediment

**Connie Mulligan** (University of Florida, Gainesville, FL, USA): Epigenetic signatures of psychosocial stress and trauma

**Eskeatnaf Mulugeta** (Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands): Single-cell omics for forensic usage

**Walther Parson** (Medical University of Innsbruck, Innsbruck, Austria): Solving the mystery of Kaspar Hauser

**Mechthild Prinz** (John Jay College of Criminal Justice, New York, NY, USA): After more than 20 years: Update on the DNA identification of 9/11 victims

**Antti Sajantila** (University of Helsinki, Helsinki, Finland): Investigation of human persistent DNA viruses for use in archaeology and forensics

**Anne Stone** (Arizona State University, Tempe, AZ, USA): The origins of Hansen's disease (leprosy)

**Mark Stoneking** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): Genes, culture, and human evolution

**Andreas Tillmar** (University of Linköping, Linköping, Sweden): Inferring relationships from whole-genome sequences - A forensic perspective

**Athina Vidaki** (Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands): Developments towards personalized epigenomic profiling in forensics

**Hugo Zeberg** (Karolinska Institute, Solna, Sweden): Neandertal gene variants and COVID-19





### *Special Plenary Lectures*

**Garry Kasparov** (Office of Garry Kasparov, New York, New York, USA): Deep thinking: Where machine intelligence ends and human creativity begins

**Greger Larson** (University of Oxford, Oxford, UK): The origins, dispersal, and pathogen history of chickens

### *Joint Event ISABS and Ministry of the Interior - Crime Scene Investigation Training Course*

Mystery on the ship —Investigation of the water-related crime scene

### Moderators:

**Henry Lee** (College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Henry C. Lee Institute, West Haven, CT, USA)

**Dragan Primorac** (ISABS & St. Catherine Specialty Hospital, Zagreb, Croatia; Universities of Split, Osijek and Rijeka, Croatia; Eberly College of Science, The Pennsylvania State University, University Park, State College, PA, USA; The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Regiomed Kliniken, Coburg, Germany)

**Andrea Ledić** (Forensic Science Centre „Ivan Vučetić", Zagreb, Croatia)

**Šimun Anđelinović** (University Hospital Center, Split, Croatia)

**Peter Ausili** (Chair, AAFS International Affairs Committee, AAFS, Colorado Springs, CO, USA)

**Andrea Zaferes** (Lifeguard Systems Ink., Shokan, NY, USA)

### Topics:

- Homicide, suicide, and accident investigation of the water-related crime scene
- Narcotics smuggling on ship

### *Interdisciplinary Session of American Academy of Forensic Sciences and ISABS*

**Carl R. McClary** (Past President, AAFS, Colorado Springs, CO, USA): Standards development and the progress of the Academy Standards Board (ASB) development organization

**Peter Ausili** (Chair, AAFS International Affairs Committee, AAFS, Colorado Springs, CO, USA): Fentanyl: History, abuse, danger

**Laura Fulginiti** (AAFS President, AAFS, Colorado Springs, CO, USA): Collaboration to effect identification for unknown individuals recovered in a forensic context

**Henry Lee** (College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Henry C. Lee Institute, West Haven, CT, USA): The past, current, and future of forensics in the US. Crime Scene Training

**Damir Primorac** (Faculty of Law, University of Mostar, Mostar, Bosnia and Herzegovina; University Department of Forensic Sciences, University of Split, Split, Croatia), Andrej Bozhinovski (Faculty of Law, University of Zagreb, Zagreb, Croatia) and Lucija Sokanović (Faculty of Law, University of Split, Split, Croatia): Correcting miscarriages of justice - Innocence Project of Croatia

**Victor W. Weedn** (District of Columbia Office of the Chief Medical Examiner, Washington, DC, USA; George Washington University, Washington, D.C., USA): Arrest-related

death by prone restraint cardiac arrest
**Andrea Zaferes** (Lifeguard Systems Ink., Shokan, NY, USA): Aquatic death and dive accidents

### *IRI 2 Workshop: Osteoarthritis: A personalized approach to diagnosis and treatment*

**Dejan Blažević** (Clinical hospital for traumatology, Clinical hospital center "Sestre Milosrdnice", Zagreb, Croatia)
**Igor Borić** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Petar Brlek** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Ana Cindrić** (Genos Ltd., Zagreb, Croatia)
**Borut Čeh** (Bia Separations CRO and Labena Ltd. Ljubljana, Slovenia)
**Martin Čemerin** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Fabijan Čukelj** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Helena Deriš** (Genos Ltd., Zagreb, Croatia)
**Tomislav Dujmović** (Clinical Hospital Merkur, Zagreb, Croatia)
**Damir Hudetz** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Željko Jeleč** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Marina Kristek** (Something Different Consulting Ltd., Zagreb, Croatia)
**Gordan Lauc** (University of Zagreb and Genos, Ltd., Zagreb, Croatia)
**Vid Matišić (**St. Catherine Specialty Hospital, Zagreb, Croatia)
**Vilim Molnar** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Eduard Stjepan Pavelić** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Uršula Prosenc Zmrzljak** (Bia Separations CRO and Labena Ltd. Ljubljana, Slovenia)
**Dragan Primorac** (ISABS & St. Catherine Specialty Hospital, Zagreb, Croatia; Universities of Split, Osijek and Rijeka, Croatia; Eberly College of Science, The Pennsylvania State University, University Park, State College, PA, USA; The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Regiomed Kliniken, Coburg, Germany)
**Eduard Rod** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Srećko Sabalić** (Clinical hospital for traumatology, Clinical hospital center "Sestre Milosrdnice", Zagreb, Croatia)
**Bine Stančič** (Labena Ltd., Ljubljana, Slovenia)
**Neven Starčević** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Mario Staresinić** (Clinical Hospital Merkur, Zagreb, Croatia)
**Vedrana Škaro** (Genos Ltd., Zagreb, Croatia).
**Saša Šterpin** (Bia Separations CRO and Labena Ltd., Ljubljana, Slovenia)
**Josip Štivičić** (Clinical Hospital Merkur, Zagreb, Croatia)
**Kristijan Vrdoljak** (St. Catherine Specialty Hospital, Zagreb, Croatia)
**Dinko Vidović** (Clinical hospital for traumatology, Clinical hospital center "Sestre Milosrdnice", Zagreb, Croatia)
**Marija Zekušić** (Clinical hospital for traumatology, Clinical hospital center "Sestre Milosrdnice", Zagreb, Croatia)





### *Joint Session: ISABS, St. Catherine Hospital, Regiomed Kliniken, Germany, University of Split, Croatia*

**Johannes Brachmann** (Medical School REGIOMED, Coburg, Germany; University of Split Medical School, Split, Croatia): Sudden cardiac death - A new insight into potentially fatal genetic markers
**Georg Breuer** (Medical School REGIOMED, Germany; University of Split Medical School, Split, Croatia): The pathway to personalized anesthesiology
**Katarina Vukojević (**University of Split Medical School, Split, Croatia; Medical School REGIOMED, Coburg, Germany): Involvement of epithelial to mesenchymal transition factors during the human eye embryogenesis and tumorigenesis

### *Bioanthropology and global health in the times of crisis*

**Luka Bočkor** (Institute for Anthropological Research, Zagreb, Croatia): Integrative approaches to global health
**Aleksandra Buha Đorđević** (Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia): Toxicology in the context of global health
**Noël Cameron** (School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom): Child growth and armed conflict
**Miran Čoklo** (Institute for Anthropological Research, Zagreb, Croatia): Unravelling Data for Rapid Evidence-Based Response to COVID-19 – INANTRO experience from the unCoVer project
**Vlatka Čubrić-Čurik** (University of Zagreb Faculty of Agriculture, Zagreb, Croatia): Domestication of cattle: current status and ancient DNA perspectives
**Ino Čurik** (University of Zagreb Faculty of Agriculture, Zagreb, Croatia): Runs of homozygosity as an important concept in population genomics
**Struan F.A. Grant** (Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA) Implicating effector genes at COVID-19 GWAS loci in disease-relevant immune cell types
**Florin Grigorescu** (Institut de Convergences Migration – Collège de France, Paris, France): Gender differences and genetic geographical variation in the Mediterranean area of COVID-19 disease in relation with infertility – output of European MEDIGENE program
**Damir Marjanović** (International Burch University, Sarajevo, Bosnia and Herzegovina and Institute for Anthropological Research, Zagreb, Croatia): SARS-CoV-2 among Sarajevo inhabitants - the pre-vaccination period
**Mait Metspalu** (University of Tartu and Estonian Biocenter, Tartu, Estonia): Bringing genetics to the medical system - Estonian experience
**Saša Missoni** (Institute for Anthropological Research, Zagreb, Croatia): Anthropological research on Croatian islands – CRIBS birth cohort
**Jelena Šarac** (Institute for Anthropological Research, Zagreb, Croatia): Mediterranean diet adherence and its association with biological markers and health in Dalmatia
**Serena Tucci** (Yale University, New Haven, CT, USA): The genetic legacy of archaic hominin admixture
**John E. Vena** (Medical University of South Carolina, SC, USA): Obesity in childhood related to maternal factors during Pregnancy in the ECHO-FGS Cohort Study
**Maria Zafiropolou** (European Commission, Expert in the Healthcare and Social Sector): Regional Sensitivity Barometer: Studying the effects of the pandemic on development

### *Selected Lectures*

**Irena Abramović** (School of Medicine, University of Zagreb, Zagreb, Croatia): MiR-182-5p and MiR-375-3p in blood plasma as biomarkers for prostate cancer

**Sree Kanthaswamy** (Arizona State University, Tempe, AZ, USA): Expanded CODIS STR allele frequencies - evidence for the irrelevance of race-based DNA databases

**Dorian Laslo** (Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia): Enrichment of rare variants in genes involved in mitochondrial metabolism in patients with early-onset or familial Parkinson's disease

**Sasho Risteski** (Faculty of Medicine, Ss. Cyril and Methodius University in Skopje; Institute of Forensic Medicine, Criminalistics and Medical Deontology; Faculty of Natural Sciences and Mathematics, Institute of Biology, Skopje, North Macedonia): Development and validation of a new multiplex methylation-specific PCR assay for forensic body fluid identification

**Lana Salihefendić** (Alea Genetic Center and International Burch University, Sarajevo, Bosnia and Herzegovina): The effect of host genetics on COVID-19 susceptibility and severity: A study of 16 coding genes on a subset of Bosnian-Herzegovinian patients

**Stefanie Scheiper-Welling** (University Hospital Frankfurt, Goethe University Frankfurt, Institute of Legal Medicine, Frankfurt, Germany): Molecular Autopsy: Identification, classification and reporting of sequence variations in young sudden unexpected death victims and affected families

**Aleksandar Vojta** (Faculty of Science, University of Zagreb, Zagreb, Croatia): Cell line models with stably integrated CRISPR/DCAS9 fusions for studying the epigenetics of IgG glycosylation

**Nikola Vuković** (Uppsala University, Evolutionsbiologiskt Centrum EBC, Uppsala, Sweden): West country story - a detailed investigation of neolithic & bronze age individuals from south-western England

### *YIA Lectures*

**Elena Essel** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): Non-destructive extraction of ancient DNA from bone and tooth artifacts

**Karlo Miškec** (Faculty of Science, University of Zagreb, Zagreb, Croatia): Functional validation of GWAS hits associated with IgG glycosylation using CRISPR/CAS9 transient expression system

**Amira Nabil** (Medical Research Institute, Alexandria University, Egypt): Molecular characterization of oculoauriculovertebral spectrum in a group of Egyptian patients

**Vid Matišić** (St. Catherine Specialty Hospital, Zagreb, Croatia): A comprehensive pharmacogenomic multi-gene panel analysis in clinical practice, experience from St. Catherine hospital

**Rachelle Turiello** (University of Virginia, Charlottesville, VA, USA): A centrifugal microfluidic solution for the automation of forensic epigenetic sample preparation

### *Sponsor Lecture*

**Rea Dabelic** (10x Genomics, San Diego Metropolitan Area, CA, USA): High-resolution characterization of the immune system with Single Cell Immune Profiling





### *Satellite Joint Event 8th Croatian Human Genetics Conference & 1st Croatian Personalized and Precision Medicine Conference, June 21, Hotel Palace Dubrovnik*

**Darko Antičević** (St. Catherine Specialty Hospital, Zagreb, Croatia): Acetabular protrusion - underestimated but frequent deformity in patients with osteogenesis imperfecta

**Lidija Bach-Rojecky** (Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia): The role of pharmacogenomics in evaluating the efficacy and safety of drugs

**Damir Marjanović** (International Burch University, Sarajevo, Bosnia and Herzegovina and Institute for Anthropological Research, Zagreb, Croatia): Croatian Genetic Heritage: Renewed Y Chromosome Story Two Decades Later

**Natalija Novokmet** (Institute for Anthropological Research, Zagreb, Croatia): Influence of sub-structuring of statistical forensic parameters on genetic STR markers in South-Eastern Europe

**Ljubica Odak** (Children's Hospital Zagreb, Zagreb, Croatia): Exome sequencing and autism spectrum disorder- diagnostic challenges and future directions

**Denis Polančec** (Srebrnjak Children's Hospital, Zagreb, Croatia): Sex-related immunophenotype differences in human stromal vascular fraction from lipoaspirate and microfragmented lipoaspirate revealed by polychromatic flow cytometry

**Martina Smolić** (Faculty of Dental Medicine and Health and Faculty of Medicine, University of Osijek, Osijek Croatia): Evaluation of direct mechanisms of action of GLP-1 receptor agonists in cell-culture models of nonalcoholic and drug-induced fatty liver disease

**Katarina Vulin** (Children's Hospital Zagreb, Zagreb, Croatia): Chromosomal microarray in clinical diagnosis of cerebral palsy

# SCIENTIFIC PROGRAM



Please note that the program and speakers are subject to change.

## Tuesday, June 21

### Sattelite Joint Event: 8th Croatian Human Genetics Conference & 1st Croatian Personalized and Precision Medicine Conference

**Hall „Dubrava" (9th floor)**

**16:00 Opening (D. Primorac)**
**16:10 Darko Antičević** (St. Catherine Specialty Hospital, Zagreb, Croatia): Acetabular protrusion - underestimated but frequent deformity in patients with osteogenesis imperfecta
**16:30 Lidija Bach-Rojecky** (Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia): The role of pharmacogenomics in evaluating the efficacy and safety of drugs
**16:50 Martina Smolić** (Faculty of Medicine, and Faculty of Dental Medicine and Health, University of Osijek, Osijek Croatia): Evaluation of direct mechanisms of action of GLP-1 receptor agonists in cell-culture models of nonalcoholic and drug induced fatty liver disease
**17:10 Damir Marjanović** (International Burch University, Sarajevo, Bosnia and Herzegovina and Institute for Anthropological Research, Zagreb, Croatia): Croatian Genetic Heritage: Renewed Y Chromosome Story Two Decades Later

**17:30 Break**

**17:40 Natalija Novokmet** (Institute for Anthropological Research, Zagreb, Croatia): Influence of sub-structuring of statistical forensic parameters on genetic STR markers in South-Eastern Europe
**18:00 Denis Polančec** (Srebrnjak Children's Hospital, Zagreb, Croatia): Sex-related immunophenotype differences in human stromal vascular fraction from lipoaspirate and microfragmented lipoaspirate revealed by polychromatic flow cytometry
**18:20 Ljubica Odak** (Children's Hospital Zagreb, Zagreb, Croatia): Exome sequencing and autism spectrum disorder - diagnostic challenges and future directions
**18:40 Katarina Vulin** (Children's Hospital Zagreb, Zagreb, Croatia): Chromosomal microarray in clinical diagnosis of cerebral palsy
**19:00 Closing remarks (D. Primorac)**

**19:15 Adjourn**

# Wednesday, June 22

## Mayo Clinic Short Course on Epigenomics

**Hall „Dubrava" (9th floor)**

***Session 1: Fundamentals (K. D. Robertson, moderator)***

**8:30** **Keith D. Robertson** (Mayo Clinic, Rochester, MN, USA): Fundamental mechanisms underlying the regulation of gene transcription
**9:00** **Julie M. Cunningham** (Mayo Clinic, Rochester, MN, USA): Molecular basis of epigenetic control: Epigenetic regulation by post-synthetic DNA and RNA modifications
**9:30** **Zong Wei** (Mayo Clinic, Scottsdale, AZ, USA): Molecular basis of epigenetic control: Epigenetic regulation through histone proteins and chromatin-modifying complexes
**10:00** **Haojie Huang** (Mayo Clinic, Rochester, MN, USA): Molecular basis of epigenetic control: Epigenetic regulation by the RNA world
**10:30** **Feda Hamdan** (Mayo Clinic, Rochester, MN, USA): Molecular basis of epigenetic control: Chromatin architecture and nuclear organization
**11:00** **Tamas Ordog** (Mayo Clinic, Rochester, MN, USA): Epigenetic states and inheritance: epigenetic inheritance and environmental epigenetics

**11:30** **Lunch Break**

***Session 2: Applications (K. D. Robertson, moderator)***

**12:30** **Alexandre Gaspar-Maia** (Mayo Clinic, Rochester, MN, USA): Epigenetic states and inheritance: Cellular reprogramming
**13:00** **Tamas Ordog** (Mayo Clinic, Rochester, MN, USA): Epigenetics in the lab: Epigenomic methodologies
**13:30** **Moritz Binder** (Mayo Clinic, Rochester, MN, USA): Epigenetics in the lab: Bioinformatic analysis of epigenomic data
**14:00** **Eva Morava-Kozicz** (Mayo Clinic, Rochester, MN, USA): Epigenomics in medicine: Epigenetic syndromes
**14:30** **Mrinal Patnaik** (Mayo Clinic, Rochester, MN, USA): Epigenomics in medicine: Epigenetic basis of human disease - Cancer
**15:00** **Veronique Belzil** (Mayo Clinic, Jacksonville, FL, USA): Epigenomics in medicine: Epigenetic basis of human disease - neurodegenerative diseases

***Sponsor lecture***

**15:30** **Rea Dabelic** (10x Genomics, San Diego Metropolitan Area, CA, USA): High resolution characterization of the immune system with Single Cell Immune Profiling

**15:50** **Adjourn**





***Interdisciplinary Session of American Academy of Forensic Sciences and ISABS 2022***

**Hall „Mare IV" (10th floor)**

**12:30** **Carl R. McClary** (Past President, AAFS, Colorado Springs, CO, USA): Standards development and the progress of the Academy Standards Board (ASB) development organization
**12.50** **Peter Ausili** (Chair, AAFS International Affairs Committee, AAFS, Colorado Springs, CO, USA): Fentanyl: History, abuse, danger
**13:10** **Laura Fulginiti** (AAFS President, AAFS, Colorado Springs, CO, USA): Collaboration to effect identification for unknown individuals recovered in a forensic context
**13:30** **Henry Lee** (College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Henry C. Lee Institute, West Haven, CT, USA): The past, current, and future of forensics in the US. Crime Scene Training
**13:50** **Damir Primorac** (Faculty of Law, University of Mostar, Mostar, Bosnia and Herzegovina; University Department of Forensic Sciences, University of Split, Split, Croatia), **Andrej Bozhinovski** (Faculty of Law University of Zagreb, Zagreb, Croatia) and **Lucija Sokanović** (Faculty of Law, University of Split, Split, Croatia): Correcting miscarriages of justice - Innocence Project of Croatia
**14:10** **Andrea Zaferes** (Lifeguard Systems Ink., Shokan, NY, USA): Aquatic death and dive accidents
**14:30** **Victor W. Weedn** (District of Columbia Office of the Chief Medical Examiner, Washington, D.C., USA; George Washington University, Washington, D.C., USA): Arrest-related death by prone restraint cardiac arrest

***Joint Event - ISABS and Ministry of the Interior's Training Course (by invitation), on the boat***

***Crime Scene Investigation Training Course: Mystery on the ship - Investigation of the water-related crime scene***

**16:00** **Henry Lee** (College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Henry C. Lee Institute, West Haven, CT, USA) and **Dragan Primorac** (ISABS & St. Catherine Specialty Hospital, Zagreb, Croatia; Universities of Split, Osijek and Rijeka, Croatia; Eberly College of Science, The Pennsylvania State University, University Park, State College, PA, USA; The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Regiomed Kliniken, Coburg, Germany) - Briefing and organization. Students will divide into 4 groups.
**16:30** **Šimun Anđelinović** (University Hospital Center, Split, Croatia), **Andrea Ledić** (Forensic Science Centre „Ivan Vučetić", Zagreb, Croatia), **Peter Ausili** (AAFS International Affairs Committee, AAFS, Colorado Springs, CO, USA), **Andrea Zaferes** (Lifeguard Systems Ink., Shokan, NY, USA) - Crime scene rotation and work on the quizzes. Rotations will be around four crime scenes in the first hour (15 min. each scene).
**17:30** **Šimun Anđelinović** (University Hospital Center, Split, Croatia), **Andrea Ledić** (Forensic Science Centre „Ivan Vučetić", Zagreb, Croatia), **Peter Ausili** (AAFS International Affairs Committee, AAFS, Colorado Springs, CO, USA), **Andrea Zaferes** (Lifeguard Systems Ink., Shokan, NY, USA) - Crime scene teams will start search, processing and reconstruct the scene; one scene at a time (30 min for each scene). Students will observe the team process of the scene.

**19:30 Henry Lee** (College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Henry C. Lee Institute, West Haven, CT, USA) and **Dragan Primorac** (ISABS & St. Catherine Specialty Hospital, Zagreb, Croatia; Universities of Split, Osijek and Rijeka, Croatia; Eberly College of Science, The Pennsylvania State University, University Park, State College, PA, USA; The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Regiomed Kliniken, Coburg, Germany) - Workshop Summation. Questions and Answers (all instructors).





## Thursday, June 23

**9:00 Opening Ceremony, Hall „Mare I" (main hall, 10th floor)**

***Opening Session (D. Primorac, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**10:30 Julie G. Allickson** (Mayo Clinic, Rochester, MN, USA): Building the ecosystem of regenerative medicine in the Mayo Clinic enterprise
**11:05 Mark Stoneking** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): Genes, culture, and human evolution
**11:40 Angel Carracedo** (University of Santiago de Compostela, Santiago de Compostela, Spain): Impact of omics on forensics

**12:15 Break for Lunch**

**12:15** ***Joint Session: ISABS, St. Catherine Hospital, Regiomed Kliniken, Germany, University of Split, Croatia (by invitation)***

***Hall „Mare IV", 10th floor***

**Johannes Brachmann** (Medical School REGIOMED, Coburg, Germany; University of Split Medical School, Split, Croatia): Sudden cardiac death - A new insight into potentially fatal genetic markers
**Georg Breuer** (Medical School REGIOMED, Germany; University of Split Medical School, Croatia): The Pathway to personalized anesthesiology
**Katarina Vukojević** (University of Split Medical School, Split, Croatia; Medical School REGIOMED, Coburg, Germany): Involvement of epithelial to mesenchymal transition factors during the human eye embryogenesis and tumorigenesis

***Oral session 1: Integrated; Genetic Variation and its Implications for Human Disease, Forensics and Anthropology (S. Vuk-Pavlović, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**14:00 Manolis Kellis** (Massachusetts Institute of Technology, The Broad Institute, Cambridge, MA, USA): From genomics to therapeutics: Single-cell dissection and manipulation of disease circuitry
**14:25 Yi Xing** (Children's Hospital of Philadelphia, Philadelphia, PA, USA): Long-read strategies to study the human transcriptome
**14:50 Struan F. A. Grant** (Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA): 3D genomic strategies to understand complex trait genetic architecture
**15:15 Adrijana Kekić** (Pharmacy Clinical Practice, Mayo Clinic Arizona, Phoenix, AZ, USA): Pharmacogenomics in clinical practice: Lessons from the Individualized Medicine Clinic

**15:25 Break**

**15:40 Liewei Wang** (Mayo Clinic, Rochester, MN, USA): Cancer pharmacogenomics: discovery and translation

**16:05 Andreas Tillmar** (University of Linköping, Linköping, Sweden): Inferring relationships from whole-genome sequences - A forensic perspective
**16:30 Janet Kelso** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): The functional impacts of gene flow between archaic and modern humans
**16:55 Stefanie Scheiper-Welling** (University Hospital Frankfurt, Goethe University Frankfurt, Institute of Legal Medicine, Frankfurt, Germany): Molecular Autopsy: Identification, classification and reporting of sequence variations in young sudden unexpected death victims and affected families

**17:05 Adjourn**

***Bioanthropology and global health in the times of crisis (session parallel to the main program)***

***Hall „Mare IV" (10th floor)***

**15:25 Welcome address** (S. Missoni)
**15:30 Noël Cameron** (School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom): Child growth and armed conflict
**15:50 John E. Vena** (Medical University of South Carolina, SC, USA): Obesity in childhood related to maternal factors during pregnancy in the ECHO-FGS cohort study
**16:10 Florin Grigorescu** (Institut de Convergences Migration – Collège de France, Paris, France): Gender differences and genetic geographical variation in the Mediterranean area of COVID-19 disease in relation with infertility – output of European MEDIGENE program
**16:30 Saša Missoni** (Institute for Anthropological Research, Zagreb, Croatia): Anthropological research on Croatian islands – CRIBS birth cohort
**16:50 Jelena Šarac** (Institute for Anthropological Research, Zagreb, Croatia): Mediterranean diet adherence and its association with biological markers and health in Dalmatia





# Friday, June 24

***Bioanthropology and global health in the times of crisis (session parallel to the main program)***

***Hall „Mare IV", 10th floor***

**8:30 Struan F. A. Grant** (Children's Hospital of Philadelphia Research Institute, USA): Implicating effector genes at COVID-19 GWAS loci in disease-relevant immune cell types
**8:50 Mait Metspalu** (University of Tartu and Estonian Biocenter, Tartu, Estonia): Bringing genetics to the medical system - Estonian experience
**9:10 Damir Marjanović** (International Burch University, Sarajevo, Bosnia and Herzegovina and Institute for Anthropological Research, Zagreb, Croatia): SARS-CoV 2 among Sarajevo inhabitants - the pre-vaccination period
**9:30 Serena Tucci** (Yale University, Department of Anthropology, New Haven, CT, USA): The genetic legacy of archaic hominin admixture

**9:50 Break**

**10:05 Vlatka Čubrić-Čurik** (University of Zagreb, Faculty of Agriculture, Zagreb, Croatia): Domestication of cattle: current status and ancient DNA perspectives
**10:25 Ino Čurik** (University of Zagreb, Faculty of Agriculture, Zagreb, Croatia): Runs of homozygosity as an important concept in population genomics
**10:45 Aleksandra Buha Đorđević** (Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia): Toxicology in the context of global health
**11:05 Maria Zafiropolou** (European Commission, Expert in the Healthcare and Social Sector): Regional Sensitivity Barometer: Studying the effects of the pandemic on development
**11:25 Luka Bočkor** (Institute for Anthropological Research, Zagreb, Croatia): Integrative a pproaches to global health
**11:45 Miran Čoklo** (Institute for Anthropological Research, Zagreb, Croatia): Unravelling Data for Rapid Evidence-Based Response to COVID-19 – INANTRO experience from the unCoVer project

***Oral session 2: Integrated; High-definition OMICS (T. Kozicz, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**8:30 Tae Hyun Hwang** (Mayo Clinic, Jacksonville, FL, USA): Machine learning- and AI-driven approaches to dissect tumor immune microenvironment using digital pathology and spatial transcriptome for novel immune and cellular therapy development in gastrointestinal cancer
**8:55 Ulrika Marklund** (Karolinska Institute, Solna, Sweden): Development of neuronal cell diversity in the gut revealed by single-cell transcriptomics
**9:20 Alexandre Gaspar Maia** (Mayo Clinic, Rochester, MN, USA): Single-cell multiomic analysis to identify cell-specific transcriptional dependencies in cancer and COVID-19 inflammatory response
**9:45 Elias Puchner** (University of Minnesota, Minneapolis, MN, USA): Characterizing locus-specific chromatin structure and dynamics with correlative conventional and super-resolution imaging in living cells

**10:10 Break**

**10:25 Eskeatnaf Mulugeta** (Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands): Single-cell omics for forensic usage
**10:50 Tomislav Maričić** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): Genome editing, stem cells and Neanderthals
**11:15 Karlo Miškec** (Faculty of Science, University of Zagreb, Zagreb, Croatia): Functional validation of GWAS hits associated with IgG glycosylation using CRISPR/CAS9 transient expression system
**11:25 Vid Matišić** (St. Catherine Specialty Hospital, Zagreb, Croatia): A comprehensive pharmacogenomic multi-gene panel analysis in clinical practice, experience from St. Catherine hospital

**11:35 Break for Lunch**

***Nobel Laureate Session (D. Primorac, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**12:30 Aaron Ciechanover** (Technion – Israel Institute of Technology, Haifa, Israel): The road for cure and prevention of a pandemic is strewn with bioethical issues: lessons from the COVID-19 pandemic
**13:15 Sir Richard John Roberts** (Northeastern University, Boston, MA, USA & New England Biolabs, Ipswich, MA, USA): Combating hunger and climate change with biotechnology
**14:00 Thomas Südhof** (University of Stanford, Palo Alto, CA, USA): Towards an understanding of Alzheimer's disease as a synaptic disorder

**14:45 Break**

***Oral session 3: Individualized medicine; Omics of neurodegenerative and psychiatric diseases (M. Kellis, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**15:00 Ian Maze** (Icahn School of Medicine at Mount Sinai, New York, NY, USA): Protein monoaminylation in brain: Novel mechanisms of neural development, plasticity and disease
**15:25 Veronique Belzil** (Mayo Clinic, Jacksonville, FL, USA): Single-cell profiling of the human M1 and DLPFC in ALS and FTLD
**15:50 Mark A. Frye** (Mayo Clinic, Rochester, MN, USA): Bipolar disorder from a multiomic perspective

**16:15 Adjourn**

**18:00 *Nobel Spirit - televised session with Nobel Laureates; Croatian Radiotelevision (by invitation)***

**20:00 *Conference Banquet and Award Ceremony***





## Saturday, June 25

***Oral Session 4: Anthropological genetics (M. Stoneking, moderator) and FSA***

***Hall „Mare I" (main hall, 10th floor)***

**8:30 Matthias Meyer** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): In the absence of skeletal remains: studying ancient human DNA preservation in sediment
**8:55 Mateja Hajdinjak** (The Francis Crick Institute, London, UK): Stone Age encounters: Neanderthal ancestry of early humans in Eurasia
**9:20 Joachim Burger** (University of Mainz, Mainz, Germany): News from the Neolithic
**9:45 Elena Essel** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): Non-destructive extraction of ancient DNA from bone and tooth artifacts

**9:55 Break**

**10:10 Mattias Jakobsson** (University of Uppsala, Uppsala, Sweden): Archaic and modern humans in island Southeast Asia
**10:35 Wolfgang Haak** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany): Population genetics, kinship practices and social organization in prehistoric societies of Europe
**11:00 Mait Metspalu** (University of Tartu and Estonian Biocenter, Tartu, Estonia): The genesis of the genetic landscape of northeast Europeans
**11:25 Nikola Vuković** (Uppsala University, Evolutionsbiologiskt Centrum EBC, Uppsala, Sweden): West country story - a detailed investigation of Neolithic & Bronze age individuals from south-western England

**11:35 Break for Lunch**

**11:35 *Poster Session 1: Anthropological Genetics (M. Jakobsson, moderator)***

***Sunset Lounge (hotel lobby)***

**11:35 *IRI 2 Workshop: Osteoarthritis: A personalized approach to diagnosis and treatment (by invitation; IRI 2 partners only)***

***Hall „Mare IV", 10th floor***

***Oral Session 5: Individualized Medicine: Omics of Common and Rare Diseases (U. Marklund, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**13:30** **Henry A. Erlich** (Children's Hospital Oakland Research Institute, Oakland, CA, USA): Non-invasive prenatal testing for the beta-hemoglobinopathies using next-generation sequencing and probe capture
**13:55** **Gordan Lauc** (University of Zagreb & Genos, Ltd., Zagreb, Croatia): Glycans as biomarkers and functional effectors in cardiometabolic diseases
**14:20** **Eva Morava-Kozicz** (Mayo Clinic, Rochester, MN, USA): Metabolic repair: A new approach to the therapy of abnormal glycosylation
**14:45** **William A. Faubion** (Mayo Clinic, Rochester, MN, USA): Epigenomics of inflammatory bowel disease

**15:10** **Break**

**15:25** **Purna Kashyap** (Mayo Clinic, Rochester, MN, USA): Multi-omics to mechanisms: The road to microbiome-driven precision medicine
**15:50** **Zong Wei** (Mayo Clinic, Scottsdale, AZ, USA): Signal-dependent chromatin remodeling in metabolism and inflammation
**16:15** **Dragan Primorac** (ISABS, Croatia): Osteoarthritis: From molecular pathways to therapeutic advances
**16:40** **Dorian Laslo** (Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia): Enrichment of rare variants in genes involved in mitochondrial metabolism in patients with early-onset or familial Parkinson's disease
**16:50** **Amira Nabil** (Medical Research Institute, Alexandria University, Alexandria, Egypt): Molecular characterization of oculoauriculovertebral spectrum in a group of Egyptian patients

***Evening lecture 1 (M. Kayser, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**20:00** **Greger Larson** (University of Oxford, Oxford, UK): The origins, dispersal, and pathogen history of chickens





## Sunday, June 26

***Oral Session 6: Forensic Genetics (M. Kayser, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**8:30** **Walther Parson** (Medical University of Innsbruck, Innsbruck, Austria): Solving the mystery of Kaspar Hauser
**8:55** **Mitchell Holland** (Pennsylvania State University, State College, PA, USA): A forensic genomics approach for the identification of Sister Marija Krucifiksa Kozulić of Rijeka
**9:20** **Charla Marshall** (Armed Forces DNA Identification Laboratory, Dover, DE, USA): Capture and MPS for human identification
**9:45** **Sree Kanthaswamy** (Arizona State University, Tempe, AZ, USA): Expanded CODIS STR allele frequencies - evidence for the irrelevance of race-based DNA databases

**9:55** **Break**

**10:10** **Jodi Irwin** (Federal Bureau of Investigation (FBI), USA): Recovery of nuclear DNA from hair shafts associated with the Romanov family
**10:35** **Mechthild Prinz** (John Jay College of Criminal Justice, New York, NY, USA): After more than 20 years: Update on the DNA identification of 9/11 victims
**11:00** **Jack Ballantyne** (University of Central Florida and National Center of Forensic Science, Orlando, FL, USA): RNA sequencing applications in forensic genetics
**11:25** **Rachelle Turiello** (University of Virginia, Charlottesville, Virginia, USA): A centrifugal microfluidic solution for the automation of forensic epigenetic sample preparation

**11:35** **Break for Lunch**

**11:35** ***Poster Session 2: Forensic Genetics (M. Holland, moderator)***

***Sunset Lounge (hotel lobby)***

***Oral Session 7: Individualized Medicine; Cancer and Cancer Precursor "Omics" (E. Morava-Kozicz, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**13:30** **Natalia Tretyakova** (University of Minnesota, Minneapolis, MN, USA): Regulation and deregulation of DNA epigenetic marks
**13:55** **Haojie Huang** (Mayo Clinic, Rochester, MN, USA): Massive epigenetic reprogramming triggered by a single genetic alteration
**14:20** **Keith D. Robertson** (Mayo Clinic, Rochester, MN, USA): Targeting epigenetic regulator mutations in kidney cancer
**14:45** **Irena Abramović** (School of Medicine, University of Zagreb, Department of Medical Biology, Zagreb, Croatia): MiR-182-5p and MiR-375-3p in blood plasma as biomarkers for prostate cancer

**14:55** **Break**

**15:10** **Zhenkun Lou** (Mayo Clinic, Rochester, MN, USA): Sensitizing cancer cells to DNA damage-inducing agents and anti-tumor immunity
**15:35** **Steven A. Johnsen** (Robert Bosch Center for Tumor Diseases, Stuttgart, Germany): Dynamic enhancer-promoter interactions mediate chemoresistance in PDAC
**16:00** **John "Al" Copland** (Mayo Clinic, Jacksonville, FL, USA): Therapeutic targeting of the FOXO3 cis-regulome in anaplastic thyroid cancer

**16:25** **Adjourn**

***Evening lecture 2 (D. Primorac, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**20:00** **Garry Kasparov** (Office of Garry Kasparov, New York, New York, USA): Deep thinking: Where machine intelligence ends and human creativity begins





# Monday, June 27

***Oral Session 8: Integrated; Interdisciplinary Applications of Epigenomics (N. Tretyakova, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**8:30** **Vijay Shah** (Mayo Clinic, Rochester, MN, USA): Multiomics reveals new pathobiologic targets for alcohol-associated hepatitis
**8:55** **Aleksey Matveyenko** (Mayo Clinic, Rochester, MN, USA): Circadian etiology of Type 2 diabetes
**9:20** **Tamas Ordog** (Mayo Clinic, Rochester, MN, USA): Impact of disordered metabolism on 3D gene regulation in diabetes
**9:45** **Aleksandar Vojta** (Faculty of Science, University of Zagreb, Zagreb, Croatia): Cell line models with stably integrated CRISPR/DCAS9 fusions for studying the epigenetics of IgG glycosylation

**9:55** **Break**

**10:10** **Weibo Luo** (University of Texas, Southwestern Medical Center, Dallas, TX, USA): Crosstalk between hypoxia and epigenetics in breast cancer
**10:35** **Connie Mulligan** (University of Florida, Gainesville, FL, USA): Epigenetic signatures of psychosocial stress and trauma
**11:00** **Athina Vidaki** (Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands): Developments towards personalized epigenomic profiling in forensics
**11:25** **Sasho Risteski** (Faculty of Medicine, Ss. Cyril and Methodius University in Skopje; Institute of Forensic Medicine, Criminalistics and Medical Deontology; Faculty of Natural Sciences and Mathematics, Institute of Biology, Skopje, North Macedonia): Development and validation of a new multiplex methylation specific PCR assay for forensic body fluid identification

**11:35** **Break for Lunch**

**11:35** ***Poster Session 3: Individualized Medicine (V. Belzil, moderator)***

***Sunset Lounge (hotel lobby)***

***Special plenary session***

***Hall „Mare I" (main hall, 10th floor)***

**13:30** **Konstantinos N. Lazaridis** (Mayo Clinic, Center for Individualized Medicine, Rochester, MN, USA): Transforming clinical practice through individualized medicine today and tomorrow

***Oral Session 9: Integrated; COVID-19 and the Genetics of Pandemics (W. Haak, moderator)***

***Hall „Mare I" (main hall, 10th floor)***

**14:05** **Johannes Krause** (Max Planck Institute for the Science of Human History, Jena, Germany): The genetic history and origin of the Black Death
**14:30** **Anne Stone** (Arizona State University, Tempe, AZ, USA): The origins of Hansen's disease (leprosy)
**14:55** **Antti Sajantila** (University of Helsinki, Helsinki, Finland): Investigation of human persistent DNA viruses for use in archaeology and forensics

**15:20** **Break**

**15:35** **Hugo Zeberg** (Karolinska Institute, Solna, Sweden): Neandertal gene variants and COVID-19
**16:00** **Lana Salihefendić** (Alea Genetic Center and International Burch University, Sarajevo, Bosnia and Herzegovina): The effect of host genetics on covid-19 susceptibility and severity: A study of 16 coding genes on a subset of Bosnian-Herzegovinian patients

**16:15** **Closing remarks (D. Primorac)**

**16:25** **Adjourn**

# NOBEL LAUREATE SESSION

# THE ROAD TO CURE IS STREWED WITH BIOETHICAL ISSUES – PERSPECTIVES FROM THE COVID-19 PANDEMIC

**Aaron Ciechanover**

Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Bioethics and medical practice have always been intertwined, and the growing role technology plays in medicine has tightened the linkage. From the end-of-life (the time that surrounds death), euthanasia, and abortions all the way to genetic editing and the information about our future health embedded in our individual genomes – the road to development of therapeutic modalities has been strewed with bioethical blocks. The recent Pandemic has floated to the surface few, among them: (i) how to prioritize whom to ventilate; (ii) postponement of other burning global issues like climate change or treatment of patients with other maladies; (iii) the anti-vaccines movement; (iv) the infodemic – the pandemic of disinformation and misinformation; and last but not least, (v) the rise of discrimination and racism. Recognizing these problems is the first step to attempt solving them, though the solutions might be different in different nations, as historical, traditional, religious and national considerations always play a role in handling such sensitive issues.





# COMBATING HUNGER AND CLIMATE CHANGE WITH BIOTECHNOLOGY

**Richard J. Roberts**

CSO New England Biolabs, Ipswich, MA, USA

As the earth warms and its population rises there is an urgent need for technical solutions to tackle both. Fortunately, improvements in plant and agricultural techniques can be extremely helpful, both in improving crop yield and helping to mitigate CO2 and CH4 emissions and increasing yields. Already we have seen many ways in which biotechnology can improve the productivity of plants by incorporating pest resistance genes and improving their nutritional value. The main problem at the moment is the non-scientific disdain afforded to GMOs, by the so-called green movements. The political interference they have caused has led to ridiculously stiff regulations governing the introduction of GM crops. I will present examples of both current and potential improvements that are within reach. One thing is very clear. Europe will need these improved crops and the political influence of non-scientists needs to cease, if the whole world is to benefit from biotechnology in the way they benefitted from its application to vaccine production during the COVID-19 crisis.

# TOWARDS AN UNDERSTANDING OF ALZHEIMER'S DISEASE AS A SYNAPTIC DISORDER

**Thomas C. Südhof**

Howard Hughes Medical Institute and Dept. of Molecular Physiology, Stanford University Medical School, Stanford CA, USA

In many neurodegenerative disorders, including Alzheimer's Disease (AD), synapses are affected early during pathogenesis. Multiple mutations in APP and presenilin genes cause rare cases of familial AD, while the ApoE4 variant of the ApoE gene represents the strongest genetic risk factor for sporadic AD in the general population. APP and presenilin gene mutations are thought to induce AD pathogenesis by overproducing pathogenic Abeta variants, and ApoE4 is thought to influence Abeta clearance, but how Abeta might incite AD pathogenesis and how ApoE4 might predispose to AD pathogenesis remains incompletely understood. Moreover, compelling evidence implicates microglial and possibly astrocytic dysfunction in AD pathogenesis. We have taken a cell-biological approach to addressing these questions with a focus on synapses because of their prominent role in AD, recognizing that synapse impairments in AD may be primary results of the pathogenic process in AD, but could also be secondary to microglial dysfunction. We have examined how pathogenic APP mutations, chronic impairments of presenilin function, or ApoE4 may act on synapses, using human neurons trans-differentiated from ES and iPS cells as a model. Instead of searching for potential Abeta receptors (of which many have been reported) or studying the effects of ApoE4 on Abeta (which still remain unclear despite decades of study), we have examined the signaling processes associated with pathogenic mutant forms of APP, by inhibition of presenilins, or by ApoE. Our studies suggest that APP, presenilins, and ApoE4 dramatically affect synapse function, albeit in a differential manner, indicating that synapses may represent a common pathway for different genetic conditions promoting AD pathogenesis.



# ABSTRACTS OF INVITED LECTURES

# RNA SEQUENCING APPLICATIONS IN FORENSIC GENETICS

**Jack Ballantyne[1],** Cordula Haas[2], Erin Hanson[1]

[1]University of Central Florida, National Center for Forensic Science, Orlando, Florida, United States, [2]University of Zurich, Zurich Institute of Forensic Medicine, Zurich, Switzerland

The application of RNA profiling in forensic genetics has experienced tremendous growth and development in the past decade. The earliest studies, and main applications, have been applied to body fluid and tissue identification, using tissue-specific RNA transcripts and, principally, reverse transcription endpoint PCR and subsequent capillary electrophoretic (CE) separation. Several markers have been identified for the forensically most relevant body fluids and tissues and the method has been successfully used in casework. The introduction of Massively Parallel Sequencing (MPS) methodology has provided several benefits that continue to advance the field. Specifically, RNA sequencing permits a more quantitative nuanced approach to gene expression analysis compared to CE since transcripts are counted via the number of reads, resulting in a digital gene expression profile that is amenable to sophisticated statistical methods. Additionally, more targets can be tested in the same sample and RNA sequence variation within transcripts can be interrogated and used for high-resolution assignment of body fluids to donors in mixed in body fluid/tissue samples. This presentation will give an overview on forensic transcriptome analyses and applications, including whole transcriptome sequencing (WTS) as well as targeted MPS approaches. Using data from the authors' laboratories, detailed examples will include RNA biomarker selection, body fluid and organ tissue identification and, via the recent development of an improved high-resolution MPS assay, the assignment of DNA donors to body fluids via coding region SNPs. Recent development in other applications will be briefly mentioned including the potential for determination of the age of stains, the age of the donor, the post-mortem interval and to aid post-mortem death investigations.





# SINGLE-CELL PROFILING OF THE HUMAN M1 AND DLPFC IN ALS AND FTLD

Sebastian Pineda[1], Erica Cook[2], Hyeseung Lee[3], Brent Fitzwalter[3], Shahin Mohammadi[3], Luc Pregent[2], Bjorn Oskarsson[4], Jaimin Shah[4], Ronald Petersen[4], Neil Graff-Radford[4], Bradley Boeve[4], David Knopman[4], Keith Josephs[4], Michael DeTure[2], Melissa Murray[2], Dennis Dickson[2], Myriam Heiman[3], **Veronique Belzil[2],** Manolis Kellis[1]

[1]Department of Electrical Engineering and Computer Science, MIT, Cambridge, MA, USA, [2]Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, USA, [3]Broad Institute of MIT and Harvard, Cambridge, MA, USA, [4]Department of Neurology, Mayo Clinic, Jacksonville, Florida, USA [5]Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, USA

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are two devastating and fatal neurodegenerative conditions. While distinct, they share many clinical, genetic, and pathological characteristics, and both show selective vulnerability of layer 5b extratelencephalic-projecting cortical populations, including Betz cells in ALS and von Economo neurons (VENs) in FTLD. Here, we report the first high-resolution single-cell atlas of the human primary motor cortex (M1) and dorsolateral prefrontal cortex (DLPFC) and their transcriptional alterations in ALS and FTLD across 75 individuals, including 17 control samples and 58 sporadic and C9orf72-associated ALS and FTLD patient samples. We identify 47 transcriptionally distinct cellular subtypes including two Betz-cell subtypes, and we observe a previously unappreciated molecular similarity between Betz cells and VENs. Many of the dysregulated genes and pathways are shared across excitatory neurons, with Betz cells and VENs being the most transcriptionally affected. Our results suggest that transcriptional similarity between Betz cells and VENs underline the cell-specific vulnerability observed in ALS and FTLD, and explain their concomitant diagnoses.

# NEWS FROM THE NEOLITHIC

**Joachim Burger**

University of Mainz, Mainz, Germany

The transition from foraging to a sedentary and agricultural way of life is the decisive step in human history, without which most of the human populations living today would not exist. This breathtaking cultural transition began 11,000 years ago and has been called the "Neolithic Revolution". It has been researched for over 100 years, in the last 17 years also with the help of palaeogenetic data. Here I present our recent analysis of high-quality palaeogenomes from predominantly Neolithic contexts. For the first time, we have explicitly modeled the population history of late hunter-gatherers and early farmers. In this way, we have created a "demogenomic model" of human populations in southwest Asia and parts of Europe between 30,000 and 7,000 years ago. The study provides new insights into pre-Neolithic population dynamics during the Late Ice Age in Europe and Anatolia. It also clarifies basic demographic processes that led to the differentiation of early Neolithic populations (and present-day Western Mediterraneans). But what do we learn from this for the Neolithic itself?





# IMPACT OF OMICS ON FORENSICS

**Angel Carracedo**

Institute of Forensic Sciences, University of Santiago de Compostela, Santiago de Compostela, Spain

Genomics is revolutionizing many areas of forensic science at a similar extend as it is happening in clinical medicine and in a similar way as clinical genetics is moving towards genomics medicine; fforensic genetics is slowly transitioning into forensic genomics. The evolution of next generation or massively parallel sequencing is allowing the use of genomic, transcriptomic, and epigenomics to address various forensic questions that cannot be answered, or only in a limited way, using classical forensic genetics approaches. Forensic genetics has directly benefited of the advances in genomics and new applications as the determination of external physical characteristics, the geographic origin of samples or the estimation of the age from minute biological material are now possible. Human transcriptome data of different tissues generated are allowing the identification of RNA markers to determine the cellular source of crime scene samples. This is forensically relevant for reconstructing the course of events that may have happened at the scene of crime and to support the use of DNA at the activity level of evidence interpretation. Non-human genomic and transcriptomic are useful in different forensic contexts and forensic microbiome is now an important emerging field. Proteomics has also some important specific applications. Integration of data is challenging but key for the progress of the field. Forensic toxicology and forensic pathology are also benefiting of the advances in genomics with a special impact in the genetic diagnosis of cardiac sudden death and the so-called molecular autopsy is essential complement to classical autopsy.

# THERAPEUTIC TARGETING OF THE FOXO3 CIS-REGULOME IN ANAPLASTIC THYROID CANCER

**John "Al" Copland,** Tamas Ordog

Mayo Clinic, Cancer Biology Department, San Pablo Road, Jacksonville, Florida, USA

Anaplastic thyroid cancer (ATC) is the most aggressive and deadliest human cancer (median survival: 4 months). Metastatic ATC is uniformly fatal. ATC is a rare disease (800-1000 U.S. cases/year) that develops in less than 2% of differentiated thyroid cancers (DTCs). Most DTC patients have excellent prognosis in response to surgery, thyroid stimulating hormone suppression, radioiodine and sometimes radiation therapy; and even metastatic disease is often curable. Several mutations have been found to contribute to the striking transformation of DTCs to ATC. However, therapies based upon these mutations have only improved outcomes in specific cases. We previously found that FOXO3, a transcription factor (TF) with an established tumor suppressor function in DTCs, becomes an oncogene in ATC, reflecting increased nuclear retention arising from dysregulated AKT signaling. However, the spectrum of FOXO3 transcriptional targets and downstream pathways, and the epigenetic mechanisms underlying these effects remain unclear. Therefore, our goal is to identify the ATC-specific cis-regulome of FOXO3, discover the epigenetic mechanisms recruited by FOXO3 to regulate these target genes, and investigate the therapeutic utility of agents rationally selected based on their targets' involvement in FOXO3 actions and for their ability to kill ATC cells in vitro and in vivo. Our overall hypothesis is that due to increased DNA binding, FOXO3 becomes a master TF critical for establishing the ATC phenotype, making ATC cells specifically vulnerable to pharmacological agents targeting FOXO3-regulated mechanisms, genes and pathways. We are combining multi-omics with epigenetic pharmacology to define the ATC-specific FOXO3 cis-regulome, to investigate the role of FOXO3-induced chromatin decondensation and reconfiguration in enabling and augmenting the expression of oncogenic gene networks, and to determine the utility of pharmacological agents targeting FOXO3-regulated mechanisms. Our multiomics-supported, mechanistically informed approach may identify key ATC vulnerabilities and validate the therapeutic potential of their targeting with focus on combinations of clinically relevant agents to ultimately transform the therapy of ATC, the deadliest of cancers.





# MOLECULAR BASIS OF EPIGENETIC CONTROL: EPIGENETIC REGULATION BY POST-SYNTHETIC DNA AND RNA MODIFICATIONS

**Julie Cunningham**

Mayo Clinic, Rochester, Minnesota, USA

Post translational modifications of DNA and RNA are critical components regulating chromatin and RNA processes. In this presentation, I will present what we know about the chemistry underlying these modifications, some thoughts on how they may have evolved, and touch upon how perturbations of these mechanisms can impact health.

# NON-INVASIVE PRENATAL TESTING FOR THE BETA-HEMOGLOBINOPATHIES USING NEXT-GENERATION SEQUENCING AND PROBE CAPTURE

**Henry Erlich**

Children's Hospital Oakland Research Institute and UCSF Benioff Children's Hospital Oakland, Oakland, CA, USA

The presence of fetal DNA in maternal plasma and the massively parallel and clonal features of Next Generation Sequencing (NGS) have made non-invasive prenatal testing (NIPT) a reality. The analysis by NGS of fetal DNA in maternal plasma has been applied to the diagnosis of chromosomal aneuploidies but the NIPT of autosomal recessive diseases has been more challenging. We have applied NGS to the NIPT of the autosomal recessive diseases, sickle cell anemia (SCA) and b-thalassemia, by using a capture probe panel that covers 4 Kb of the b-globin gene and linked SNPs as well as >450 genomic polymorphisms used to estimate the fetal fraction. The hybrid capture method is well suited to the analysis of the small DNA fragments present in the maternal plasma. The fetal fraction is estimated by counting paternally transmitted sequence reads in the plasma library for alleles present in the fetus but absent in the mother. If the mother and father's mutations differ, the paternally transmitted mutation can be detected qualitatively as a sequence in the plasma that is absent in the mother but the mother's transmitted allele is determined by quantitative analysis of the sequence read proportions in the plasma. If the maternal and paternal mutations are the same, as in a pregnancy at risk for SCD, the fetal b-globin genotype is inferred by counting sequence reads corresponding to the mutation and wild type alleles. The observed proportions are compared to those expected for each of the three possible fetal genotypes (Mut/Mut; Mut/WT; WT/WT) to infer fetal genotype. The expected values are calculated based on the fetal fraction estimate. An algorithm assigns probability values to each of the potential fetal genotypes. We have used the bioinformatic strategy of in silico size selection for the maternal plasma reads to increase the fetal fraction. We have also used haplotype information, when available, to consider the observed ratios at linked SNPs to help predict the fetal genotype at the mutation site. This probe capture/NGS system promises to provide a robust non-invasive test for sickle cell anemia and b-thalassemia and represents a model approach for other autosomal recessive diseases.





# EPIGENOMICS OF INFLAMMATORY BOWEL DISEASE

**William A. Faubion**

Mayo Clinic, Rochester, Minnesota, USA

Inflammatory bowel disease is a chronic intestinal inflammatory condition affecting 1 in 300 individuals in the developing world for which there is insufficient insight into pathogenicity or precision therapeutics. While there are genetic traits associated with disease, the rise in incidence over the last 3 decades supports an environmental influence to disease susceptibility and behavior. We will review environmental signals received by particular immune subsets (FOXP3+ CD4+ lymphocytes) that couple to epigenetic complexes regulating immune responses in the human IBD, Crohn's disease.

# SINGLE-CELL MULTIOMIC ANALYSIS TO IDENTIFY CELL-SPECIFIC TRANSCRIPTIONAL DEPENDENCIES IN CANCER AND COVID-19 INFLAMMATORY RESPONSE

**Alexandre Gaspar-Maia**

Center for Individualized Medicine, Mayo Clinic, Rochester, MN, USA

The human body is composed of an estimated 37 trillion cells that live harmoniously among their neighbors. An equilibrium between differentiated cells, progenitor cells and stem cells has to be achieved at any point of development to enable homeostasis and proper tissue organization. However, in cancer, a single cell can lead to the downfall of an entire organism. To ensure that all the signals and processes are coordinated, cells need to regulate the expression of genes using a multitude of factors (transcription factors that bind to the DNA itself) and chromatin regulators (proteins that surround the DNA). Novel single cell technologies have enabled us to define both the expression and the chromatin landscape. Here, we will discuss how single cell multiomic analysis has enable us to define cell-specific transcriptional dependencies in cancer and in inflammatory response upon COVID19 infection.





# 3D GENOMIC STRATEGIES TO UNDERSTAND COMPLEX TRAIT GENETIC ARCHITECTURE

**Struan F. A. Grant**

Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA

We are employing cutting-edge 3D genomic approaches to facilitate understanding of genetic loci for common complex disease. There is a significant need to discover and validate new genetic targets that influence such traits to advance therapies to prevent and treat disease. We are focused on the functional significance of genome wide association study (GWAS) signals associated with various complex traits. While numerous GWAS efforts have been successful in discovering key genetic variants, this approach only reports genomic signals associated with a given phenotypic trait and not necessarily the precise localization of culprit effector genes. Approaches are now emerging to make these determinations; however, they typically suffer from low-resolution and inaccuracies. Using bone mineral density (BMD) as an example, we recently published our high-resolution genome-wide 'variant to gene mapping' efforts, where we integrated RNA-seq, ATAC-seq and chromatin conformation capture (promoter-focused Capture C) in primary human osteoblasts to implicate culprit effector genes for osteoporosis, including validating two novel effector genes, EPDR1 and ING3. ~30% of GWAS signals were found to reside in enhancers with direct physical contact with genes expressed in osteoblasts, totaling 86 leads - many being novel and warranting functional follow-up. However, this also means that many GWAS loci remain to be resolved, so in order to uncover additional aspects of the genetic architecture of bone density determination we are now studying temporally specific roles that are dependent on the stage of differentiation Crucially, our pipeline does not involve large sample sizes, but rather uses primary healthy human cells to triangulate key enhancers coinciding with, and signposted by, putatively causal variants. The ultimate aim is to provide the community with new, high value targets to aid in understanding mechanism, and eventually therapies, for common complex diseases.

# POPULATION GENETICS, KINSHIP PRACTISES AND SOCIAL ORGANISATION IN PREHISTORIC SOCIETIES OF EUROPE

**Wolfgang Haak**

Max Planck Institute for Evolutionary Anthropology, Department of Archaeogenetics, Leipzig, Germany

The continuously growing record of ancient human genomic data reveals ever more detailed insights into the population history of prehistoric societies. By focussing on regional transects, closing spatial and temporal gaps, and through a proper integration of the archaeological context, archaeogenetic studies now exceed simplistic migration scenarios and can provide nuanced accounts of genetic and cultural transformations. Increasing numbers of intra-site studies add further details by shedding light on kinship practices and forms of social organisation in prehistoric societies. I will present a selection of recent case studies from various regions in Neolithic and Early Bronze Age Europe, which further advance our understanding of prehistoric societies and the formation of the European gene pool.





# STONE AGE ENCOUNTERS: NEANDERTAL ANCESTRY OF EARLY HUMANS IN EURASIA

**Mateja Hajdinjak**[1,2]

[1]Ancient Genomics Laboratory, The Francis Crick Institute, London, United Kingdom, [2]Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

Our closest evolutionary relatives, the Neandertals, appeared in the European fossil record around 430,000 years ago (~430ka). Before their disappearance, Neandertals lived throughout Europe, western and central Asia, and the Near East. The ancestors of all modern humans emerged in Africa at least ~300ka and, sometime between ~100ka and ~50ka, a subset of those humans migrated out-of-Africa and spread across the world. However, by ~40ka Neandertals disappeared. The reasons of Neandertal disappearance, the extent to which they overlapped with modern humans, and the nature of interactions between the two hominin groups have been intensively debated for decades. Comparisons between the Neandertal genomes and the genomes of modern humans showed that Neandertals contributed ~2% to the genomes of all people living outside of sub-Saharan Africa. Based on the size of Neandertal segments in modern human genomes, the time of this admixture was narrowed to between 58ka and 52ka, and most likely in the Near East. However, where, when and how often these two groups came into contact was not well understood. Despite genomic data being recovered from more than 6,500 ancient humans to date, genome-wide data of individuals close in time when modern humans could have met some of the last Neandertals are still extremely sparse. Through the combination of minimally destructive sampling, decontamination with mild hypochlorite solution and hybridisation captures, we obtained genome-wide data of sixteen new and improved coverage of further nine previously published modern humans from western Eurasia older than 30ka. In addition to shedding light on the genetic diversity of past populations, these data allow us to start reconstructing the fine-scale dynamics of the interactions between late Neandertals and early humans in Eurasia. Our data suggest that small groups of first humans to arrive in Eurasia interacted intimately with Neandertals, having had close Neandertal ancestors, and were eventually either absorbed into their populations or became extinct. Moreover, our new data further raise the possibility of Neandertal populations becoming assimilated in more numerous human populations that arrived later, indicating a far more dynamic population history and turnovers during this time period than previously appreciated.

# A FORENSIC GENOMICS APPROACH FOR THE IDENTIFICATION OF SISTER MARIJA KRUCIFIKSA KOZULIĆ OF RIJEKA

Charla Marshall[1,2,3], Kimberly Sturk-Andreaggi[1,2], Erin M. Gorden[1,2], Jennifer Daniels-Higginbotham[1,2], Sidney Gaston Sanchez[1,2], Željana Bašić[4], Ivana Kružić[4], Šimun Anđelinović[5,6], Alan Bosner[7], Miran Čoklo[8], Anja Petaros[9], Timothy P. McMahon[1], Dragan Primorac[3,5,10,11,12,13,14,15,16,17], **Mitchell M. Holland[3]**

[1]Armed Forces Medical Examiner System (AFMES), Dover Air Force Base, Dover, DE, USA, [2]SNA International, Contractor Supporting the AFMES, Alexandria, VA, USA, [3]Department of Biochemistry & Molecular Biology, Forensic Science Program, The Pennsylvania State University, University Park, PA, USA, [4]University Department of Forensic Sciences, University of Split, Split, Croatia, [5]Medical School, University of Split, Split, Croatia, [6]Clinical Department for Pathology, Legal Medicine and Cytology, Clinical Hospital Center Split, Split, Croatia, [7]Department of Forensic Medicine and Criminalistics, University of Rijeka School of Medicine, Rijeka, Croatia, [8]Institute for Anthropological Research, Center for Applied Bioanthropology, Zagreb, Croatia, [9]National Board of Forensic Medicine, Department of Forensic Medicine, Linköping, Sweden, [10]St. Catherine Specialty Hospital, Zagreb, Croatia, [11]School of Medicine, University of Osijek, Osijek, Croatia, [12]Faculty of Dental Medicine and Health, University of Osijek, Osijek, Croatia, [13]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, New Haven, CT, USA, [14]School of Medicine, University of Rijeka, Rijeka, Croatia, [15]Medical School REGIOMED, Coburg, Germany, [16]University of Split, University Department of Forensic Sciences, Split, Croatia, [17]The National Forensic Sciences University, Gandhinagar, Gujarat, India

Sister Marija Krucifiksa Kozulić (1852-1922) was a nun who dedicated herself to helping the poor and less fortunate. In light of her generous and virtuous life, Sister Kozulić is being considered for sainthood by the Vatican. However, this process could not proceed without the identification of her remains, which has required the skills of many experts, including pathologists, anthropologists, and molecular biologists. This presentation will highlight the efforts made to support the identification of Sister Kozulić with a forensic genomics approach; targeting both mitochondrial and nuclear DNA markers using capture and massively parallel sequencing (MPS) methods. Sister Kozulić was buried in a tomb in Rijeka with her biological sister, Tereza Kozulić, along with the commingled remains of other nuns from the Society of Sisters of the Sacred Heart of Jesus. In search for Sister Kozulić amongst the many skeletal remains recovered from the tomb, mitochondrial genome (mitogenome) sequencing was performed on femoral samples using capture and MPS methods developed for degraded DNA. The obtained mitogenome sequences revealed DNA averaging less than 100 bp in length that is typical of historical samples. The results revealed two individuals sharing the same H1 haplotype with an uncommon 13327 G/A heteroplasmy. This finding was taken as a preliminary indicator of the biological sisters' remains, who were the only known maternal relatives buried in the tomb. Additionally, one of the haplotypes exhibited a private 12337 C insertion at ~30% frequency, which distinguished the two haplotypes from one another. Due to the uniqueness of the haplotype, combined with the rarity of the shared heteroplasmy, it was assumed that these remains represented the sisters. The next step was to perform autosomal DNA testing on the remains, along with a buccal swab from a known paternal niece, to evaluate their genetic relationship. The reference sample of the now-deceased niece was collected in 2011, and the extracted DNA was low quality. Due to the degree of DNA degradation, kinship analysis was attempted after MPS of identity informative SNPs and autosomal STRs using the Precision ID Identity SNP and GlobalFiler NGS STR panels. Although only partial SNP and STR profiles were obtained from the sisters, comparison of their genotypes with that of the paternal niece supported the expected kinship scenario. Based on the SNP and STR results, the likelihood ratio (LR) exceeded 93,000 for a full sibling relationship between the presumed remains of Marija and Tereza versus being unrelated, with a posterior probability of 98.1% when considering the degrees of relatedness tested. In addition, the findings were >574,000 times more likely in favor of the sisters as 2nd degree relatives of the niece than from individuals who were unrelated. Given the supported kinship relationship with a known family reference, the two sets of skeletal remains are believed to belong to Sisters Marija and Tereza Kozulić. In the absence of direct reference samples from the two sisters, it is not possible to identify which set of remains belongs to Sister Marija. Nonetheless, the findings have allowed for the Vatican to move forward with the beatification process. The points of view in this abstract are those of the authors and do not reflect the views of their respective agencies. In addition, this publication in no way reflects an endorsement of products, instruments, or software.

Citation:

A forensic genomics approach for the identification of Sister Marija Crucifiksa Kozulić. C Marshall, K Sturk-Andreaggi, EM Gorden, J Daniels-Higginbotham, SG Sanchez, Ž Bašić, I Kružić, Š Andelinović, A Bosner, M Čoklo, A Petaros, TP McMahon, D Primorac, MM Holland (2020), Genes, 11, 938-950



Abstracts of invited lectures

12th ISABS Conference on Forensic and Anthropological Genetics
and Mayo Clinic Lectures in Individualized Medicine
June 22-27, 2022, Dubrovnik, Croatia

# MASSIVE EPIGENETIC REPROGRAMMING TRIGGERED BY A SINGLE GENETIC ALTERATION

**Haojie Huang**

Mayo Clinic, Rochester, MN, USA

SPOP is a Cullin3-based E3 ubiquitin ligase adaptor (substrate-binding) protein. Whole genome and exome sequencing studies including The Cancer Genome Atlas (TCGA) invariably show that SPOP is the most frequently mutated gene in human primary prostate cancers. In addition to the discovery that prostate cancers with SPOP mutations have highest androgen receptor (AR) transcriptional activity among all genotypically distinct subsets of prostate cancer, this subtype of tumors are reportedly associated with genome-wide DNA hypermethylation in prostate cancer although the underlying mechanisms were elusive. Recent studies from Dr. Haojie Huang's laboratory at Mayo Clinic have shown that SPOP binds and promotes polyubiquitination and degradation of histone methyltransferase and DNMT interactor GLP. SPOP mutation induces stabilization of GLP and its partner protein G9a and aberrant upregulation of global DNA hypermethylation in cultured prostate cancer cell lines and primary specimens of patients. Genome-wide DNA methylome analysis shows that a subset of tumor suppressor genes (TSGs) including FOXO3, GATA5, and NDRG1, are hypermethylated and downregulated in SPOP-mutated prostate cancer cells. DNA methylation inhibitor 5-azacytidine effectively reverses expression of the TSGs examined, inhibits SPOP-mutated PCa cell growth in vitro and in mice, and enhances docetaxel anti-cancer efficacy. Together, these findings identify the GLP/G9a-DNMT module as a mediator of DNA hypermethylation in SPOP-mutated prostate cancer. These data also suggest that SPOP mutation could be a biomarker for effective treatment of prostate cancer with DNA methylation inhibitor alone or in combination with taxane chemotherapeutics.





# RECOVERY OF NUCLEAR DNA FROM HAIR SHAFTS ASSOCIATED WITH THE ROMANOV FAMILY

**Jodi Irwin**

Federal Bureau of Investigation (FBI), Quantico, VA, USA

To optimize the recovery of probative DNA from shed hair, an extraction protocol targeting ultrashort DNA fragments was applied to hair shafts found in items associated with the Romanov family. Published mitochondrial DNA genome sequences of Tsar Nicholas II and his wife, Tsarina Alexandra, made these samples ideal to assess DNA extraction techniques and evaluate the types of genetic information that can be recovered from aged hair. Using this method, the mtGenome of the Tsarina's lineage was identified in hairs that were concealed in a pendant made by Karl Fabergé for Alexandra Feodorovna Romanov. In addition, to determine if the lock originated from more than one individual, two single hairs from the locket were extracted independently and the autosomal SNP data used to assess relatedness. Testing of hairs found in a second artifact, a framed photograph of Louise of Hesse-Kassel, Queen of Denmark and maternal grandmother of Tsar Nicholas II, revealed that the hair belonged to a woman who shared Tsar Nicholas' maternal lineage, including the well-known point heteroplasmy at position 16169.

# ARCHAIC AND MODERN HUMANS IN ISLAND SOUTHEAST ASIA

**Mattias Jakobsson**

University of Uppsala, Uppsala, Sweden

Multiple lines of evidence show that modern humans interbred with archaic Denisovans. Denisovans were initially thought to have a simple shared demographic history with modern humans, through a single admixture event with the ancestor of Australasians, but later work suggest that Denisovan ancestry can also be detected in varying degree across Australasians and at lower levels in East Asian, South Asian, Siberian and Native American populations. I will discuss recent finds of additional admixture between Australasians and Denisovans distinctively in Island Southeast Asia and the Philippines. In total, 118 ethnic groups of the Philippines including 25 diverse self-identified Negrito populations were investigated and we show that some groups, for instance the Ayta Magbukon, possess the highest level of Denisovan ancestry in the world ~30-40% greater than the level in Australians and Papuans. This finding is consistent with an independent admixture event into Negritos from Denisovans. Together with the recently described fossils claimed to be a new species (Homo luzonensis), we propose diverse archaic groups inhabiting the Philippines prior to the arrival of modern humans, likely genetically similar to Denisovans. Altogether, our findings unveil a complex intertwined history of modern and archaic humans in the Asia-Pacific region, where distinct Islander Denisovan populations differentially admixed with incoming Australasians across multiple locations and at various points in time.





# DYNAMIC ENHANCER-PROMOTER INTERACTIONS MEDIATE CHEMORESISTANCE IN PDAC

**Steven A. Johnsen**

Robert Bosch Center for Tumor Diseases, Stuttgart, Germany

Pancreatic ductal adenocarcinoma (PDAC) displays a remarkable propensity towards therapy resistance. However, molecular epigenetic and transcriptional mechanisms enabling this are poorly understood. We integrated epigenome, transcriptome, nascent RNA and chromatin topology data and identified a novel subgroup of enhancers that mediate transcriptional reprogramming and chemoresistance in PDAC. These Interactive Hubs (iHUBs) display characteristics typical for active enhancers (H3K27ac enrichment) in both therapy sensitive and resistant states but exhibit increased interactions and production of enhancer RNA (eRNA) in the resistant state. Notably, deletion of individual iHUBs was sufficient to decrease transcription of target genes and sensitize resistant cells to chemotherapy. Moreover, targeting either eRNA production or signaling pathways upstream of iHUB activation using clinically tested small molecule inhibitors decreased eRNA production and interaction frequency, and restored chemotherapy responsiveness in vitro and in vivo. Thus, our findings identify iHUBs as important regulators of chemotherapy response and demonstrate their targetability in sensitization to chemotherapy.

# MULTI-OMICS TO MECHANISMS: THE ROAD TO MICROBIOME-DRIVEN PRECISION MEDICINE

**Purna C. Kashyap**

Mayo Clinic, Rochester, Minnesota, USA

The current treatment paradigm of one-size fit all does not consider interindividual variability in an individual's exposome including diet, lifestyle and environment and genetics including host and microbial genes, which underlie the pathogenesis, susceptibility, and outcomes of most chronic diseases to varying levels. While there has been a significant interest in host genetics since with sequencing of the host genome, there is increasing realization that microbial genes also contribute to several pathophysiologic mechanisms. To move towards a personalized medicine approach, we need to consider nonlinear contributions from patient genetics, microbiome and exposome. In my presentation, I will discuss the contribution of the microbiome in the pathophysiology of functional gastrointestinal disorders like irritable bowel syndrome, in the context of host omics and physiological responses, as well as environmental factors. This approach can facilitate personalized treatment strategies by providing more meaningful mechanism-based stratification.





# DEEP THINKING: WHERE MACHINE INTELLIGENCE ENDS AND HUMAN CREATIVITY BEGINS

**Garry Kasparov**

Office of Garry Kasparov, New York, New York, USA

As our machines become capable of more complex tasks, they are evolving from tools to partners—if we use them wisely. There is little doubt that the combination of human plus machine is the key to unlocking the power of artificial intelligence. The key is finding ways to work together, to develop processes that get the best from both. Instead of being afraid of automation encroaching into the intellectual world, we must embrace the potential to discard rote cognitive tasks to focus on the uniquely human elements of life: leadership, creativity, and the pursuit of happiness. Much as a telescope extends human vision, artificial intelligence, or augmentedintelligence, as Kasparov prefers it, will extend our mental abilities. Also like a telescope, we must point our powerful new AI tools in the right direction, ambitiously and imaginatively.

# PHARMACOGENOMICS IN CLINICAL PRACTICE: LESSONS FROM INDIVIDUALIZED MEDICINE CLINIC

**Adrijana Kekić**

Pharmacy Clinical Practice, Mayo Clinic Arizona, Phoenix, AZ, USA

Pharmacogenomics combines sciences of clinical pharmacology and human genomics to predict drug response phenotypes. This presentation will highlight current use of PGx in a clinical practice, its benefits and limitations, and future direction.





# THE FUNCTIONAL IMPACTS OF GENE FLOW BETWEEN ARCHAIC AND MODERN HUMANS

**Janet Kelso**

Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

The genomes of archaic and ancient modern humans offer a unique window into their histories. However, the sequencing and analysis of DNA from ancient humans is complicated by DNA degradation, chemical modifications and contamination. Recent technological advances have made it possible retrieve and sequence DNA from bones and other remains found at archaeological excavations, and we have been able to reconstruct the genomes of several Neandertals. We have also identified, based on their genome sequences, Denisovans, a previously unknown extinct Asian hominin group related to Neandertals.

By comparing these archaic genome sequences to the genome sequences of ancient and present-day modern humans we have shown that gene flow between archaic and modern humans occurred at multiple times, and that this gene flow has shaped the genomes of both Neandertals and of modern humans. For example, the ancestors of some modern humans interbred with Neandertals and Denisovans such that all present-day people outside of Africa carry approximately 2% Neandertal DNA, and that some populations, largely in Oceania, also carry DNA inherited from Denisovans. This introgressed DNA has been shown to have both positive and negative outcomes for present-day carriers: underlying apparently adaptive phenotypes such as high altitude adaptation, as well as influencing immunity and disease risk. In recent work we have identified Neandertal haplotypes that are likely of archaic origin and determined the likely functional consequences of these haplotypes using public genome, gene expression, and phenotype datasets.

# THE GENETIC HISTORY AND ORIGIN OF THE BLACK DEATH

**Johannes Krause**

Max Planck Institute for the Science of Human History, Jena, Germany

High throughput DNA sequencing has revolutionized the field of archaeogenetics in the past decade, providing a better understanding of human genetic history, past population dynamics and host pathogen interactions through time. Targeted DNA capture approaches have allowed reconstructing complete ancient bacterial genomes providing direct insights into the evolution and origin of some of the most infamous bacterial pathogens known to humans such as Yersinia pestis, the causative agent of plague. Ancient Y. pestis genomes spanning over 5000 years of human history from the Stone Age to modern times provide novel insights into the evolution of one of the most infamous human pathogens. They provide direct evidence for the timing and emergence of major virulence factors essential for the transmission of Y. pestis by fleas. The oldest reconstructed genomes of Y. pestis fully capable of causing the bubonic form from the Eastern European Bronze Age provides evidence for prehistoric epidemics in prehistory. Suggesting that the emergence of this form of the disease happened more than 1000 years earlier than previously suggested. Temporal studies of pathogens might thus throw new light on the origin of human diseases and potentially allow predicting and preventing further transmissions and dissemination in the future.





# THE ORIGINS, DISPERSAL, AND PATHOGEN HISTORY OF CHICKENS

**Greger Larson**

University of Oxford, Oxford, UK

There are approximately 80 billion chickens on Earth which makes them by far the most numerous domestic animals. Despite their ubiquity there has been little consensus regarding the timing or location or circumstances of their domestication. In order to understand not just when, where, and how they first became associated with human societies, but also what happened next and how their close proximity to people has driven the evolution of Marek's disease, a highly contagious viral neoplastic disease, I will discuss three recent studies. The first critically assessed the domestic status of chicken remains described in >600 sites in 89 countries, alongside an evaluation of zoogeographic, morphological, osteometric, stratigraphic, contextual, iconographic, and textual data. A second study predicated on the direct radiocarbon dating of >20 ancient European chickens reframes the arrival and dispersal of chickens across Eurasia, and a third study uses an ancient DNA approach to sequence the Marek's virus to understand how decades of vaccines have driven the evolution and virulence of this disease. Combined, these studies establish a new and comprehensive foundation for understanding humanity's most important bird.

# GLYCANS AS BIOMARKERS AND FUNCTIONAL EFFECTORS IN CARDIOMETABOLIC DISEASES

**Gordan Lauc**[1, 2]

[1]University of Zagreb Faculty of Pharmacy and Biochemistry, [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia

The majority of proteins that evolved after appearance of multicellular life are glycosylated and glycans significantly affect structure and function of these proteins. However, due to structural complexity of glycans and the absence of a direct genetic template, the analysis of protein glycosylation is much more complicated than the analysis of DNA or proteins. Consequently, the knowledge about the importance of individual variation in glycans for both normal physiological processes and diseases is still limited. By generating glycomic data for over 100,000 individuals from some of the best characterized clinical and epidemiological cohorts we enabled glycomics to meet other 'omics. Changes in glycosylation have been observed in numerous diseases, often even before other symptoms of a disease appeared, indicating that they may reflect early steps in the molecular pathophysiology of many complex diseases. Initial data from intervention studies and animal models suggest that reversing changes in glycosylation may decrease the disease risk.





# SENSITIZING CANCER CELLS TO DNA DAMAGE-INDUCING AGENTS AND ANTI-TUMOR IMMUNITY

Qin Zhou, Robert Mutter and **Zhenkun Lou**

Mayo Clinic, Rochester, MN, USA

Spleen-associated tyrosine kinase (Syk) is a non-receptor tyrosine kinase that regulates immunity, cell adhesion, and vascular development. Here we found a new role of Syk in DNA repair regulation. We discovered that Syk is activated by ATM and recruited to DNA double-strand breaks where Syk activates the key enzyme in homologous recombination (HR) to promote resection and HR. Furthermore, Syk upregulation and promotion of resection and HR is a potential mechanism of platinum and PARP inhibitor resistance in ovarian and breast cancer with high expression of Syk. This resistance can be overcome by Syk inhibition or deletion for Syk-high expressing cancer cells. In addition, Syk inhibition could promote anti-tumor immunity by inhibiting M2 macrophages. We propose that Syk is a new target to inhibit HR and sensitize resistant tumors to DNA targeted therapy and immunotherapy.

# CROSSTALK BETWEEN HYPOXIA AND EPIGENETICS IN BREAST CANCER

**Weibo Luo**

University of Texas Southwestern Medical Center, Dallas, TX, USA

Hypoxia, a common feature of the tumor microenvironment, regulates various cancer biological processes to drive tumor progression. The hypoxia response is primarily controlled by the transcription factor hypoxia-inducible factor (HIF). HIF enhances thousands of downstream target genes and regulates many hypoxia-induced pathological processes in human cancers, including angiogenesis, metabolism, immune evasion, pH homeostasis, cell survival, maintenance of stem cells, and cell migration/invasion. The transcription activity of HIF is dynamically regulated by multiple epigenetic regulators including p300, JMJD2C, ZMYND8, G9a, GLP, CHD4, and BRD4 in cancer cells. HIF regulators are upregulated in breast cancer and contribute to breast cancer progression by augmenting hypoxia response. Apart from protein-coding genes, HIF also globally induces long non-coding RNAs in human breast cancer cells. These hypoxia-induced long non-coding RNAs represent another layer of mechanism of hypoxia-dependent breast cancer progression. Taken together, HIF and epigenetic regulators are mutually regulated and their crosstalk is crucial for breast cancer progression.





# GENOME EDITING, STEM CELLS AND NEANDERTALS

**Tomislav Maričić**

Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, Leipzig, Germany

Neandertals are our closest extinct relatives and using their genomic sequences together with sequences of thousands of humans living today, we were able to define all genomic positions that are unique to modern humans. Out of those, only around one hundred positions have changed an amino acid and the biological significance of those is largely unknown. Since those changes are shared among all humans, we cannot study them in living humans and have to rely on other model systems, such as pluripotent stem cells (PSCs) and genome editing. This allows us to introduce the Neandertal substitution into a human PSC, and study the effect of the introduced substitution in differentiated tissue of interest, e.g., human brain organoids. In my talk, I will first present methodological improvements in genome editing in which we were able to increase editing efficiency and detect and reduce unwanted editing side effects. Then I will present our study of six amino acid changes in proteins that have key roles in kinetochore function and chromosome segregation. We could show that cells with Neandertal substitutions have shorter metaphase and more chromosome segregation errors when differentiated to human brain organoids, than cells without those substitutions. This suggests that one unique feature of modern humans is that the fidelity of chromosome segregation has improved during neocortex development.

# DEVELOPMENT OF NEURONAL CELL DIVERSITY IN THE GUT REVEALED BY SINGLE-CELL TRANSCRIPTOMICS

Khomgrit Morarach, Anastassia Mikhailova, Viktoria Knoflach, Wei Li, Ziwei Liu, Fatima Memic, **Ulrika Marklund**

Karolinska Institute, Solna, Sweden

As largest part of the peripheral nervous system, the enteric nervous system (ENS) spans the entire gastrointestinal tract and organises into irregular ganglia of intermingled neural subtypes. Owing to these challenging anatomical features, research on ENS composition and development has lagged behind that of the central nervous system (CNS). Meticulous work over decades have identified enteric neural subtypes including motor-, inter- and sensory neurons but the ambiguous molecular markers have hampered elucidation of the full cellular complexity, and thus functionality, of the ENS. We have utilised single cell RNA-sequencing to establish unbiased molecular definitions of enteric neurons in the murine small intestine. Analysis of the myenteric plexus identified 12 enteric neuron classes (ENCs) that were validated in tissue by histochemical detection of unique marker combinations. Further transcriptome analysis of the fetal ENS presented a novel neural diversification mechanism. Unlike in the developing CNS, where spatial patterning of stem cells predominates cell fate decisions, myenteric neuron diversity seems primarily formed via identity conversion of postmitotic neurons. We anticipate that the mapping of enteric neuron classes may help to better define enteric neural circuits, while the developmental blueprint could pave the way for efficient derivation of specific enteric neuron types for the purpose of cell-based regenerative medicine or ENS disease modelling.





# CAPTURE AND MPS FOR HUMAN IDENTIFICATION

**Charla Marshall[1,2],** Kimberly Sturk-Andreaggi[1,2], Jacqueline Tyler Thomas[1,2], Courtney Cavagnino[1,2], Katelyn Kjelland[1,3], Jennifer Daniels-Higginbotham[1,2], Suzanne Barritt-Ross[1], Kerriann K. Meyers[1,2], Ellen Greytak[4], Janet Cady[4], CeCe Moore[4], Steven Armentrout[4], Timothy P. McMahon[1]

[1]Armed Forces Medical Examiner System's Armed Forces DNA Identification Laboratory (AFMES-AFDIL), Dover Air Force Base, Delaware, USA, [2]SNA International, LLC, Alexandria, Virginia, USA [3]Pacific Architects and Engineers, Falls Church, Virginia, [4]Parabon NanoLabs, Inc., Reston, Virginia, USA

In 2015, the Armed Forces Medical Examiner System's Armed Forces DNA Identification Laboratory (AFMES-AFDIL) became the first accredited forensic DNA laboratory worldwide to validate massively parallel sequencing (MPS) methods for routine casework. These MPS methods were needed to analyze DNA fragments from chemically treated, historical bone samples that were too degraded for traditional PCR enrichment, averaging only 70 base pairs in size. For example, MPS with hybridization capture (capture-MPS) enabled mitochondrial DNA (mtDNA) analysis of Korean War unknowns that were immersed in formalin baths in the 1950s as a post-mortem preservation treatment prior to burial. STR typing of these samples was not possible, and the success rates for Sanger sequencing of mtDNA were very low (~8%). However, with capture-MPS, mitochondrial genome analysis was successful for approximately 60% of all samples tested. This allowed for comparison of mtDNA between profiles obtained from unknowns and family reference specimens from maternal relatives. While the capability for mtDNA analysis led to hundreds of MPS-based identifications of missing U.S. service members from conflicts dating back to the mid-20th century, unsolved cases still remain. These include cases involving consistent mtDNA profiles, those of service members lacking mtDNA references, and others with hypothesized mutational events between the unknown and the family member's mtDNA sequence. Therefore, SNP capture is necessary to expand the capability for DNA-based identifications. This presentation will broadly cover the SNP capture and MPS methods developed and/or evaluated at the AFMES-AFDIL. These targets include a large SNP panel (850,000 SNPs), two medium SNP panels (25,000 SNPs and 95,000 SNPs), a small SNP panel (5,000 SNPs), as well as whole genome enrichment approaches with some comparisons to untargeted whole genome sequencing.

# CIRCADIAN ETIOLOGY OF TYPE 2 DIABETES

**Aleksey Matveyenko**

Mayo Clinic, Department of Physiology and Biomedical Engineering, Rochester, Minnesota, USA

Type 2 diabetes mellitus (T2DM) is one of the major health challenges facing today's society and projected to afflict nearly 1 in 3 people by year 2050. T2DM is associated with an increase in population morbidity/mortality, and more recently, has been shown to exacerbate adverse outcomes associated with COVID-19. The pathophysiology of T2DM is mediated by complex interactions among diverse environmental and genetic susceptibilities, which ultimately culminate in the development of pancreatic islet failure characterized by impaired insulin secretory function. Although underlying genetics contribute to the pathogenesis of T2DM, environmental and epigenetic factors appear to be the primary drivers of this disease. Specifically, recent evidence suggests that disruptions of circadian light/dark and fasting/feeding cycles have contributed to the induction of pancreatic islet failure and an overall increase in the predisposition to T2DM. The aim of the presentation is to describe emerging physiological, genetic and epigenetic insights into the role of the circadian system in regulating pancreatic islet function and failure in health and disease.





# PROTEIN MONOAMINYLATION IN BRAIN: NOVEL MECHANISMS OF NEURAL DEVELOPMENT, PLASTICITY AND DISEASE

**Ian Maze**

Icahn School of Medicine at Mount Sinai, Department of Neuroscience, New York, NY, USA

Histone H3 monoaminylations at glutamine (Q) 5 [e.g., serotonylation (H3Q5ser) and dopaminylation (H3Q5dop)] have recently been identified as epigenetic markers in neurons. These monoaminylation states appear to play critical roles in the mediation of permissive gene expression supported, in part, by adjacent lysine 4 (K4) methylation and have been implicated in diverse biological and disease processes, ranging from neuronal differentiation to the precipitation of drug-seeking behaviors in adult animals. Our previous work demonstrated that H3Q5ser and H3Q5dop are catalyzed by the Transglutaminase 2 (TGM2) enzyme, both in vitro and in vivo. Here, we discuss the identification of a new class of histone monoaminylation, H3Q5 histaminylation (H3Q5his), which displays dynamic expression in brain in the context of sleep-wake cycles. We further find that H3Q5his, unlike H3Q5ser, electrostatically inhibits recruitment of the chromatin reader protein WDR5 and attenuates MLL1 complex methyltransferase activity on H3K4. Importantly, we demonstrate that H3Q5 monoaminylation dynamics are determined by local concentrations of monoamines, which can be sensed by TGM2. This noncanonical erasure/'rewriting' mechanism suggests a previously unreported biochemical process through which certain post-translational modifications can be established and removed by a single enzyme based upon its sensing of local cellular microenvironments.

# THE GENESIS OF THE GENETIC LANDSCAPE IN NORTHEAST EUROPE

**Mait Metspalu[1],** Lehti Saag[1,2], Lena Kushniarevich[1], Toomas Kivisild[1,3], Aivar Kriiska[4], Kristiina Tambets[1]

[1]Institute of genomics, University of Tartu, Tartu, Estonia, [2]Research Department of Genetics, Evolution, and Environment, University College London, London, UK, [3]Department of Human Genetics, KU Leuven, Leuven, Belgium, [4]Institute of Archaeology, University of Tartu, Tartu, Estonia

It has been well established that postglacial peopling of Europe has involved three major demic expansions. Hunter-gatherers (HG) and early agriculturalists both from West Asia and pastoralists from the Pontic steppe. In addition, there is a more easterly influence in NE Europe detected in the Iron Age. Here we refine this broad narrative in NE Europe. We first explore the dynamics of western and eastern HGs in the Baltics, suggesting more genetic connections between people associated with different cultures/periods than previously thought. Then we document both abrupt and continuous patterns of genetic change during and after adoption of agriculture in the northern East European Plain. A genetic change with the arrival of steppe ancestry can be seen all over the region, whereas later changes are more subtle and region-specific. Finally, we explore patterns of genetic sharing between Estonians and Finns using large scale biobank datasets and novel methods. Despite substantive differentiation in allele frequencies, the two populations sport unexpectedly many segments of long shared allele intervals dating roughly to around 5th/6th century AD. This shows the importance of relatively recent events for the formation of contemporary populations.





# IN THE ABSENCE OF SKELETAL REMAINS: STUDYING ANCIENT HUMAN DNA PRESERVATION IN SEDIMENT

**Matthias Meyer**

Max Planck Institute for Evolutionary Anthropology, Evolutionary Genetics Department, Leipzig, Germany

In 2017, we reported the recovery of Neandertal and Denisovan mitochondrial DNA from Pleistocene cave sediments, suggesting that the analysis of sediment DNA may overcome our dependency on the scarce fossil record for investigating the human past. Yet, this work also raised a number of questions that are important to determine the relevance of this newly discovered source of DNA for future research: How common is ancient human DNA preservation in sediment? What are the temporal limits of ancient DNA preservation in sediment? What is the source of this DNA? Do single samples contain DNA from one or several individuals? Can human DNA move between layers? What are the limits of resolution that can be achieved in the analysis of DNA sequences from sediment? To address these questions, we have undertaken several large studies on different scales. On a global scale, we screened sediment samples from more than 200 archaeological sites, mostly in Eurasia, to assess the preservation of ancient human and faunal DNA. On a local scale, we reconstructed genetic time-series data from archaeological sites based on hundreds of sediment samples. For example, at Denisova Cave in Russia, we detected the DNA of Neandertals, Denisovans and modern humans in 175 out of 728 samples, enabling the reconstruction of the occupational history of the site at an unprecedented level of resolution. In Galería de las Estatuas in Spain, we expanded our search of DNA in sediment from mitochondrial to nuclear DNA, which allowed us to detect a turnover in the Neandertal populations that occupied the site. On a microscale, we analyzed DNA from blocks of sediment that had been impregnated in resin for micromorphological analysis, targeting microstructures derived from bone fragments, coprolites and minerals. Together, the results of these studies show that ancient human DNA is a very small yet frequently detectable component of DNA in Pleistocene cave sediment, that the isolation of the DNA from single individuals is possible from sediment, and that the movement of DNA across archaeological layers is not a common phenomenon. Current efforts focus on the improvement and simplification of methods for the recovery and analysis of DNA sequences from sediment, with the ultimate goal of making sediment DNA analysis a more widely used tool in prehistoric archaeology.

# METABOLIC REPAIR: A NEW APPROACH TO THE TREATMENT OF ABNORMAL GLYCOSYLATION

**Eva Morava-Kozicz**

Mayo Clinic, Department of Clinical Genomics, Rochester, Minnesota, USA

Congenital disorders of glycosylation are a group of genetic disorders that affect protein and lipid glycosylation and glycosylphosphatidylinositol synthesis. For most affected individuals, only symptomatic and preventive treatments are used. Innovative diagnostic approaches using functional glycomics, novel biomarkers, animal models have been developed and our emerging experience with organ transplantation led us to new therapeutic approaches. Most metabolic enzymes demonstrate substrate specificity, including most enzymes of the monosaccharide activation pathways, sugar nucleotide synthesis and transport; pathways, which are very important in the glycosylation process. In most of the cases, one compound is a much better substrate (usually 100- to 1000-fold) than the other structurally similar molecules. The study of inborn errors of metabolism thought us that enzyme specificity is not perfect. Numerous examples exist of "promiscuous" reactions in the context of inborn errors of metabolism, when the concentration of a substrate is highly elevated due to a metabolic block, leading to the production of unexpected metabolites. However, we can also make a use of such a flexibility in treating of inborn errors. The administration of a molecule (e.g., a specific monosaccharide) in high concentration can turn a molecule into a substrate for alternative pathways. In the lack of absolute specificity of metabolic enzymes increasing certain sugar metabolites can activate enzymes of decreased activity leading to suboptimal, but measurable kinetics. Proteins are flexible and amino acid side chains may alter their secondary structure to accept a molecule that is slightly different from the ideal substrate. Emerging metabolic therapies are using this concept in several glycosylation disorders (MPI-CDG, SLC35A2-CDG, SLC35C1-CDG and PGM1-CDG). High throughput drug screens led to unexpected discoveries like the demonstrating the efficacy of epalrestat in altering metabolic flux and enzyme activity in the most common congenital disorders of glycosylation, PMM2-CDG. Here we focus on recent advances in potential therapeutic approaches for CDG.





# EPIGENETIC SIGNATURES OF PSYCHOSOCIAL STRESS AND TRAUMA

**Connie J. Mulligan**

Department of Anthropology, Genetics Institute, University of Florida, Gainesville, FL, USA

The field of social and behavioral epigenetics examines how social and behavioral experiences, such as psychosocial stress, can lead to epigenetic changes. We investigate how psychosocial stress or trauma that is experienced during pregnancy may induce epigenetic marks in future generations. Specifically, we test for associations between maternal stress or trauma, changes in DNA methylation, and health outcomes. Results from two collaborative projects are reported: 1) associations of maternal stress, newborn birthweight, and newborn epigenetic changes in a longitudinal study of mother-infant dyads from the Democratic Republic of Congo (DRC) and 2) investigation of epigenetic signatures of violence trauma in three groups of three-generation Syrian families with contrasting exposures to war violence. DNA methylation (DNAm) data were generated using the Illumina MethylationEPIC BeadChip. In the DRC project, babies at birth (n=66), 6 months (n=7), 1 year (n=34), 2 years (n=31), and 3 years (n=16) were investigated. We identified a signal of epigenetic age acceleration that was significantly correlated with low birthweight and only emerged over time. There was no correlation of maternal stress with newborn DNAm. These results suggest that the impact of low birthweight on adult health may be mediated by epigenetic changes that emerge over the life course. In the Syria project, 45 individuals directly exposed to war violence, 30 prenatally exposed, 15 germ-line exposed, and 45 control individuals were analyzed. Methylation at multiple CpG sites was associated with violence trauma at all three levels of exposure even after strict Bonferroni correction for multiple testing. These results suggest that exposure to violence experienced during pregnancy may impact future generations at the epigenomic level.

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# SINGLE-CELL OMICS FOR FORENSIC USAGE

**Eskeatnaf Mulugeta**

Department of Cell Biology, Department of Genetic Identification, Erasmus University Medical Center Rotterdam (Erasmus MC), Rotterdam, The Netherlands

The separation of individuals from biological mixture and the subsequent genetic characterization and individual identification steps are crucial components of forensic investigation that still pose a significant challenge to the field despite several attempts. Single-cell (multi)omics approaches are an emerging set of tools that are providing an unprecedented level of understanding about cellular identity and processes. In the past few years, several single-cell techniques have emerged that can capture the transcriptome (scRNA-seq), epigenome (e.g., chromatin accessibility, scATAC-seq; DNA-methylation), and genome. These techniques have the potential to solve the challenges that are faced in forensic research, but their adaptation is still lagging behind. Thus, we are exploring the possibility of adapting existing single-cell approaches and develop novel ones for forensic research. First, by using existing scRNA-seq and by developing a novel SNP-based mixture deconvolution bioinformatics pipeline, we succeeded to separate individuals from multi-person blood mixtures according to the individual contributors. In subsequent steps, we were able to determine the sex and biogeographic ancestry (maternal, paternal, and bi-parental ancestry) of the separated individuals and the tissue of origin of the biological mixture. In addition, by comparing the individual SNP profile (from the scRNA-seq) with a reference set (exome-seq), we were able to achieve individual identification of the separated contributors. Next, in order to increase the number of SNPs that can be used for mixture deconvolution and increase the ability of separating more complex mixtures, we tested the possibility of using single-cell scATC-seq and obtained robust separation. At the current state, our novel approach has the potential to identify perpetrators of violent crime from blood mixtures found at crime scenes, while further adaptations may allow moving to other types of biological mixtures. Driven by this success, we are now developing other novel and affordable forensic-specific single-cell methods that will allow determining appearance, ancestry, sex, and other forensically important information.





# IMPACT OF DISORDERED METABOLISM ON 3D GENE REGULATION IN DIABETES

**Tamas Ordog**

Mayo Clinic, Rochester, Minnesota, USA

Diabetes mellitus involves systemic alterations in metabolic functions, which contribute to end-organ dysfunction leading to diabetic complications. We previously reported that reduced mitochondrial gene expression and mitochondrial density in type 1 and type 2 diabetic patients were associated with peripheral autonomic neuropathy and delayed gastric emptying. Investigating the underlying mechanisms, we found impaired oxidative metabolism to associate with reduced physiologimarical hypoxic signaling in human and mouse enteric neurons. Genetic and pharmacological manipulations of hypoxia-inducible factor 1 alpha (HIF1A) revealed a role for HIF1A in the regulation of expression of neuronal nitric oxide synthase (NOS1), the source of the gaseous inhibitory neurotransmitter nitric oxide, an established regulator of gastric emptying. Genome-wide analysis of HIF1A binding in conjunction with chromosome conformation capture identified a role for HIF1A in the regulation of target genes including Nos1 by modifying short- and long-range cis-regulatory interactions in part via cohesin recruitment to loop anchors. Pharmacological upregulation of HIF1A levels with a drug approved for use in humans reversed diabetic gastroparesis in female streptozotocin-diabetic mice. In a parallel line of studies, we investigated the role of mitochondrial dysfunction in interstitial cells of Cajal (ICC), electrical pacemaker and neuromediator cells of the gut, which have also been implicated in diabetic gastroparesis. Genetic deletion of the mitochondrial tricarboxylic acid cycle and electron transfer chain enzyme complex succinate dehydrogenase in ICC altered repressive histone and DNA modifications and Kit expression without any deleterious effects on gastric emptying in normal mice but dramatically increased the prevalence and severity of gastroparesis in female but not male diabetic mice. Together, our studies highlight transcriptional and epigenetic mechanisms downstream of impaired mitochondrial function in diabetic enteric neuropathy and gastroparesis.

# SOLVING THE MYSTERY OF KASPAR HAUSER

**Walther Parson**

Medical University of Innsbruck, Institute of Legal Medicine, Innsbruck, Austria

Molecular genetic identification of historic individuals, also known as Celebrity Genetics, has become a recognized discipline in forensics. These investigations are not only finding interest among a broad audience, the challenging biological material involved often requires the development of alternative technical solutions to yield successful results. Thus, new concepts stimulated by difficult cases have contributed to the field in significant ways. The molecular investigations on remains attributed to Kaspar Hauser perfectly add to this tradition. Kaspar Hauser was a celebrity, the center of curiosity of Germany's Biedermann society, a feature in newspapers and an object of interest to visitors of the city of Nuremburg in the early 19th century. In May 1828, he appeared in Nuremberg seemingly out of nowhere, a lubberly appearance that was barely able to speak and walk. According to his own account, for as long as he could remember, he sat in a small, dark dungeon without ever getting to see anybody else. His only companions were two horses and a dog, all made of wood. While it still remains unclear whether or not his story holds true, the fate of a child growing up in lack of any social contact has been a focus of academic research throughout the past two centuries. As a matter of fact, evidence was brought forward that Kaspar Hauser could have been an abducted prince of the Grand Duchy of Baden, South Germany. In an attempt to shed more light on this assertion, forensic genetic analyses were conducted on samples attributed to him and samples from pedigree members of the House of Baden in the late 1990s and early 2000's. These analyses led to contradictory results. Some of these results were scientifically published, others were only discussed in the media, which left the case unsolved and provided room for speculations. With the emergence of novel forensic genetic methods, including Massively Parallel Sequencing, the case was reopened again in 2019. Old and new samples were investigated using these methods. These analyses not only yielded significant results; they also serve as basis to explain the discrepant data obtained 20 years ago. The new methods and conclusions further provide foreground for the field of forensic genetics and answer some of the questions regarding Kaspar Hauser's descent.





# OSTEOARTHRITIS: FROM MOLECULAR PATHWAYS TO THERAPEUTIC ADVANCES

**Dragan Primorac**[1,2,3,4,5,6,7,8,9,10,11]

[1]St. Catherine Specialty Hospital, Zagreb, Croatia, [2]Medical School, University of Zagreb, Zagreb, Croatia, [3]University of Osijek Faculty of Dental Medicine & Health, Osijek, Croatia, [4]University of Split, School of Medicine, Split, Croatia, [5]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA, [6]The Henry C Lee College of Criminal Justice & Forensic Sciences, University of New Haven, West Haven, CT, USA, [7]The National Forensic Sciences University, Gandhinagar, Gujarat, India, [8]University of Rijeka, School of Medicine, Rijeka, Croatia, [9]Medical School REGIOMED, Coburg, Germany, [10]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [11]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina

Being the most common musculoskeletal progressive condition, osteoarthritis is an interesting target for research. It is estimated that the prevalence of knee osteoarthritis (OA) among adults 60 years of age or older is approximately 10% in men and 13% in women, making knee OA one of the leading causes of disability in the elderly population. Today, it is clear that osteoarthritis is not a disease characterized by loss of cartilage due to mechanical loading only, but a condition that affects all tissues in the joint, causing detectable changes in tissue architecture, metabolism, and function. The pathogenesis of OA is primarily determined by the imbalance of pro-inflammatory and anti-inflammatory mediators, leading to low-grade inflammation, which is responsible for cartilage degradation, bone remodeling, and synovial proliferation. The pathogenesis of this degenerative process is not completely understood; however, a low-grade inflammation leading to an imbalance between anabolic and catabolic processes is a well-established factor. The complex network of cytokines regulating these processes and cell communication has a central role in the development and progression of osteoarthritis. In addition, concentrations of both proinflammatory and anti-inflammatory cytokines were found to be altered depending on the osteoarthritis stage and activity. At the moment, biological treatments such as platelet-rich plasma, bone marrow mesenchymal stem cells, and autologous micro-fragmented adipose tissue (MFAT) containing stromal vascular fraction (SVF) are ordinarily used. The cell-based treatment options seem to be the only methods so far that increase the quality of cartilage in osteoarthritis patients. Mesenchymal stem cell (MScs) research offers new opportunities for osteoarthritis treatment as their paracrine effect exhibits clinical improvement in osteoarthritis patients, providing much-needed minimally invasive treatment options. In my lecture, I will present several prospective, non-randomized, interventional, single-center, open-label clinical studies performed at St Catherine hospital where patients with OA, were treated with the intraarticular application of autologous micro-fragmented adipose tissue (MFAT) containing stromal vascular fraction (SVF). After the treatment, dGEMRIC sequencing was used to analyze the contents of cartilage glycosaminoglycans (GAGs) in specific areas of the treated knee joint as the anionic, negatively charged contrast gadopentetate dimeglumine (Gd-DTPA2–) infiltrates into the cartilage, thus indirectly showing the amount of GAGs in areas of interest at different time points. Our results showed a stable dGEMRIC index in the first 12 months after application of MFAT and a mild decrease in dGEMRIC index after 24 months of application. We believe that molecular changes in the cartilage in patients with OA are mediated by a complex interplay of pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors, and adipokines. Therefore, we recently launched

one of the most comprehensive multi-omic study (Clinical and molecular phenotypization of OA: personalized approach to diagnostics and treatment) on OA patients, aiming to explore cytokines, chemokines, N-glycans, phenylalanine, and miRNA changes in the plasma and synovium before and after intraarticular application of MFAT with SVF in knees of the patients with OA. Simultaneously, we will observe changes in the glycosaminoglycans level (GAG) by using delayed gadolinium (Gd)-enhanced magnetic resonance imaging of cartilage (dGEMRIC), but also, we will perform a standard orthopedic physical examination including KOOS, WOMAC, VAS, CESD-R assessments as well as MRI Osteoarthritis Knee Score (MOAKS).





# AFTER MORE THAN 20 YEARS: UPDATE ON THE DNA IDENTIFICATION OF 9/11 VICTIMS

**Mechthild Prinz**

John Jay College of Criminal Justice, New York, NY, USA

The terrorist attack on the World Trade Center in New York City on September 11, 2001 took the lives of at least 2,753 individuals on the ground and on the two airplanes. As of March 2022, 1,647 or approximately 60% of the victims have been identified. This mass fatality incident was characterized by severe fragmentation, degradation, and destruction of the human remains, which explains why so many of the victims seem to have disappeared. With only 289 intact bodies, but over 20,000 fragments of human remains, the World Trade Center victim identification became a DNA driven effort. Short tandem repeat (STR) analysis was the main tool for typing muscle tissue and bone samples, as well as ante mortem personal effects and buccal references. The project triggered the development of DNA matching software tools and quality review and anthropological verification procedures. Victim identification took place in several distinct phases and is still ongoing today. Systematic resampling of previously unidentified remains has led to additional identifications, with the most recent ones having been reported in September 2021. The talk will provide an overview on lessons learned during the project and present the most recent developments.

# CHARACTERIZING LOCUS SPECIFIC CHROMATIN STRUCTURE AND DYNAMICS WITH CORRELATIVE CONVENTIONAL AND SUPER-RESOLUTION IMAGING IN LIVING CELLS

Dushyant Mehra[1,2], Santosh Adhikari[1], **Elias M. Puchner[1]**

[1]School of Physics and Astronomy, University of Minnesota, Minnesota, USA, [2]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota, USA

The spatio-temporal organization of chromatin is critical for gene regulation. However, simultaneously mapping the structure of chromatin and its dynamics remains a challenge. Conventional fluorescence microscopy in combination with CRISPR/dCas9 labeling has been able to image the spatial distribution and dynamics of loci in living cells, but cannot resolve structural features below ~250 nm. The advent of super-resolution photoactivated localization microscopy (PALM) presented a breakthrough for resolving intracellular structures with up to ~20 nm resolution in fixed cells. However, the motion of chromatin during the long data acquisition time precludes any structural characterization of chromatin in living cells due to motion blurring. Here I will present our correlative conventional fluorescence and PALM imaging approach to quantitatively map time-averaged structure and dynamics of chromatin below the optical diffraction limit in living cells. By employing a repetitive telomere sequence as a well studied model system and by assigning localizations to a telomere as it moves, we reliably discriminate between bound and unbound dCas9 molecules, whose mobilities overlap. Our approach accounts for changes in DNA mobility and relates local chromatin motion to larger scale domain movement. In our experimental system, we show that compacted telomeres move faster and have a higher density of bound dCas9 molecules, but the relative motion of those molecules is more restricted than in less compacted telomeres. Correlative conventional and PALM imaging therefore improves the ability to analyze the mobility and time-averaged nanoscopic structural features of locus specific chromatin with single molecule sensitivity and yields unprecedented insights across length and time scales.





# TARGETING EPIGENETIC REGULATOR MUTATIONS IN KIDNEY CANCER

Ryan T Wagner, Ryan A Hlady, Xiaoyu Pan, Liguo Wang, Sungho Kim, Jeong-Heon Lee, Thai Ho, **Keith D Robertson**

Mayo Clinic, Rochester, Minnesota, USA

Clear cell renal cell carcinoma (ccRCC) accounts for ~75% of kidney cancers and is the 8th leading cause of cancer death in the United States. After completion of The Cancer Genome Atlas (TCGA) Project, clinically actionable mutations were identified in virtually every solid tumor. One major exception, however, is RCC, where the current standard of care, checkpoint inhibitor and anti-VEGF therapy, does not take into account that ~50% of RCCs have mutations in chromatin regulators. After first-line therapy, response rates are 20% and there are no FDA-approved therapies that target chromatin regulators, highlighting the need to identify how epigenome regulator mutations can be therapeutically targeted. The epigenome is profoundly disrupted in cancers including ccRCC, including altered DNA and histone methylation patterns that promote oncogenic transcriptional patterns and elevated DNA damage. Aside from the near ubiquitous loss of VHL, the mutational landscape of ccRCC is dominated by loss-of-function mutations in epigenetic regulators, including SETD2, BAP1, and PBRM1. SETD2, the sole factor responsible for trimethylating the histone H3 lysine 36 position, has been firmly linked to poor outcome and the promotion of metastasis. SETD2 and its mark H3K36me3 have been linked to diverse processes ranging from transcriptional regulation, mRNA splicing, nucleosome positioning, and DNA repair, yet exactly how this regulator and its mark drive cancer phenotypes, particularly in ccRCC, remains unknown. Using a combination of engineered cell line models, biochemical methods, and primary patient tumors, coupled with transcriptome/epigenome analysis and interaction studies, we describe novel ways that SETD2 loss-of-function contributes to cancer initiation and progression. We also probe the interplay among multiple regulators of methylation at the H3K36 position to define novel pharmacologic paradigms that may lead to individualized therapies that target SETD2 mutant tumors.

# MULTIOMICS REVEALS NEW PATHOBIOLOGIC TARGETS FOR ALCOHOL ASSOCIATED HEPATITIS

**Vijay H. Shah**

Mayo Clinic, Rochester, MN, USA

Alcoholic hepatitis (AH) is associated with liver neutrophil infiltration through activated cytokine pathways leading to elevated chemokine expression. Super-enhancers are expansive regulatory elements driving augmented gene expression. Here, we explore the mechanistic role of super-enhancers linking cytokine TNF**α** with chemokine amplification in AH. Our findings highlight the role of super-enhancer in propagating inflammatory signaling by inducing chemokine expression and the therapeutic potential of BET inhibition in AH treatment. Alcohol-associated liver disease (ALD) in its earliest form is evidenced as hepatic steatosis which may progress to liver cirrhosis. The mechanisms behind this initiating insult are poorly understood and therapeutics to treat ALD are limited. Liver is a specialized organ with cells exhibiting heterogeneity along the porto-central axis. Periportal preponderance of lipid droplet accumulation was noted in human ALD livers compared to other clinical causes of hepatic steatosis. Using single-cell multiomics technology, we studied transcriptional mechanisms across the hepatic lobule that could account for liver zonation of lipid droplets in a murine ALD model. We utilized multiomics data to provide novel insight into HNF4**α** and PPAR**α** mediated, zone-specific regulation of HSD17**β**13. We conclude that mechanisms underlying ALD initiate in a zonated manner leading to spatially distinct establishment of hepatic steatosis and provide novel insight into disease pathogenesis.





# THE ORIGINS OF HANSEN'S DISEASE (LEPROSY)

Adele Crane[1], Kelly Blevins[2,3], Felicia King[1], Christopher Lum[4], Kanako Furuta[5], Luciano Nakazato[6], Mrinalini Watsa[7,8], Gideon Erkenswick[7,9], Keolu Fox[10], **Anne Stone[2,11]**

[1]School of Life Sciences, Arizona State University, Tempe, AZ, USA, [2]Center for Bioarchaeological Research, Arizona State University, Tempe, AZ, USA, [3]Department of Archaeology, Durham University, Lower Mount Joy South Road, Durham, UK, [4]Department of Pathology, John A Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, [5]Hawaii Pathologists Laboratory, Honolulu, Hawaii, [6]Department of Veterinary Medicine, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brazil, [7]Field Projects International, Escondido, CA, USA, [8]San Diego Zoo Wildlife Alliance, San Diego, CA, USA, [9]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA, [10]Department of Anthropology, University of California, San Diego, La Jolla, CA, USA, [11]School of Human Evolution and Social Change, ASU, Tempe, AZ, USA

Hansen's disease, also known as leprosy, is caused by the pathogens Mycobacterium leprae and the more recently discovered M. lepromatosis, which is primarily found in Mexico and the Caribbean. Hansen's disease is one of the oldest known human diseases and remains a public health issue today, with over 200,000 new cases reported yearly. Ancient genome analyses show that M. leprae lineages have a most recent common ancestor approximately five thousand years ago. However, the global pattern of genomic variation in M. leprae is not well defined. This is particularly true in the Pacific Islands, where the origins of the pathogen in humans in relation to Colonialism are disputed, and in animals, which are poorly surveyed for this pathogen. To investigate this, we have extracted DNA from 98 formalin-fixed paraffin-embedded biopsy blocks collected between 1992 to 2016 from patients living in the Pacific and from PCR positive samples from 11 species of animals from Brazil. We have also survey small mammals in the Amazon basin of Peru. To date, we have successfully used whole-genome enrichment and next-generation sequencing to generate 12 Pacific M. leprae genomes ranging from 1.6 - 63x depth of coverage. Phylogenetic analyses place these strains in branches 0 and 5, the basal lineages of the M. leprae phylogeny. The phylogeographical patterning and evolutionary dating analysis of these strains support a pre-modern introduction of M. leprae into the Pacific Islands. We have also expanded this work by including time-series samples from patients during treatment and will use both empirical data and modelling to identify ongoing selection. In Peru, the 92 small mammals tested to date have been negative using the RLEP qPCR assay, while initial capture of the Brazilian samples is in progress. This research provides insight into the evolutionary history of M. leprae and the exchanges of this pathogen among species.

# GENES, CULTURE, AND HUMAN EVOLUTION

**Mark Stoneking**[1,2]

[1]Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, [2]Université Lyon 1, CNRS Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne, France

A commonly-held view is that humans have stopped evolving because we rely on culture to adapt to changing circumstances. However, an alternative view is that culture can also influence human evolution. I will show, by way of examples, that some cultural practices have directly impacted specific genes, while others have indirectly influenced patterns of genetic variation. Furthermore, given that some cultural practices have genetic consequences, we can use genetic analyses to learn more about such cultural practices; as an example, I will discuss a genetic approach to dating the origin of clothing.





# INFERRING GENETIC RELATIONSHIPS FROM WHOLE GENOME SEQUENCES - A FORENSIC PERSPECTIVE

**Andreas Tillmar**[1,2]

[1]National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden, [2]Linköping University, Faculty of Medicine and Health Sciences, Department of Biomedical and Clinical Sciences, Linköping, Sweden

Being able to determine genetic relationships between individuals has long been important in legal medicine and forensic applications, like paternity testing and missing person identification. Current practice typically involves DNA analysis of a small number (around 20) of short tandem repeat markers, which usually is sufficient to establish close relationships. Recent advances in DNA typing technologies have however made it possible to a relatively low cost, with increased sensitivity and with high quality, obtain much more DNA information from a single DNA analysis. This opens up new possibilities for inferring distant relationships but also for introducing new forensic applications such as investigative genetic genealogy. Large DNA datasets may require new methods for the relationship inference and for the assessment of the statistical weight. This presentation will provide an overview of such methods and show how large DNA datasets, obtained from whole genome sequencing, could be used to infer genetic relationships in legal and forensic settings.

# REGULATION AND DEREGULATION OF DNA EPIGENETIC MARKS

**Natalia Tretyakova**

University of Minnesota, Minneapolis, MN, USA

Reversible DNA methylation allows for the precise activation and inactivation of genes in a tissue-specific manner by mediating DNA-protein interactions and influencing chromatin structure. 5-Methylcytosine (5mC) marks are introduced by DNA methyltransferases (DNMT), while DNA demethylation is initiated by ten eleven translocation (TET) dioxygenases which oxidize 5mC to hydroxymethyl-C (hmC), formyl-C (fC) and carboxy-C. While DNA methylation patterns are stable in healthy somatic tissues, they can become scrambled as a result of inflammation, environmental stress and chemical exposures, contribution to cancer initiation. Recent studies revealed that many other diseases including asthma, Alzheimer's disease, and autism have epigenetic drivers. My laboratory investigates the potential role of DNMT and TET proteins in inflammation-mediated colon cancer and smoking induced lung cancer. We have conducted animal studies to show that chronic inflammation and chronic exposure to cigarette smoke cause dramatic changes in DNA hydroxymethylation, gene expression changes, and aberrant protein expression. Mechanistic studies with recombinant proteins probes potential mechanisms for the observed epigenetic changes, while CRISPR-cas 9 gene editing and RNA interference studies are starting to reveled the functional roles of the affected genes in lung cancer. We employed structure-based design to develop small molecule inhibitors of TET proteins, which can be used as chemical probes to investigate the functions of DNA demethylating enzymes in lung cancer and could comprise initial leads for future drug design. Finally, we are investigating reversible cross-linking between fC in DNA and histone proteins as a potential novel mechanism of epigenetic regulation.





# DEVELOPMENTS TOWARDS PERSONALIZED EPIGENOMIC PROFILING IN FORENSICS

**Athina Vidaki**

Erasmus MC, University Medical Center Rotterdam, Department of Genetic Identification, Rotterdam, The Netherlands

Human genetic variation is a major resource in forensics, but does not always allow us to answer crucial forensically relevant questions. Since the epigenome acts as an interphase between the fixed genome and our dynamic environment, it offers possibilities to address many questions regarding an individual's phenotype. Back in 2017, we proposed that, together with genetic prediction of appearance and biogeographical ancestry, DNA methylation-based ageing and lifestyle habit prediction is expected to increase the ability of narrowing down suspect pools. However, there are still various challenges to be addressed prior to implementing such approach in forensics. During this talk, I aim to present our recent developments towards offering a personalized epigenomic fingerprint. First, to consider the issue of dealing with heterogeneous material, we focused our efforts investigating age-associated patterns of the Y-chromosome, in both blood and sperm. We envision that a future Y-chromosome based age prediction tool would allow us to estimate the age of males from mixed samples, often encountered in sexual assault cases. Secondly, to apply already existing knowledge on lifestyle-associated DNA methylation, we built and thoroughly validated statistical models for the prediction of both tobacco smoking and alcohol consumption habits. In the case of smoking, we also developed a targeted lab method based on next-generation sequencing, which we optimized and tested in a population cohort. Thirdly, to improve DNA methylation detection towards standardization, we developed and validated a novel, patent-pending tool for assessing the initial step of bisulfite conversion, which is currently the golden standard. Finally, to deal with the limited amount of available human biological material, we are currently focusing in developing a new technology, CpGtracer that will soon allow us to simultaneously analyze hundreds of DNA methylation markers from trace amounts of DNA. Overall, while there are still additional considerations to tackle, including privacy, ethical and legal concerns, we are confident that a broadened DNA-based forensic intelligence including epigenomic profiling will soon become valuable, especially during criminal investigations with unknown suspect(s).

# CANCER PHARMACOGENOMICS: DISCOVERY AND TRANSLATION

**Liewei Wang**

Mayo Clinic, Rochester, MN, USA

A major challenge facing genomics science is understanding and predicting how sequence variation in noncoding regions of the genome might contribute to variation in gene regulation, variation that could result in variations in various phenotypes either cellular or clinical phenotypes. This challenge is highlighted by the fact that approximately 90% of genome-wide association study (GWAS) single nucleotide polymorphism (SNP) signals map outside of protein coding genes (1-3). A significant advance has been our recognition that many of these GWAS SNP signals locate in the non-coding regions, regulating gene expression through enhancer, so called "expression quantitative trait loci" (eQTLs), SNPs that are associated with variation in gene expression (3, 4). Here, we will present a novel mechanism that we have identified repeatedly for these SNPs using breast cancer clinical trial DNA samples to understand how the SNP might influence drug response, both efficacy and toxicity. What we have found is that the SNP effect on gene expression, leading to different clinical outcome, is dependent on the presence of drugs/hormones mediated though nuclear receptors such as the estrogen and glucocorticoid receptors. This type of SNP-gene expression relationship is often not present or is much less significant at baseline—ie before ligand (either agonists or antagonists) exposure, and occurs only in the presence of a drug or hormone. This type of SNP- gene expression relationship highly depends on the presence of individual endogenous or exogenous compounds, so called PGx eQTL, which can occur often and can be highly significant functionally. As a result, we could potentially take advantage of specific SNP genotypes to manipulate gene expression by exposure to various compounds. These SNPs could be also used as biomarkers associated with various clinical phenotypes.





# SIGNAL-DEPENDENT CHROMATIN REMODELING IN METABOLISM AND INFLAMMATION

Liu Wang, SM Bukola Obayomi, **Zong Wei**

Mayo Clinic, Department of Physiology and Biomedical Engineering, Scottsdale, AZ, USA

In type 2 diabetes (T2D), inflammation induces massive changes in the transcriptome and epigenome of islet endocrine and immune cells, resulting in eventual dysfunction of islets. We identified a novel mechanism of vitamin D-dependent chromatin accessibility dynamics, orchestrated by the balance between two SWI/SNF chromatin remodeling complexes, BRD9-BAF and BRD7-PBAF, in regulating islet dysfunction. In beta cells, the balance between BAF-BRD9 and PBAF-BRD7 determines the VDR-driven anti-inflammatory and pro-survival response. Pharmacologically potentiated VDR signaling by a synthetic ligand in combination with a BRD9 inhibitor can partially restore beta cell function and glucose homeostasis in various T2D mouse models. Tissue specific genetic models further demonstrated the functional role of VDR and BRD9 in beta cell stress response in vivo. Recently, we also identified BRD9 as a modulator for glucocorticoid responses in macrophages. Pharmacologic inhibition of BRD9 potentiated the anti-inflammatory responses of dexamethasone. Mechanistically, BRD9 co-localized at a subset of GR genomic binding sites, and depletion of BRD9 enhanced GR occupancy primarily at inflammatory-related genes to potentiate GR-induced repression. Together, our results revealed the context-dependent function of specific SWI/SNF subunits on VDR/GR activity, and demonstrated the therapeutic potential of targeting bromodomain readers to synergistically enhance NR function.

## NEANDERTAL GENE VARIANTS AND COVID-19

**Hugo Zeberg**[1,2]

[1]Karoliniska Institutet, Department of Neuroscience, Stockholm, Sweden, [2]Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, Leipzig, Germany

Early in the COVID-19 pandemic, it was clear that SARS-CoV-2 infection tend to have drastically different outcome in different people. Whereas some patients suffer only mild disease, others become critically ill. Some risk factors, such as high age and metabolic syndrome, can explain some of the variability but far from all of it. Therefore, host genetic risk factors have been investigated by several large studies, which have been successful in identifying more than 20 risk genetic risk factors influencing the outcome of COVID-19. In this talk I will describe the evolutionary history and the Neandertal origin of two of the major genetic variants influencing the outcome of COVID-19.

# HIGH SCHOOL STUDENT FUTURE SCIENTIST AWARD PRESENTATIONS

Presentation number: FSA 1

# DAMAGE TO HAIR CELLS DURING SPINNING IN RELATION TO NUTRITION (VESTIBULOCOCHLEAR ORGAN MODEL)

**Filip Bulat**

VII. Gymnasium, Zagreb, Croatia

The sensory receptors of vestibulocochlear organ in humans are hair cells which transmit the stimulus via nerves to the brain. Evolutionary, they are of ectodermal origin. Many diseases affect these cells resulting in degradation and loss of function in vestibulocochlear system. We were interested in knowing how starvation, i.e., malnutrition affects ciliates Paramecium sp. The purpose of the research was to experimentally test the reaction of malnourished Paramecium sp. (starving Paramecium) to spinning, which is a physiological stimulus for the vestibular organ. The same volume of Paramecium culture medium of good physiological condition with optimal concentrations of oxygen in the water was placed in 10 test tubes marked with letters A1 to A5 and B1 to B5. The culture (Paramecium sp.) that was fed was marked group A, while the malnourished culture was marked group B. The test tubes were kept on a rack. The same volume of sample was added to each test tube. Immediately before spinning each tube was tightly screwed and put in a separate small transparent bag tied at the top with the wool string 10 cm long. Bags were evenly spun for 5 minutes. The test tubes were then placed back on a rack and after 10 minutes a sample from the test tube was taken with a pipette and put in a Petri dish on the bottom of which a net for counting Paramecium sp. sized 1cm x 1cm was drawn. The Petri dish was then filled with a sample of Paramecium sp. culture medium to the 3 mm mark. The experiment was conducted in the following order: A1 and B1 were not exposed to trauma, and they represent control samples, while other samples were exposed to spinning: A2 and B2: on the 1st, 4th, and 8th day of the experiment, A3 and B3: on the 1st, 3rd, 5th, 7th, and 8th day, A4 and B4: one spin each day, A5 and B5 two consecutive spins each day. The proportion of dead Paramecium in a given sample volume during 8 days after spinning was measured in relation to the control sample. Compared to control sample in test tube A2, 18,33% specimens die. In test tube B2 20,83% specimens die. In both samples the majority of Paramecium survives and has proper mobility. The total number of live specimens in sample B2, on the eighth day of the experiment, is smaller in relation to the total number of live specimens in sample A2 on the eighth day of the experiment. 40.67% of Paramecium die in sample B3 after eight days, while 28,21% of Paramecium die in sample A3. 43,75% of Paramecium die in sample B4, and 44,83% of Paramecium die in sample A4. Test tube B5 has 50,21% of dead Paramecium, while 42,54% of Paramecium die in sample A5. Results show that better nutrition leads to better resistance to physiological, and especially to extreme stimuli, and the survival of the cells is thus greater. Since there is no data on the subject that was analyzed, it is assumed that better condition of well-nourished specimens results in better resistance to damage; therefore, sensory receptors that have proper nutrition (blood flow) are more resistant to damage and degradation of function in vestibulocochlear system.

Presentation number: FSA 2

# THE COMPARISON BETWEEN CURRENT TREATMENTS FOR EARLY ALZHEIMER'S DISEASE

**Ante Čolak**

II. Gymnasium, Zagreb, Croatia

Detailed study and analysis of the accession literature on the basis of which the discussion of the comparison was made. Aducanumab is effective in removing **β**-amyloid plaque, but as an anti-AD drug it is not effective in preventing or alleviating symptoms. On the other hand, symptomatic therapies are effective in alleviating the cognitive decline caused by neurodegeneration, but are not effective in preventing the development of AD. The first, the PRIME test, showed that aducanumab reduces amyloid PET SUVR findings 15 within 54 weeks of treatment. Due to the promising results, Biogen decided to conduct two new studies: EMERGE and ENGAGE. Moreover, EMERGE met its goal: subjects treated with the highest dose, 10 mg/kg, had a significantly better result (30% better) measured on the Clinical Dementia Rating Scale (CDR-SB). Furthermore, participants in the ENGAGE study improved their cognitive performance by 27% measured by CDR-SB. Current treatments for Alzheimer's disease vary from symptomatic treatments to the drug known as aducanumab which reduces the **β**-amyloid plaques that are considered to be one of the causes of this neurodegenerative disease. Due to the insufficient improvement of cognitive abilities among patients, aducanumab has proved to be insufficient treatment for cognitive manifestations of the disease. Comparatively, symptomatic treatments, like acetylcholinesterase inhibitors and NMDA receptor antagonists, help patients improve their cognitive capabilities, meaning they are more effective than aducanumab regarding the symptoms. An indirect benefit of the discovery of aducanumab is shaping the course of Alzheimer's research.





Presentation number: FSA 3

# THE IMPACT OF DISINFECTANTS ON DIFFERENT TYPES OF BACTERIA AND BACTERIAL RESISTANCE

**Mei Đulabić Chalfe**

V. Gymnasium, Zagreb, Croatia

The aim was to determine how everyday and ubiquitous disinfectants affect different types of bacteria and whether different cosmetic ingredients in some antibacterials such as hand gel affect the growth of different types of bacteria positively or negatively. Recognize if the resistance of different bacteria to the same disinfectants differs. Also, the aim of the study was to determine whether Salmonella's resistance to disinfectants differs from other gram-negative bacteria. Collecting swabs from 7 places then applying diluted solutions of these swabs to various selective and differential nutrient substrates (Plate Count agar, Mac Conkey agar, BD Salmonella Shigella Agar). Placing Petri dishes in an incubator and calculating the CFU before and after using the disinfectant. Gram-negative bacteria were on average more susceptible to disinfectants than other bacteria. The most effective disinfectant was 70% ethanol alcohol. The least effective disinfectant was antibacterial wipes. Salmonella was more resistant to disinfectants than other gram-negative bacteria. The main conclusion of the study is that all types of bacteria have shown some kind of resistance to the disinfectants used, which coincides with the read literature and hypothesis.

Presentation number: FSA 4

# THE INFLUENCE OF 21 CENTURY ON HIGH SCHOOL STUDENTS' MENTAL HEALTH

**Robertina Filković**

V. Gymnasium, Zagreb, Croatia

This research is carried out to confirm the fact that social networks and school have negative influence on adolescents' mental health. The fact is proven by two hypotheses: 1. Social networks have negative influence on high school students' mental health. 2. The pressure made for better academic accomplishment leads to higher number of suicidal high school students. The research is carried out with two questionnaires. First questionnaire, which was carried out at the end of the January, was solved by 374 high school students. Second questionnaire is carried out to establish how much does school affect students' mental health. It was carried out in mid-April and was solved by 238 students. Results from first questionnaire show that today many high school students have symptoms of mental illnesses. The main cause is technology. According to answers social networks cause development of anorexia nervosa and bulimia nervosa. On average, school success is important to examinees and among those who said that school success is very important 23 think about suicide and 14 resort to self-harm. Depression symptoms mostly appear at the examinees who engage with sport. The results show that high school students are getting suicidal, and they are finding help in illicit substances. They are isolating themselves and their social life and academic success are failing. To reduce the number of high school students who are suffering from mental illnesses, parents and students' education is needed.





Presentation number: FSA 5

# REVIEW OF MICROPLASTICS AND NANOPLASTICS EFFECT ON HUMAN HEALTH AND EPIGENETICS MODIFICATIONS

**Lucija Glavičić Marović**

XV. Gymnasium, Zagreb, Croatia

This essay aims to review the current body of knowledge on the effects of microplastics and nanoplastics on human health. The additional objective is to discuss the possible impact of microplastics and nanoplastics on human epigenetic modifications. An electronic search of published articles was conducted in PubMed and Google Scholar using the following keywords: "microplastics human health", "microplastics epigenetic", and "microplastics epigenetic modification human". Microplastics can pose risks to human health in three ways: as physical particles, chemicals, or microbial pathogens found in biofilms on microplastic particles. The most important entry route to the human body is ingestion. Only small microplastic particles can be absorbed (<150 μm can penetrate the gastrointestinal epithelium, <100 nm can penetrate the dermal barrier, <10 μm can be absorbed through the alveolar epithelium). Potential harmful pathways to human health include gut and lung inflammation, oxidative stress and cytotoxicity, translocation, metabolism and energy homeostasis, neurotoxicity, reproductive toxicity and carcinogenicity. Prenatal and neonatal exposure to certain chemicals forming plastics (bisphenol A, phthalates, and others) can cause not only epigenetic changes, but also genetic and morphological changes. The potentially harmful effects are determined mainly by levels of exposure, concentration and individual susceptibility. Growing evidence suggests that increased exposure to microplastics can increase incidence in gut and lung inflammation, obesity, immunological disorders, neurological diseases, cardiovascular diseases and cancer. The increasing exposure of humans to microplastics calls for further research to understand its impact better.

Presentation number: FSA 6

# INFLUENCE OF NATURAL AND COMMERCIAL DISINFECTANTS ON BACTERIAL GROWTH AND DEVELOPMENT

**Đurđica Kovačić**

III. Gymnasium, Split, Croatia

The aim of the research was to determine which of the agents used has antibacterial properties and which disinfectant is the most effective when it comes to bacterial growth inhibition. An experiment was conducted comparing the effects of commercial disinfectants (soap, antibacterial soap, antibacterial hand gel) and natural disinfectants (aqueous clove extract, aqueous garlic extract, lavender oil, immortelle oil) on bacterial growth and development. Samples were taken from the classroom handle. Swabs were inoculated into tripton soy broth, after incubation decimal dilutions were made which were then inoculated on nutrient agar. After incubation of nutrient agar, colonies were counted. The following parameters were changed in the experiments: incubation time of tripton soy broth and nutrient agar, type of disinfectant and volume of disinfectant. The results showed that lavender oil was the most effective in inhibiting the growth of bacterial colonies, while antibacterial hand gel was the least effective. Disinfectants are more effective when placed in a larger volume (1 ml) than in a smaller one (0.2 ml), and pentanol-based antibacterial soap inhibits bacterial growth better than regular sodium benzoate-based soap. In conclusion, all disinfectants used showed antimicrobial properties. In further research, it would be desirable to investigate the disinfectant effect of lavender oil on specific species of bacteria and compare the results with other natural disinfectants. The impact of the active substance on soap effectiveness could also be researched in the future.





Presentation number: FSA 7

# EFFICIENCY AND FUNCTION OF SUNSCREEN

**Nika Adriana Marijanović**

Classical Gymnasium, Zagreb, Croatia

Sunlight consists of different spectrums of radiation such as visible light, ultraviolet (UV) and infrared light. Light is measured in wavelengths (**λ**), and the unit of measurement is nanometer (nm) and millimeter (mm). Different light radiations in the spectrum have different wavelengths. Sunlight can have positive and negative effects on human health. Negative effects can overshadow the positive effects with a lack of caution when exposed to sunlight for longer periods of time. Some of the consequences can be burns, premature skin aging, hyperpigmentation, allergic reactions and more serious conditions such as skin cancer. These kind of skin damages caused by the sun are mainly due to UV rays. There are many different products on the market that can protect our skin from damaging rays. The sun protection factor rating (SPF) system for sunscreens is based on the level of UVB protection offered by the product. SPF multiplied by natural skin protection in minutes determines the maximum length of time you can expose your skin to the sun without the risk of UVB-induced skin damage. However, SPF protection depends on the use of the right amounts (2 $mg/cm^2$) of the product, therefore 60% of the time allowed by SPF is recommended. The aim of this experiment was to examine the effectiveness and quality of different sunscreens. The hypothesis of this research was that sunscreens with a higher factor will provide better cell protection. For this experiment, a total of 6 different sunscreens was used, of which 3 with a factor of 50 and the other 3 with a factor of 30. The MTT test, a standard test that measures the survival (viability) of cells after a treatment was used in the study to measure cell survival after exposure to UVB radiation. The best cell protection was provided by Eucerin 30 cream with a viability of 111.35%, followed by Vichy 50 (101.39%), the third Avon 50 (98.59%), followed by Eucerin 50 (95.64). %) and with lower viability Nivea 30 (79.3%) and Clarins 30 (78.02%). After all the information gathered from this experiment, I can conclude that the sunscreen Eucerin 30 provides the best protection against UV radiation, and Clarins 30 the worst, even though it is the most expensive product. I have also noticed that creams with a thicker composition also have a better effect. Spray sunscreens such as Nivea 30 and Clarins 30 provided the weakest protection.

Presentation number: FSA 8

# INFLUENCE OF EUTROPHICATION ON CETINA RIVER MICROORGANISMS

**Frane Marušić**

Jure Kaštelan High School, Omiš, Croatia

The aim of the study was determining the effects of anthropological eutrophication on microorganisms in the Cetina River. Methods used were the following: smear method, incubation, rapid KOH test (determination per gram), flow cytometry and morphological characterization of bacteria. Compared to the data from the water sample collected five kilometers before the mouth of Cetina, quantitative data from the flow cytometer showed a decrease in the total amount of bacteria at the mouth of the river Cetina, where the city of Omis is located, but also a marked increase in bacterial predators (HNF) and cyanobacteria. Comparing these data with other research, it was found that eutrophication is a possible impact on the differences observed in the entire aquatic ecosystem and the food chain of the Cetina River. On the other hand, the characterization of bacteria showed that there is no significant difference between the types of bacteria at the stations, which leads to the conclusion that eutrophication does not affect the diversity of bacteria at the Cetina river. Humans have a great impact on the ecosystem of the Cetina river and are one of the causes of significant changes in the community of microorganisms. Although eutrophication is not yet strongly expressed in the Cetina River, it is necessary to protect Cetina so that more negative consequences do not follow, such as the development of infectious diseases, algae blooms, and degradation of recreational opportunities.





Presentation number: FSA 9

# S IS FOR STIRRING THE SENSES (NOT THE MUSHROOM SOUP)

**Nika Miličević**

V. Gymnasium, Zagreb, Croatia

The aim of the research is to determine whether the application of different types of synesthesia will improve the performance of three tasks related to memory, concentration, and visual perception in high school students. The intention is also to compare the effects of synesthesia exercises in 1st and 3rd-grade students and to compare the results of girls and boys in the experimental groups. The study involved 200 high school students: 100 1st grade students and 100 3rd grade students with equal proportions of boys and girls. Students were divided into control and experimental groups and solved tasks for examining memory, concentration, and visual perception in separate classrooms. The first experiment examined whether colors help with grapheme memorization, the second whether drawing and eating candy help concentrate-, and the third experiment whether sound and touch improve visual perception. Each experiment lasted 60 seconds. The results were processed by a two-way t-test in Excel. Processing the results, it turned out that the differences between the 1st-grade groups were not, and between the 3rd-grade groups were statistically significant, and that the 1st-grade girls in the concentration and visual perception experiments had higher results than the 1st-grade boys. Synesthesia was found to help 3rd graders solve tasks, but not the 1st graders. It turned out that only older boys were significantly better in all experiments compared to younger 1st graders and that 1st-grade girls were significantly better only in memory and concentration experiments than 1st-grade boys.

Presentation number: FSA 10

# DIFFERENCES IN THE HUMAN JAW OF THE MODERN HUMAN FROM AUSTRALOPITHECUS AFRICANUS

**Goran Narančić**

XV. Gymnasium, Zagreb, Croatia

The aim of this investigation is to establish the presence of metric trends in hominid dental evolution by comparing the teeth measurements of gypsum jaw models of modern humans from Western Balkans with the measurements of Australopithecus from Wolpoff's data. The method of measuring the dimensions of the teeth is based on the Lundstrom's method. Mesiodistal (in the direction of the tongue, "from the back to the front") width of tooth's crown and its breadth (from right to left) were measured with a caliper. For the teeth in the same part of the jaw (upper and lower) and of the same type and position (for instance the second incisor or first molar) the arithmetic mean of all measurements for that tooth was calculated and the standard deviation as well. The incisors in the modern human samples were significantly smaller than the ones in Australopithecus, while the difference was significantly larger in the second incisor than in the first one. In Australopithecus the canines are as much as 18% larger than in the modern human sample. The premolars of Australopithecus are significantly greater than those of the modern man, but the second premolar is much smaller than the first one in modern human. The first molar is significantly larger in Australopithecus than in the modern man. There is a clear metric trend of a decrease in teeth size in the evolution of the human jaw on the sample of the people from the Western Balkans and some teeth have shrunk more than others. It is to be assumed that the same trend will keep up in the future if the assumed causes of this trend are true and if the sedentary lifestyle carries on as it is.





Presentation number: FSA 11

# COELIAC DISEASE

**Rea Pešušić**

Vladimir Prelog Science School, Zagreb, Croatia

The aim of the study was designing a preparation with a therapeutical effect on coeliac disease based on genetically modified lactobacilli in mixture with bifidobacteria. The methods used were genetic modification of selected LABs (e.g. L. gasseri, L. johnsonii L. reuteri, L. acidophilus) caring genes for gluten proteases and preparation of mixture ratios of modified LABs and bifidobacterial BIF with prebiotic (Bifidobacterium longum and Bifidobacterium bifidum) for testing in mice animal model for coeliac disease. The results included genetic modification of LABs: cloning gene for gluten processing proteases; expression vector (such as an RCR – rolling cycle replication, which rapidly synthesizes plasmids - replicon) containing cloned gene; inducible promotor nisin-controlled gene expression system (NICE), transcriptional terminator and ribosome binding site to ensure optimal transcription and translation; cloned genes for glycosylation to enable post-translational modifications and functionality of produced enzymes. Mixtures of LABs and BIF in different ratios together with prebiotic for their metabolic stimulation orally applied in mice should be tested over time tracking the changes of the gut microbiome and gluten degradation, as well as regular blood tests to check the antigen levels. Genetically modified LABs orally inserted into the gut biome could be the best and safest step in the direction of developing a drug for celiac disease. I propose a cloning vector based on a RCR replicon, inserted by electroporation, with a promoter belonging to NICE, which would allow LABs to produce gluten-specific proteases, and would possibly help replenish the gut biome, if they were introduced in a cocktail with bifidobacteria.

# YOUNG SCIENTIST AWARD PRESENTATION

Presentation number: YIA 1

# NON-DESTRUCTIVE EXTRACTION OF ANCIENT DNA FROM BONE AND TOOTH ARTEFACTS

**Elena Essel**[1], Elena I. Zavala[1], Ellen Schulz-Kornas[2,5], Anna Schmidt[1], Birgit Nickel[1], Julia Richter[1], Sarah Nagel[1], Mateja Hajdinjak[1,3], Marie Soressi[4], Matthias Meyer[1]

[1]Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, Leipzig, Germany, [2]University of Leipzig, Department of Cariology, Endodontology and Periodontology, Leipzig, Germany, [3]Francis Crick Institute, London, UK, [4]Faculty of Archaeology, Leiden University, Leiden, the Netherlands, [5]Max Planck Institute for Evolutionary Anthropology, Department of Human Evolution, Leipzig, Germany

In contrast to skeletal remains and sediments, which are widely used sources of ancient faunal and human DNA, human-made artefacts from Palaeolithic sites are not commonly utilized for genetic investigations into the past. We hypothesized that tools and ornaments prepared from faunal bones and teeth may not only preserve DNA from the animals they belonged to, but could also trap DNA from the hominin individuals who made and/or used them. As such artefacts are often too precious for destructive sampling, we developed a non-destructive DNA extraction method that uses a temperature-controlled release of DNA by immersing artefacts in phosphate buffer. We then applied this method to a set of ten Pleistocene bones and teeth that were similar in size and shape to materials typically used for artefact production. Quantitative 3D surface texture measurements conducted before and after DNA extraction showed no substantial surface alterations, in contrast to another method previously suggested for non-destructive DNA extraction. When applied to a set of eleven artefacts from the Châtelperronian layers of the Quinçay site in France (excavated and cleaned more than 30 years ago), our method enabled the recovery of ancient mammalian mitochondrial DNA from two of the samples. DNA sequences were assigned to Cervidae and Elephantidae, in agreement with the morphological identification of the artefacts. Alas, the majority of sequences (70.9 and 98.3%) originated from recent human DNA contamination introduced during and after excavation, hampering our ability to detect traces of ancient hominin DNA that may point to the makers/users of these artefacts. In summary, we present a method for isolating DNA from ancient artefacts prepared from bones and teeth while preserving not only their visual appearance, but also their structural integrity. Moreover, we demonstrate that our method is in principle suitable for identifying the source material of artefacts in cases where morphological identification is difficult. Further investigations would require both, material excavated under cleaner conditions (e.g., using gloves) as well as limited subsequent handling to increase the probability that DNA from the makers/users of the artefacts can be recovered.

**Keywords:** Non-destructive DNA extraction, temperature-controlled extraction, artefacts, ancient DNA

Presentation number: YIA 2

# A COMPREHENSIVE PHARMACOGENOMIC MULTI-GENE PANEL ANALYSIS IN CLINICAL PRACTICE, EXPERIENCE FROM ST. CATHERINE HOSPITAL

**Vid Matišić**[1], Petar Brlek[1], Vilim Molnar[1], Eduard Pavelić[1], Martin Čemerin[2], Kristijan Vrdoljak[2], Andrea Skelin[1,3], Damir Erceg[1,4,5,6], Davor Moravek[1], Ivana Erceg Ivkošić[1,6], Dragan Primorac[1,6,7,8,9,10,11,12,13,14]

[1]St. Catherine Specialty Hospital, Zagreb, Croatia, [2]Medical School, University of Zagreb, Zagreb, Croatia, [3]Genos Glycoscience Research Laboratory, Zagreb, Croatia, [4]Srebrnjak Children's Hospital, Zagreb, Croatia, [5]Croatian Catholic University, Zagreb, Croatia, [6]University of Osijek Faculty of Dental Medicine & Health, Osijek, Croatia, [7]University of Split School of Medicine, Split, Croatia , [8]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA, [9]The Henry C Lee College of Criminal Justice & Forensic Sciences, University of New Haven, West Haven, CT, USA , [10]The National Forensic Sciences University, Gandhinagar, Gujarat, India, [11]University of Rijeka, School of Medicine, Rijeka, Croatia, [12]Medical School REGIOMED, Coburg, Germany, [13]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [14]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina

The field of pharmacogenomics is still in its early stages. However, multi-gene panel-based pharmacogenomic tests are readily available for both clinicians and patients. In the Republic of Croatia, single-gene testing has been available for over a decade; however, commercial panel-based tests targeting multiple genes known to influence drug response is a new concept that was implemented in 2018 at St. Catherine Hospital. This cross-sectional study aimed to report the prevalence of actionable pharmacogenetic interventions in patients who had undergone pharmacogenetic testing using the RightMed 27-gene panel. Retrospective analysis of single-center electronic health records was performed, including a total of 319 patients. Patients underwent pharmacogenomic testing by the RightMed panel using a TaqMan quantitative real-time PCR method and copy number variation (CNV) analysis to determine the SNPs in the 27 targeted genes from 2018 until 2022. Actionable drug-gene pairs were found in 235 (73.7%) patients. Relevant guidelines on genotype-based prescribing were available for 133 (56.7%) patients at the time of testing. Based on the patients' genotype, 139 (43.6%) patients were using at least one drug with significant pharmacogenetic interactions, potentially predisposing them to adverse drug reactions or lack of therapeutic response. Two out of three patients in our practice were found to have at least one gene-drug interaction; therefore, the next step in personalized medicine is integrating pharmacogenomic data into patients' electronic health records to optimize drug therapy.

**Keywords:** pharmacogenomics, clinical application, adverse drug reactions





Presentation number: YIA 3

# FUNCTIONAL VALIDATION OF GWAS HITS ASSOCIATED WITH IgG GLYCOSYLATION USING CRISPR/Cas9 TRANSIENT EXPRESSION SYSTEM

**Karlo Miškec**[1], Anika Mijakovac[1], Azra Frkatović[2], Jasminka Krištić[2], Ana Vujić[1], Gordan Lauc[2,3], Aleksandar Vojta[1], Vlatka Zoldoš[1]

[1]University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Zagreb, Croatia, [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia, [3]University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

Alternative glycosylation of immunoglobulin G (IgG) Fc region has a crucial role in defining pro- or anti-inflammatory effector function of the antibody. Gene network involved in regulation of IgG glycosylation is still poorly understood because glycoyltransferases and glycosydases are not the major players in this regulatory process. In this study we functionally validated gene loci associated with IgG glycosylation in previous genome-wide association studies (GWAS). We utilized established stably transfected cell line FreeStyle™ 293-F with CRISPR/dCas9 fusions for direct regulation of genes targeted with specific sgRNAs. This cell system was designed to secrete IgG molecules so that glycans on IgG can be analysed following gene manipulations. We manipulated 22 GWAS hits, grouped according to glycosylation traits such as galactosylation, fucosylation, sialylation and bisecting GlcNAC. Following gene expression manipulations, IgG glycosylation was analysed. Out of seven genes associated with galactosylation, only MANBA, HIVEP2, TNFRSF13B and EEF1A1 showed change of agalactosylated structures when upregulated using dSaCas9-VPR. Out of six hits associated with fucosylation, only upregulation of TBX21 and TBKBP1 showed significant changes in galactosylation, but no change was observed for fucosylated glycan structures. These results suggest that a different cell model might be preferable to validate different GWA hits, such as Lymphoblastoid Cell Line (LCL) which is known to be rich in fucose glycan structures. Out of five loci associated with bisecting GlcNac, upregulation of KDELR2 resulted in an increase in biantennary glycan structures with bisecting GlcNac. The upregulation of DERL2 and RRBP1 resulted in an increase of digalactosylated biantennary glycans. Out of four GWA hits for sialylation, only downregulation of SPPL3 led to hyperglycosylation with concomitant increase in sialylated and galactosylated structures and decrease in agalactosylated glycans, despite the fact that all genes were successfully up- or downregulated. Overall, these results have proven the functional role of several GWA hits which are not glycosyltransferases but are associated with the IgG glycosylation pathway. Ongoing research on LCL cell line might unravel the exact role of these and other GWAS hits.

**Keywords:** CRISPR/dCas9, IgG glycosylation, FreeStyle™ 293-F Cells, GWAS

Presentation number: YIA 4

# MOLECULAR CHARACTERIZATION OF OCULOAURICULOVERTEBRAL SPECTRUM IN A GROUP OF EGYPTIAN PATIENTS

**Amira Nabil**[1], Aurélien Trimouille[2], Sahar El Shafei[1], Nihal El Shakankiri[3], Ahmed Habib[4], Heba Morsy[1], Angèle Tingaud-Sequeira[2], Caroline Rooryck[2]

[1]Medical Research Institute, Alexandria University, Department of Human Genetics, Alexandria, Egypt, [2]CHU de Bordeaux, Service de Génétique Médicale, Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Bordeaux, France, [3]Faculty of medicine, Alexandria University, Department of ophthalmology, Alexandria, Egypt, [4]Faculty of dentistry, Alexandria University, Department of Maxillofacial and plastic surgery, Alexandria, Egypt

Oculoauriculovertebral spectrum (OAVS) is the second most frequent malformative disorder of head and neck with highly heterogeneous etiology and pathogenesis. Genetic causes have been brought up due to the existence of familial cases and numerous chromosomal abnormalities have been associated with this spectrum. Retinoic acid (RA) signaling pathway has been implicated in various developmental processes and is essential for craniofacial development. Interestingly, MYT1; the first described candidate gene for OAVS belongs to RA induced transcriptome. The molecular study was carried out on 32 clinically suspected OAVS Egyptian cases with no history of teratogenic insult or numerical chromosomal aberrations using array comparative genomic hybridization, next generation sequencing panel testing, whole exome sequencing and Sanger sequencing of ALX genes in three clinically suspected Oculo-auriculo-frontonasal spectrum cases. Array-CGH revealed dosage anomalies in 6 patients. NGS panel testing revealed missense variants of unknown significance in ZYG11B and HMX1 genes in 2 patients. One missense de novo heterozygous probably pathogenic variant was identified in DGKD gene through WES, performed for a selected trio. This variant was not reported in any databases and is annotated probably damaging by different bioinformatic tools. ALX genes screened were negative for deletions in all patients. Sanger confirmation, segregation analysis followed by functional studies for all variants and genes of interest is highly recommended to reveal their candidacy to OAVS. This research work might represent a strong initiative for future studies based upon the various CNVs and variants of interest detected and may set a paradigm for molecular diagnosis of cases with overlapping phenotypes.

**Keywords:** OAVS, array CGH, NGS, WES, DGKD





Presentation number: YIA 5

# A CENTRIFUGAL MICROFLUIDIC SOLUTION FOR THE AUTOMATION OF FORENSIC EPIGENETIC SAMPLE PREPARATION

**Rachelle Turiello**, James P. Landers

University of Virginia, Department of Chemistry, Charlottesville, Virginia, USA

For those evidence-producing criminal cases that lack genetic reference material for comparison and are ineligible for or non-producing of database matches, the human epigenome has been suggested as a reservoir of information for female sex typing, monozygotic twin individualization, body fluid identification, behavioral trait prediction, and DNA phenotyping by estimation of human chronological age. In particular, more than 300 research studies have been published suggesting the utility of methylation status at specified genetic loci for approximation of human age. However, the most commonly employed strategies for epigenetic analysis require sodium bisulfite conversion (BSC), a sample preparation step to preferentially deaminate unmethylated cytosines to uracil, leaving methylated cytosines intact and distinguishable for downstream analysis. Unfortunately, conventional BSC techniques are characterized by extensive DNA loss and require time-consuming, labor-intensive workflows with a high propensity for contamination. We propose a microfluidic solution for forensic epigenetic sample preparation that leverages centrifugal force to enable rapid, efficient conversion of forensically-relevant DNA input masses in a closed, automated microCD (μCD) format. Faster conversion rates and increased DNA recovery are possible via the enhanced surface-area-to-volume ratio specific to the microfluidic strategy. The method was designed with multiplexing in mind and assessed with methylation standards by multiple downstream analytical processes, including real-time polymerase chain reaction (RT-PCR), high resolution melting (HRM), and pyrosequencing. Early phase goals of this project included testing the chemistry at the microfluidic scale, adjusting the parameters of the reaction step most commonly associated with DNA loss, optimizing microfluidic architecture, and completing preliminary μCD BSC. Assay characterization was completed with primers targeting age-associated loci, ELOVL2 and FHL2. Our approach enabled reduction of incubation intervals, thereby decreasing the total assay time, with increased DNA recovery and comparable conversion efficiency to a gold-standard method.

**Keywords:** Sodium Bisulfite Conversion, Forensic Epigenetics, DNA Phenotyping, Rotational Microfluidic

# ABSTRACTS SELECTED FOR PODIUM PRESENTATION

Presentation number: PP1-MG

# MiR-182-5p AND miR-375-3p IN BLOOD PLASMA AS BIOMARKERS FOR PROSTATE CANCER

**Irena Abramović**[1,2,3], Borna Vrhovec[4], Ivan Pezelj[4], Tomislav Kuliš[2,3,5], Alen Vrtarić[6], Monika Ulamec[2,3,7,8], Nino Sinčić[1,2,3]

[1]School of Medicine, University of Zagreb, Department of Medical Biology, Zagreb, Croatia, [2]Scientific Group for Research on Epigenetic Biomarkers, School of Medicine, University of Zagreb, Zagreb, Croatia, [3]Scientific Centre of Excellence for Reproductive and Regenerative Medicine, School of Medicine, University of Zagreb, Zagreb, Croatia, [4]University Clinical Hospital Centre "Sestre Milosrdnice", Department of Urology, Zagreb, Croatia, [5]University Hospital Centre Zagreb, Department of Urology, Zagreb, Croatia, [6]University Clinical Hospital Centre "Sestre Milosrdnice", Department of Clinical Chemistry, Zagreb, Croatia, [7]University Clinical Hospital Centre "Sestre Milosrdnice", Ljudevit Jurak Clinical Department of Pathology and Cytology, Zagreb, Croatia, [8]School of Medicine, University of Zagreb, Department of Pathology, Zagreb, Croatia

With prostate cancer (PCa) being the most commonly diagnosed neoplasm among men and often resembling benign prostate hyperplasia (BPH), biomarkers with a higher differential value than PSA are required. We investigated the expression of certain miRNAs from liquid biopsies as potential epigenetic biomarkers of PCa. The absolute expression of miR-375-3p and miR-182-5p were quantified in blood plasma and seminal plasma of 65 PCa and 58 BPH patients by digital droplet PCR. The sensitivity and specificity of these microRNAs were determined using ROC curve analysis. The higher expression of miR-182-5p and miR-375-3p in the blood plasma of PCa patients was statistically significant as compared to BPH ($p = 0.0363$ and 0.0226, respectively). Their combination achieved a specificity of 90.2 % for predicting positive or negative biopsy results, while PSA cut-off of 4 µg/L performed with only 1.7 % specificity. In seminal plasma, miR-375-3p, and miR-182-5p showed a statistically significantly higher expression in PCa patients with PSA >10 µg/L compared to ones with PSA >10 µg/L. MiR-182-5p and miR-375-3p in blood plasma show higher performance than PSA in differentiating PCa from BPH. Seminal plasma requires further investigation as it represents an obvious source for PCa biomarker identification.

**Keywords:** miRNA, prostate cancer, liquid biopsy, biomarkers





Presentation number: PP2-FG

# EXPANDED CODIS STR ALLELE FREQUENCIES - EVIDENCE FOR THE IRRELEVANCE OF RACE-BASED DNA DATABASES

Robert F Oldt, **Sree Kanthaswamy**

Arizona State University, AZ, USA

In the US, it is assumed that the most conservative random match probability (RMP) can be estimated using the suspect's self-declared racial reference DNA database. If the suspect's race is not known, the RMP is typically estimated across different race-based CODIS STR allele frequency databases, including African American, Asian, Caucasian, Hispanic, and Native American, and the most conservative RMP estimate is used. In a recent study, we evaluated the relationship between RMP and race based on CODIS STR profiles corresponding to the five race-specific allele frequency databases. Our analyses confirmed that most genetic differences between individuals are only to the slightest extent attributable to racial classification. Approximately 98% of the genetic variation was found to occur among individuals and not between races. We could not distinguish individuals separated by race as distinct genetic clusters based on forensic STR data. Accordingly, RMP values were exceedingly small regardless of the race-based STR allele frequency database, and the values also did not vary significantly when incorporating race-specific reference data. Therefore, our results show that the use of racial information does little to generate conservative RMP estimates. This finding implies that we do not need to include racial information in the US to get conservative estimates of RMP. Therefore, using race as a proxy for a genetic distinction to produce larger (i.e., conservative) RMPs for an individual DNA profile is irrelevant.

**Keywords:** Race, random match probability, population structure, forensic DNA database

Presentation number: PP3-MG

# ENRICHMENT OF RARE VARIANTS IN GENES INVOLVED IN MITOCHONDRIAL METABOLISM IN PATIENTS WITH EARLY-ONSET OR FAMILIAL PARKINSON'S DISEASE

**Dorian Laslo**[1], Gaber Bergant[2], Aleš Maver[2], Borut Peterlin[2]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Medicine Osijek, Osijek, Croatia, [2]Clinical Institute of Medical Genetics, University Medical Centre Ljubljana, Ljubljana, Slovenia

There is a growing body of evidence supporting mitochondrial dysfunction as a mayor driving force in Parkinson's disease (PD) pathogenesis, with the hallmark being the discovery of mitochondrial toxins inducing PD. The goal of this study was to analyze rare variants of genes involved in mitochondrial metabolism in patients with early-onset and familial PD. We collected both nuclear and mitochondrial variants for 204 Croatian, Serbian and Slovenian patients with PD who referred to our center for diagnostic whole exome sequencing. The 204 patients with PD were further selected based on the disease age of onset and their family history. Sequencing was performed using a standardized set of protocols. We used population variation resources in variant annotation, which included an in-housed background population variant frequency estimates based on compilation of over 7000 exomes, as well as Genome Aggregation Database dataset. The cutoff frequency for rare variants was 5% in both the local and gnomAD databases. We excluded variants deviating significantly from Hardy-Weinberg equilibrium. In our study we found statistically significant differences in 16 gene variants between PD patients and the control group. After analysis genes GBA, NPC1, ATP13A2, HTRA2, CP, SOD1, WARS2, CLN6, HEXA, NPC2, SYNJ1, WDR45, PANK2, POLG which are located in nucleus and MT-CO1 gene located in mitochondrial genome had $p<0.05$. After adjustment only nuclear genes still had $p<0.05$, while MT-CO1 gene had padj=0,381. All identified genes have important role in lysosomal or mitochondrial function. We confirmed previous findings that dysfunction in lysosomal metabolism plays an important role in pathogenesis of PD. We found that ATP13A2 mutation is more abundant in patients with early onset PD, not only juvenile form of PD. Mutation in HtrA2 gene results in protein accumulation and destabilization of mitochondrial membrane. WARS2 encodes tRNA synthetize and our study is the first one that links its mutation with early-onset PD. Our study is the first one that identified new gene, WARS2, as a risk factor for early-onset PD and confirmed that dysfunction of lysosomal or mitochondrial metabolism is risk factor for developing PD.

**Keywords:** Lysosomes, Mitochondria, Parkinson Disease





Presentation number: PP4-FG

# DEVELOPMENT AND VALIDATION OF A NEW MULTIPLEX METHYLATION-SPECIFIC PCR ASSAY FOR FORENSIC BODY FLUID IDENTIFICATION

**Sasho Risteski**[1,2], Zlatko Jakovski[1], Lozenka Ivanova[2], Sasho Panov[2], Gordana Dimeska[2], Hilda Jovanovikj[1], Ksenija Nikolova[1], Aleksandar Stankov[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Insitute of Forensic Medicine, Criminalistics and Medical Deontology, Skopje, Macedonia, [2]Ss. Cyril and Methodius University in Skopje, Faculty of Natural Sciences and Mathematics, Institute of Biology, Skopje, Macedonia

The identification of the body-fluid from which DNA originates, along with the STR profiling, is important in uncovering the context of deposition of biological traces and can aid in reconstruction of events. Several authors have reported MS-SNuPe assays for detecting differentially-methylated CpGs for differentiation between saliva, semen, vaginal fluid, venous and menstrual blood. The aim of our research was to develop and validate a novel multiplex methylation-specific PCR (F-MS-PCR) assay for detection of methylation in 5 body-fluid specific CpGs in a DNA sample. The test consists of PCR amplification of bisulfite-converted DNA from a sample by using 5 pairs of fluid-specific primers, one of which is fluorescently tagged at its 5'-end and specific to bisulfite-converted DNA, and the other is specific to bisulfite-converted but only methylated DNA. Thus, only amplification of the methylated body-fluid specific locus is achieved, the PCR product is detected by CE and the body-fluid is detected. To assess the performance, 27 semen, 21 saliva, 21 venous blood, 21 menstrual and 20 vaginal samples were tested. DNA was extracted by PrepFiler Forensic DNA Extraction Kit, quantified by Quantifiler Duo on 7500 Real-Time PCR System, and bisulfite-converted by using EpiJET Bisulfite Conversion Kit. PCR reactions consisted of: 5 µl Qiagen Multiplex PCR Mix, 1 µl 10x primer mix, 3 µl water and 1 µl bisulfite-converted DNA. PCR products were detected on 3500 Genetic Analyzer and data was analyzed by GeneMapper ID-X software. Validation of the limit of detection, sensitivity, specificity, application in casework, and analysis of old samples and mixtures, was also performed to assess the suitability of the test for routine use in the forensic genetic laboratory. The test showed high specificity (100%, except 96.73% in menstrual fluid) and high sensitivity (100%, except 97.6% in vaginal and 80.7% in menstrual fluid). The lower limit of detection varied between 125 pg (semen) and 1 ng (menstrual blood). It has fewer pipetting steps compared to MS-SNuPe, shorter sample to result time and lower cost. The test has easy interpretation, with presence of a peak indicating detected fluid, and it can be easily applied as a powerful tool in the forensic genetic laboratory.

**Keywords:** Body-fluids, DNA methylation, multiplex PCR, MS-SNuPe, capillary electrophoresis

Presentation number: PP5-MG

# THE EFFECT OF HOST GENETICS ON COVID-19 SUSCEPTIBILITY AND SEVERITY: A STUDY OF 16 CODING GENES ON A SUBSET OF BOSNIAN-HERZEGOVINIAN PATIENTS

**Lana Salihefendić[1,2]**, Larisa Bešić[1], Ivana Čeko[1,2], Naida Mulahuseinović[2], Dino Pećar[2], Amra Džuho[1], Selma Durgut[2], Damir Marjanović[1,3], Rijad Konjhodžić[2], Adna Ašić[1]

[1]International Burch University, Department of Genetics and Bioengineering, Sarajevo, Bosnia and Herzegovina, [2]Alea Genetic Center, Sarajevo, Bosnia and Herzegovina, [3]Institute for Anthropological Research, Zagreb, Croatia

The aim of this study was to analyze the effect of patient genetics on the severity of symptoms and susceptibility to COVID-19 infection. 60 COVID-19 patients from the General Hospital of Tešanj, Bosnia and Herezgovina, were recruited in the study, and divided into three groups (n=20 for each group) of patients exhibiting mild, moderate and severe clinical presentation of COVID-19. DNA was isolated from whole blood using QIAamp® DNA Mini Kit. Ion Torrent GeneStudio S 5 platform was used to perform the sequencing of 16 target genes and their regulatory regions, namely HLA-A, HLA-B, HLA-C, ACE2, IL-6, IL-4, TMPRSS2, IFITM3, IL-12, RIG-I/DDX58, IRF-7, IRF-9, IL-1B, IL-1A, CD55, and TNF-**α**. Selected genetic variants of interest were subjected to confirmatory Sanger sequencing on the SeqStudio Genetic Analyzer System. Our study confirmed that older age, male sex, and cardiovascular, respiratory and metabolic comorbidities are risk factors for severe COVID-19. In addition, we have identified several genetic variants that were significantly more common in severe than in mild and/or moderate groups of patients, including those on the genes IL4, IL1B, TMPRSS2, CD55, DDX58 and IRF7. We have further confirmed that the variant rs2285666 on ACE2 might be protective, as it was more common in moderate and mild than in severe clinical group, but significantly affected by the patient's sex, considering that ACE2 is found on X chromosome and escapes X inactivation in females. The results of this study have further emphasized the importance of personalized approach to each COVID-19 patient, as host genetics plays an important role in response to SARS-CoV-2 infection, including both susceptibility and severity of the clinical presentation. Future studies, most prominently GWAS using larger patient cohorts and appropriately matched controls, are expected to produce even more data on the effect of human genetic variants on the course of COVID-19. This study is offering the first such data, not only for the Bosnian-Herzegovinian population, but for the Western Balkan region as well.

**Keywords:** COVID-19, host genetics, personalized approach, SARS-CoV-2





Presentation number: PP6-MG

# MOLECULAR AUTOPSY: IDENTIFICATION, CLASSIFICATION AND REPORTING OF SEQUENCE VARIATIONS IN YOUNG SUDDEN UNEXPECTED DEATH VICTIMS AND AFFECTED FAMILIES

**Stefanie Scheiper-Welling[1]**, Britt-Maria Beckmann[1], Tina Jenewein[2], Elise Gradhand[3], Alexandra Tschäbunin[3], Christof Geisen[3], Marcel A. Verhoff[1], Silke Kauferstein[2]

[1]University Hospital Frankfurt, Goethe University Frankfurt, Insitute of legal medicine, Frankfurt, Germany, [2]University Hospital Frankfurt, German Red Cross, Institute of Transfusion Medicine and Immunohaematology, Frankfurt, Germany, [3]University Hospital Frankfurt, Goethe University Frankfurt, Dr. Senckenbergisches Institute of Pathology, Frankfurt, Germany

Sudden cardiac death (SCD) is an important public health issue. In young individuals a significant number of SCDs is caused by inherited cardiac diseases, frequently not detectable during conventional medico-legal autopsies (including histological and toxicological analyses). Therefore, these deaths are referred to as sudden unexplained deaths (SUDs). Next-generation sequencing (NGS) became an indispensable tool in molecular autopsy investigations. Nonetheless, NGS brought new challenges, especially regarding the interpretation of the large number of variants of unknown significance (VUS). Therefore, evaluation and classification of genetic findings is of great importance to establish a causal link between phenotype and genotype and for prevention of the remaining family members. We performed a structural assessment of young sudden death cases aged between 1-50 years including molecular autopsy. Detected sequence variants were assessed according to the ACMG classification standards. In addition, cardiological data of our new centre of sudden death in the young were investigated to evaluate possible genotype-phenotype correlations. We identified 53 rare protein-altering variants (MAF < 0.2%) classified as VUS or worse. 13 % of the cases exhibited a clinically actionable variant (pathogenic, likely pathogenic or VUS - potentially pathogenic) that would warrant cascade genetic screening in relatives. To date, using molecular autopsy in combination with the assessment of family members (n=149), an inherited cardiac disease could be detected in 24 % of the cases. 67 % of the families are still under investigations. Our data reveal that, despite the undeniable advantages, molecular autopsy is not a stand-alone tool. Moreover, multidisciplinary collaboration is crucial for an optimal management of sudden unexplained death cases in order to identify additional relatives at risk.

**Keywords:** Sudden death, Molecular Autopsy, inherited cardiac disease

Presentation number: PP7-MG

# CELL LINE MODELS WITH STABLY INTEGRATED CRISPR/DCAS9 FUSIONS FOR STUDYING THE EPIGENETICS OF IgG GLYCOSYLATION

**Aleksandar Vojta**[1], Anika Mijakovac[1], Karlo Miškec[1], Jasminka Krištić[2], Vedrana Vičić Bočkor[1], Gordan Lauc[2], Vlatka Zoldoš[1]

[1]Department of Biology, Division of Molecular Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia, [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia

To study alternative glycosylation of immunoglobulin G (IgG), which is functionally important in in pro- or anti-inflammatory effector function of the antibody, we developed a system for IgG production in the model cell line HEK-293F, which we modified by incorporating the CRISPR/dCas9-based molecular machinery for epigenetic regulation of gene expression. We extended our existing modular system for CRISPR/dCas9 fusions facilitating gene regulation to enable its stable integration into HEK-293F cells, thus creating derived cell lines with integrated machinery for programmed transcriptional control. A key factor for targeting the dCas9 fusions with activators and repressors, the guide RNA (gRNA) was transiently transfected into the derived cell lines on a separate plasmid, that also contains a cassette for monocistronic expression of IgG heavy and light chain. In this bipartite system, the creation of derived cell lines with CRISPR/dCas9 components eliminates the inefficiency of transfection with a large construct, while combining a key element for targeting (gRNA) with the IgG expression cassette on a small plasmid facilitates high transient transfection efficiency while virtually eliminating the background from untransfected cells. We validated the system by up- or downregulating the known glycosyltransferases. Transcriptional up- and downregulation of B4GALT1, responsible for adding galactose to the glycan core, had the expected effect on the abundance of agalactosylated and galactosylated structures, changing the glycosylation profile according to the transcript level. Downregulation of FUT8, encoding a fucosyltransferase, decreased the core-fucosylated structures, while upregulation of ST6GAL1 and MGAT3 increased sialylated structures and those with a bisecting GlcNAc, respectively, in line with our expectations. The system is now being used to further study the role of genes associated with IgG glycosylation in GWA studies. It can also be easily repurposed to serve as a model for other proteins and their posttranslational modifications, with appropriate targeting via gRNA. Finally, to eliminate the frequently raised concern about suitability of the HEK293-F system as a model for plasma cells, we are currently adapting the system for use with lymphoblastoid cell lines (LCLs).

**Keywords:** epigenetics, glycosylation, CRISPR, Cas9





Presentation number: PP8-AG

# WEST COUNTRY STORY - A DETAILED INVESTIGATION OF NEOLITHIC & BRONZE AGE INDIVIDUALS FROM SOUTH-WESTERN ENGLAND

**Nikola Vukovic**

Uppsala University, Evolutionsbiologiskt Centrum EBC, Uppsala, Sweden

Six thousand years before present (BP), the Neolithic expansion to Britain displaced local hunter-gatherer societies, introducing a radically different, more sedentary lifestyle. Two millennia later, a new migratory wave reached Britain, through the Bell Beaker folk, again transforming the established genetic and socio-cultural landscape of the island. While distinct burial rituals and material artifacts underline the strong cultural differences between the two populations, the Bronze Age practice of reusing more ancient Neolithic tombs renders the differentiation of individuals through archaeological study alone problematic. In order to finely investigate the population dynamics that occurred during the transition between the Neolithic and the Bronze Age in Britain at an individual resolution, we employed a genomic approach on 30 samples (age between 6000-3500 BP) from 7 sites in South-Western England. Our analyses reveal a pattern consistent with a genetic turnover, while signs of admixture between the populations are visible as well. First kin relationships between four individuals provide a direct insight into the makeup of a Neolithic multigenerational family. On three occasions, we find the remains of male Bronze Age individuals buried within Neolithic sites, suggesting a possible trend of burial site reuse. By integrating genetic and archaeological analyses, our study delivers a snapshot of one of the most influential demographic shifts in British history, shedding light on both the individual history of these tombs as well as the region's wider socio-cultural transformation.

**Keywords:** Neolithic, Bronze Age, Migration

# ABSTRACTS OF POSTER PRESENTATIONS

# MEDICAL GENETICS

*BEST PRACTICES IN TRANSLATIONAL AND PERSONALIZED MEDICINE*

Presentation number: MG 1

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

**Kristijan Vrdoljak**[1], Petar Brlek[1], Andrea Skelin[1,2], Šime Manola[3], Nikola Pavlović[3], Jozica Šikić[1], Gordana Matijević[1], Dorijan Jagačić[1], Josep Brugada[4], Dragan Primorac[1,5,6,7,8,9,10,11,12,13]

[1]St. Catherine Specialty Hospital, Zagreb, Croatia, [2]Genos Gycoscience Research Laboratory, Zagreb, Croatia, [3]Department for Cardiovascular Diseases, University Hospital Dubrava, Zagreb, Croatia, [4]Cardiovascular Institute, Hospital Clinic, University of Barcelona, Barcelona, Spain, [5]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [6]Medical School, University of Rijeka, Rijeka, Croatia, [7]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina, [8]Medical School, University of Split, Split, Croatia , [9]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA, [10]University of New Haven, Henry C. Lee College of Criminal Justice and Forensic Sciences, West Haven, CT, USA, [11]Medical School REGIOMED, Coburg, Germany, [12]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [13]National Forensic Sciences University, Gujarat, India

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

**Keywords:** Brugada syndrome, sudden cardiac death, SCN5A, implantable cardioverter-defibrillator, atrial fibrillation

Presentation number: MG 2

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

**Kristina Bojanić**[1,2,3], Sonja Vukadin[1,2], Kristina Kralik[2], Gordana Ivanac[4], Renata Sikora[1,2], Paula Percač[1], Žana Opačak[1], Mateja Novački[1], Justinija Steiner[2,3], Aleksandar Včev[1,2], Robert Smolić[1,2], Martina Smolić[1,2]

[1]Faculty of Dental Medicine and Health Osijek, J.J. Strossmayer University of Osijek, Osijek, [2]Faculty of Medicine Osijek, J.J. Strossmayer University of Osijek, Osijek, Croatia, [3]Health Center Osječko-baranjska County, Osijek, Croatia, [4]University Hospital Dubrava, Zagreb, Croatia

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

**Keywords:** breast density, breast cancer, risk-based screening, personalized breast cancer risk assessment



# *EPIGENETICS*

Presentation number: MG 3

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

**Nika Foglar**[1], Goran Josipović[2], Vlatka Zoldoš[1]

[1]Faculty of Science, University of Zagreb, Department of Biology, Zagreb, Croatia, [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia

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

**Keywords:** CRISPR/dCas9 technology, DNA methylation, histone modifications, epithelial to mesenchymal transition

Presentation number: MG 4

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

**Ana Vujić**[1], Marija Klasić[1], Vedrana Vičić Bočkor[1], Vlatka Zoldoš[1], Maja Pučić Baković[2], Gordan Lauc[2,3]

[1]University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Zagreb, Croatia, [2]Genos Glycoscience Research Laboratory, Genos Ltd., Zagreb, Croatia, [3]University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

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

**Keywords:** epigenome editing, gene regulation, CRISPR/dCas9, DNA methylation, protein glycosylation





Presentation number: MG 5

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

**Vedrana Vičić Bočkor**[1], Marija Klasić[1], Goran Josipović[2], Ana Vujić[1], Nika Foglar[1], Samira Smajlović[1], Toma Keser[3], Gordan Lauc[2], Aleksandar Vojta[1], Vlatka Zoldoš[1]

[1]University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Zagreb, Croatia, [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia, [3]University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

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

**Keywords:** epigenetics, CRISPR/dCas9, glycogenes, protein glycosylation

Presentation number: MG 6

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

**Tina Bareša**[1], Željana Bašić[1], Ivan Jerković[1], Krešimir Dolić[2], Marija Ćavar Borić[2], Danijela Budimir Mršić[2], Mislav Čavka[3], Elvira Krešić[3], Ivana Kružić[1]

[1]University of Split, University Department of Forensic Sciences, Split, Croatia, [2]University Hospital Center Split, Department of Interventional and Diagnostic Radiology, Split, Croatia, [3]University Hospital Center Zagreb, Department of Interventional and Diagnostic Radiology, Zagreb, Croatia

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

**Keywords:** MSCT imaging, virtual databases, metopic suture, population affiliation, Croatia





Presentation number: MG 7

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

**Goran Josipović**[1,2], Vanja Tadić[1], Marija Klasić[1], Vladimir Zanki[3], Ivona Bečeheli[1], Felicia Chung[4], Akram Ghantous[4], Toma Keser[5], Josip Madunić[1], Maria Bošković[1], Gordan Lauc[2,5], Zdenko Herceg[4], Aleksandar Vojta[1], Vlatka Zoldoš[1]

[1]Faculty of Science, University of Zagreb, Department of Biology, Zagreb, Croatia, [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia, [3]Faculty of Science, University of Zagreb, Department of Chemistry, Zagreb, Croatia, [4]International Agency for Research on Cancer (IARC), Lyon, France, [5]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

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

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

# GENETIC BASIS OF DISEASES

Presentation number: MG 8

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

**Eduard Pavelić**[1], Petar Brlek[1], Vilim Molnar[1], Darko Antičević[1,2], Damir Hudetz[1], Vide Bilić[1,3], Ivo Barić[4], Dragan Primorac[1,2,5,6,7,8,9,10,11,12]

[1]St. Catherine Specialty Hospital, Zagreb, Croatia, [2]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [3]Clinical Hospital of Traumatology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia, [4]Department of Paediatrics, University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia, [5]Medical School, University of Rijeka, Rijeka, Croatia, [6]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina, [7]Medical School, University of Split, Split, Croatia, [8]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA, [9]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [10]Medical School REGIOMED, Coburg, Germany, [11]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [12]National Forensic Sciences University, Gujarat, India

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

**Keywords:** Rickets disease, PHEX mutation, hypophosphatemia, RNA splicing

Presentation number: MG 9

# CCR5-Δ32 GENE VARIANT IN CELIAC DISEASE

**Nada Starčević Čizmarević**[1], Vanja Licul[2], Brankica Mijandrušić Sinčić[2], Anika Lorencin Pucić[1], Smiljana Ristić[1]

[1]Faculty of Medicine, Department of Medical Biology and Genetics, Rijeka, Croatia, [2]University Hospital Centre Rijeka, Department of Gastroenerology, Rijeka, Croatia

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

**Keywords:** CCR5-Δ32, gene variant, celiac disease





Presentation number: MG 10

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

**Nikolina Tomić**, Naida Lojo-Kadrić, Belmina Šarić, Jasmin Ramić, Lejla Pojskić

University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina

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

**Keywords:** Hyperbilirubinemia, Gilbert's Syndrome, genetic testing, UGT1A1 gene

Presentation number: MG 11

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

**Anita Barišić**[1], Maja Ergović Ravančić[2], Dijana Majstorović[3], Jadranka Vraneković[1]

[1]Faculty of Medicine, Department of Medical Biology And Genetics, Rijeka, Croatia, [2]Polytechnic in Požega, Department of Agriculture, Study of Food Technology, Požega, Croatia, [3]Juraj Dobrila University of Pula, Pula, Croatia

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

**Keywords:** adolescents, Down syndrome, children, macronutrients, micronutrients





Presentation number: MG 12

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

**Nora Pušeljić**[1], Ema Poznić[1], Silvija Pušeljić[1,2], Višnja Tomac[1,2], Martina Kos[1,2], Ivana Serdarušić[1], Paula Ivanšić[1], Luka Perić[3], Nika Pušeljić[2]

[1]Department of Pediatrics, Osijek University Hospital, Osijek, Croatia, [2]Faculty of Medicine, University of Osijek, Osijek, Croatia, [3]Department of Oncology, Osijek University Hospital, Osijek, Croatia

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

**Keywords:** Opitz-Kaveggia syndrome, FGS1, XYY chromoosomopathy

Presentation number: MG 13

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

**Robert Mujkić**[1], Darija Šnajder Mujkić[2], Petar Šušnjara[3], Anita Matić[4], Karla Rožac[1], Ivana Ilić[1], Anđela Grgić[1], Dalibor Divković[5], Kristina Selthofer Relatić[6]

[1]Department of Anatomy, Histology, Embryology, Pathological Anatomy and Pathological Histology, Faculty of Dental Medicine and Health, University of J. J. Strossmayer Osijek, Croatia, [2]Department of Anatomy and Neuroscience, Faculty of Medicine, University of J. J. Strossmayer Osijek, Croatia, [3]Department of Physiology and Immunology, Faculty of Medicine, University of J. J. Strossmayer Osijek, Croatia, [4]Department of Pathophysiology, Physiology and Immunology, Faculty of Dental Medicine and Health, University of J. J. Strossmayer Osijek, Croatia, [5]Department of Surgery, Urology, Orthopedics and Physical and Rehabilitation Medicine, Faculty of Medicine, University of J. J. Strossmayer Osijek, Croatia, [6]Department of Hearth and Vessel Disease, University Hospital Centre Osijek, Croatia

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

**Keywords:** gene expressions, obesity, male children, atherosclerosis





Presentation number: MG 14

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

**Ana Cindrić**[1], Dražen Petrović[1], Aoife Murray[2], Ivan Alić[2], Helena Deriš[1], Dean Nižetić[2], Gordan Lauc[3], Jasminka Krištić[1]

[1]Genos d.o.o., Zagreb, Croatia, [2]The Blizard Institute, Barts & The London School of Medicine, Queen Mary University of London, London, UK, [3]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

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

**Keywords:** Immunoglobulin G glycosylation, Down syndrome, RUNX1

Presentation number: MG 15

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

**Naida Lojo-Kadric**, Jasmin Ramic, Nikolina Tomic, Belmina Saric, Lejla Pojskic

University of Sarajevo – Institute for genetic engineering and biotechnology, Laboratory for human genetics, Sarajevo, Bosnia and Herzegovina

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

**Keywords:** NGS, dysosmia, dysgeuzia, COVID19





Presentation number: MG 16

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

**Višnja Tomac[1,2]**, Silvija Pušeljić[1,2], Martina Kos[1,2], Ivana Serdarušić[1], Nora Pušeljić[1], Ema Poznić[1], Paula Ivanšić[1], Luka Perić[3], Nika Pušeljić[2]

[1]Department of Pediatrics, Osijek University Hospital, Osijek, Croatia, [2]Faculty of Medicine, University of Osijek, Osijek, Croatia, [3]Department of Oncology, Osijek University Hospital, Osijek, Croatia

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

**Keywords:** Neurooculocardiogenitourinary syndrome, coloboma, epilepsy

Presentation number: MG 17

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

**Dijana Majstorović**[1], Anita Barišić[2], Jadranka Vraneković[2]

[1]Juraj Dobrila University of Pula, Faculty of Medicine, Pula, Croatia, [2]University of Rijeka, Faculty of Medicine, Department of Medical Biology and Genetics, Rijeka, Croatia

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

**Keywords:** congenital heart defects, DNA methyltransferase, Down syndrome, single nucleotide polymorphism





Presentation number: MG 18

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

**Silvija Pušeljić**[1,2], Višnja Tomac[1,2], Nora Pušeljić[1], Ema Poznić[1], Martina Kos[1,2], Ivana Serdarušić[1], Paula Ivanšić[1], Luka Perić[3], Nika Pušeljić[2]

[1]Department of Pediatrics, Osijek University Hospital, Osijek, Croatia, [2]Faculty of Medicine, University of Osijek, Osijek, Croatia, [3]Department of Oncology, Osijek University Hospital, Osijek, Croatia

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

**Keywords:** Saul-Wilson syndrome, dwarfism, skeletal abnormalities

Presentation number: MG 19

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

**Anita Matić**[1], Ivan Koprivčić[2,3], Ksenija Marijanović[3,4], Maja Tolušić Levak[3,5], Robert Mujkić[1], Vjekoslav Wertheimer[2,3], Nikolina Lazić[1]

[1]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [2]Department of Orthopedics and Traumatology, Osijek University Hospital, Osijek, Croatia, [3]Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [4]Clinical Department of Pathology and Forensic Medicine, Osijek University Hospital Osijek, Croatia, [5]Department of Dermatology and Venereology, Osijek University Hospital, Osijek, Croatia

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

**Keywords:** breast tumor, leptin, leptin receptor, BMI, lymph node





Presentation number: MG 20

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

**Ema Poznić**[1], Nora Pušeljić[1], Višnja Tomac[1,2], Silvija Pušeljić[1,2], Martina Kos[1,2], Ivana Serdarušić[1], Paula Ivanšić[1], Luka Perić[3], Nika Pušeljić[2]

[1]Department of Pediatrics, Osijek University Hospital, Osijek, Croatia, [3]Department of Oncology, Osijek University Hospital, Osijek, Croatia

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

**Keywords:** CHARGE syndrome, choanal atresia, heart defect

Presentation number: MG 21

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

Azra Kazazić[1], Nela Kelam[2,3], Anita Racetin[2,3], Natalija Filipović[2,3], Yu Katsuyama[4], Katarina Vukojević[1,2,3]

[1]Department of Anatomy, University of Mostar, School of Medicine, Mostar, Bosnia and Herzegovina, [2]Department of Anatomy, Histology and Embryology, University of Split School of Medicine, Split, Croatia, [3]Center for Translational Research in Biomedicine, University of Split School of Medicine, Split, Croatia, [4]Department of Anatomy, Shiga University of Medical Science, Ötsu, Japan

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

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





Presentation number: MG 22

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

Josipa Marin Lovrić[1,2,] Natalija Filipović[3], Ljubo Znaor[1,2], Anita Rančić[1,2], Josko Petričević[4], Nenad Kunac[5], Violeta Soljić[6,7], Mirna Saraga-Babić[3], **Katarina Vukojević[3,6,8,9]**

[1]Department of Ophthalmology, University of Split School of Medicine, Split, Croatia, [2]Department of Ophthalmology, University Hospital of Split, Split, Croatia, [3]Department of Anatomy, Histology and Embryology, University of Split School of Medicine, Split, Croatia, [4]Department of Pathology, University of Mostar, School of Medicine, Mostar, Bosnia and Herzegovina, [5]Department of Pathology, University Hospital of Split, Split, Croatia, [6]Department of Histology and Embryology, University of Mostar, School of Medicine, Mostar, Bosnia and Herzegovina, [7]University of Mostar, Faculty of Health Studies, Mostar, Bosnia and Herzegovina, [8]Department of Anatomy, University of Mostar, School of Medicine, Mostar, Bosnia and Herzegovina, [9]Center for Translational Research in Biomedicine, University of Split School of Medicine, Split, Croatia

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

**Keywords:** human eye, embryogenesis, tumorigenesis, epithelial to mesenchymal transition

# *INDIVIDUALIZED (PERSONALIZED) MEDICINE*

Presentation number: MG 23

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

**Petar Brlek**[1], Zrinka Djukić Koroljević[1], Ivica Grgurević[1], Jelena Bošnjak[1], Andrea Skelin[1], Tomislav Pavlović[1], Igor Borić[1,2,3], Ivo Barić[4], Dragan Primorac[1,2,3,5,6,7,8,9,10]

[1]St. Catherine Specialty Hospital, Zagreb, Croatia, [2]Medical School, University of Rijeka, Rijeka, Croatia, [3]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina, [4]Department of Paediatrics, University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia, [5]Medical School, University of Split, Split, Croatia , [6]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [7]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA, [8]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [9]Medical School REGIOMED, Coburg, Germany, [10]National Forensic Sciences University, Gujarat, India

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

**Keywords:** sterol 27-hydroxylase, cholestanol, metabolic disease, CYP27A1, chenodeoxycholic acid

Presentation number: MG 24

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

**Luise Mildeberger**, Julia Bueto, Verena Wilmes, Stefanie Scheiper-Welling, Marcel A. Verhoff, Silke Kauferstein

University Hospital Frankfurt, Institute of Legal Medicine, Frankfurt am Main, Germany

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

**Keywords:** MicroRNA, biomarkers, sudden cardiac death, myocardial infarction





Presentation number: MG 25

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

**Eduard Pavelic[1]**, Vid Matisic[1], Vilim Molnar[1], Petar Brlek[1], Tomislav Pavlovic[1], Marko Strbad[2,3], Lenart Girandon[2], Dragan Primorac[1,4,5,6,7,8,9,10,11]

[1]St Catherine Specialty Hospital, Department of Orthopedics, Zagreb, [2]Educell Ltd, Trzin, Slovenia, [3]Biobanka Ltd, Trzin, Slovenia, [4]University of Split, School of Medicine, Split, Croatia, [5]University of Split, University Department of Forensic Sciences, Split, Croatia, [6]University of Osijek, Faculty of Medicine, Osijek, Croatia, [7]University of Osijek, Faculty of Dental Medicine and Health, Osijek, Croatia, [8]University of Rijeka, School of Medicine, Rijeka, Croatia, [9]University of New Haven, Henry C. Lee College of Criminal Justice and Forensic Sciences, West Haven, CT, USA, [10]Medical School REGIOMED, Coburg, Germany, [11]The National Forensic Sciences University, Gandhinagar, Gujarat, India

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

**Keywords:** Mesenchymal stem cells, allogenic MSC, sarcoidosis

# *INFECTIOUS DISEASES*

Presentation number: MG 26

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

**Belmina Saric**, Nikolina Tomic, Naida Lojo-Kadric, Jasmin Ramic, Lejla Pojskic

University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Laboratory for Human Genetics, Sarajevo, Bosnia and Herzegovina

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

**Keywords:** molecular docking, SARS-CoV-2, Indian Traditional System of Medicine, main protease

Presentation number: MG 27

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

**Amalija Lukić**, Sanja Pleško

University Hospital Centre Zagreb, Zagreb, Croatia

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

**Keywords:** Invasive Aspergillosis, galactomannan, RT PCR, molecular biology

# *MOLECULAR DIAGNOSTICS: CURRENT TECHNOLOGY AND APPLICATIONS*

Presentation number: MG 28

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

Ana Banovac, **Livia Sliskovic**, Ivan Jerkovic

University Department of Forensic Sciences, University of Split, Split, Croatia

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

**Keywords:** genetic testing, attitude, knowledge, Croatian population, personalized medicine

Presentation number: MG 29

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

**Selma Durgut**[1], Lana Salihefendić[1], Ivana Čeko[1], Naida Mulahuseinović[1], Adna Ašić[2], Adis Kandić[1], Rijad Konjhodžić[1]

[1]Alea Genetic Centre, Health Institute Alea Dr. Kandić, Sarajevo, Bosnia and Herzegovina, [2]Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina

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

**Keywords:** celiac disease, HLA genotype, molecular testing, IBD symptoms





Presentation number: MG 30

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

**Lana Salihefendić**[1,2], Adna Ašić[1], Ivana Čeko[2], Dino Pećar[2], Larisa Bešić[1], Naida Mulahuseinović[2], Amra Džuho[1], Laura-Severina Köhn[1], Rijad Konjhodžić[2]

[1]International Burch University, Department of genetics and bioengineering, Sarajevo, Bosnia and Herzegovina, [2]Alea Genetic Center, Sarajevo, Bosnia and Herzegovina

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

**Keywords:** COVID-19, next-generation sequencing, Ion Torrent GeneStudioTM S5, immunological response

Presentation number: MG 31

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

**Matea Kos**

Clinical Hospital Dubrava, Clinical Department of Pathology and Citology, Laboratory for Immunohistochemistry and Molecular pathology, Zagreb, Croatia

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

**Keywords:** adenocarcinoma, EGFR, histology, citology





Presentation number: MG 32

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

**Ivana Čeko**, Merima Smajlhodžić-Deljo, Alma Suljić, Lana Salihefendić, Dino Pećar, Adis Kandić, Rijad Konjhodžić

ALEA Genetic Center, Health Institute ALEA Dr. Kandić, Sarajevo, Bosnia and Herzegovina

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

**Keywords:** SARS-CoV-2 virus, RNA extraction, Real-time PCR

Presentation number: MG 33

# COMPARATIVE ANALYSIS OF SARS-COV-2 DETECTION KITS

**Naida Mulahuseinović**, Lana Salihefendić, Selma Durgut, Enis Kandić, Rijad Konjhodžić

ALEA Genetic Center, Department for Molecular Biology, Sarajevo, Bosnia and Herzegovina

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

**Keywords:** SARS-CoV-2, SARS-CoV-2 detection kits, real-time PCR





Presentation number: MG 34

# PRENATAL RHD GENOTYPING BY NIPT METHOD: CROATIAN EXPERIENCE

**Jasna Bingulac-Popović**, Irena Jukić

Croatian Institute of Transfusion Medicine, Department of Molecular Diagnostics and Medical Department, Zagreb, Croatia

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

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

Presentation number: MG 35

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

Emiliano Maresi[1], Gaia Morello[1], Elisabetta Orlando[1], Giovanni De Lisi[1], Clio Bilotta[1], **Giuseppe Davide Albano[1]**, Antonina Argo[1], Ada Maria Florena[1]

[1]PROMISE Department, University of Palermo, Palermo, Italy

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

**Keywords:** Placental pathology, SARS-CoV-2 infection, vertical transmission, immunohistochemistry



# *PRENATAL DIAGNOSTICS*

Presentation number: MG 36

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

**Martin Čemerin**[1], Petar Brlek[1], Vid Matišić[1], Vilim Molnar[1], Ivana Erceg Ivkošić[1,2], Dragan Primorac[1,2,3,4,5,6,7,8,9,10]

[1]St. Catherine Specialty Hospital, Zagreb, Croatia, [2]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [3]Medical School, University of Rijeka, Rijeka, Croatia, [4]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina, [5]Medical School, University of Split, Split, Croatia, [6]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA, [7]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [8]Medical School REGIOMED, Coburg, Germany, [9]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [10]National Forensic Sciences University, Gujarat, India

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

**Keywords:** renal agenesis, CAKUT, GREB1L, miscarriage, genetic testing

*PROTEIN GLYCOSYLATION IN DIAGNOSTICS AND THERAPY*

Presentation number: MG 37

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

**Dinko Šoić[1]**, Toma Keser[1], Jerko Štambuk[2], Domagoj Kifer[1], Flemming Pociot[3,4], Gordan Lauc[1,2], Mislav Novokmet[2], Grant Morahan[5], Olga Gornik Kljaić[1]

[1]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia, [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia, [3]Steno Diabetes Center Copenhagen, Herlev, Denmark, [4]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [5]Centre for Diabetes Research, University of Western Australia, Perth, WA, Australia

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

**Keywords:** C3 glycoprotein, glycopeptides, LC-MS, N-glycosylation, Type 1 diabetes onset

Presentation number: MG 38

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

**Fran Lukšić**[1], Anika Mijakovac[1], Jasminka Krištić[2], Vedrana Vičić Bočkor[1], Ana Cindrić[2], Gordan Lauc[2,3], Vlatka Zoldoš[1]

[1]University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Zagreb, Croatia, [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia, [3]University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

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

**Keywords:** immunoglobulin G, N-glycosylation, IgG glycome, HEK293 FreeStyle, in vitro cell aging





Presentation number: MG 39

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

**Anika Mijakovac**[1], Karlo Miškec[1], Jasminka Krištić[2], Azra Frkatović[2], Gordan Lauc[2,3], Aleksandar Vojta[1], Vlatka Zoldoš[1]

[1]University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Zagreb, Croatia, [2]Genos Glycoscience Research Laboratory, Genos Ltd., Zagreb, Croatia, [3]University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

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

**Keywords:** IgG glycosylation, estrogen, gene regulation, RUNX3, CRISPR/dCas9

Presentation number: MG 40

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

**Helena Deriš**[1], Petra Tominac[1], Frano Vučković[1], Nina Briški[1], Arne Astrup[2], Ellen Blaak[3], Gordan Lauc[1,4], Ivan Gudelj[1,5]

[1]Genos Ltd, Glycoscience Research Laboratory, Zagreb, Croatia, [2]Obesity Science Dept, The Novo Nordisk Foundation, Hellerup, Denmark, [3]Maastricht University, Department of Human Biology, Maastricht, Netherlands, [4]University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia, [5]University of Rijeka, Department of Biotechnology, Rijeka, Croatia

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

**Keywords:** plasma N-glycans, weight loss, low-calorie diet, glycoproteins





Presentation number: MG 41

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

**Branimir Plavša**[1], Ivona Kološnjaj[1], Tomislav Bulum[2], Martina Tomić[3], Sandra Vučković Rebrina[2], Marijana Vučić Lovrenčić[4], Lea Duvnjak[2], Olga Gornik[1]

[1]University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia, [2]Merkur University Hospital, Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Department of Endocrinology, Zagreb, Croatia, [3]Merkur University Hospital, Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Department of Ophthalmology, Zagreb, Croatia, [4]Merkur University Hospital, Department of Laboratory Medicine, Zagreb, Croatia

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

**Keywords:** glycosylation, N-glycosylation, IgG, diabetes, type 1

Presentation number: MG 42

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

**Matej Nemčić[1]**, Marko Tijardović[1], Tomislav Bulum[2], Martina Tomić[3], Branimir Plavša[1], Sandra Vučković Rebrina[2], Renata Zadro[4], Dragan Primorac[4,5,6,7,8,9,10,11,12,13], Gordan Lauc[1,14], Grant Morahan[15,16], Marijana Vučić Lovrenčić[17], Lea Duvnjak[2], Olga Gornik[1]

[1]Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia, [2]Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital, Department of Endocrinology, Zagreb, Croatia, [3]Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital, Department of Ophtalmology, Zagreb, Croatia, [4]St. Catherine Hospital, Zagreb, Croatia, [5]University of Split, School of Medicine, Split, Croatia, [6]University of Split, University Department of Forensic Sciences, Split, Croatia, [7]University of Osijek, Faculty of Medicine, Osijek, Croatia, [8]University of Osijek, Faculty of Dental Medicine and Health, Osijek, Croatia, [9]University of Rijeka, School of Medicine, Rijeka, Croatia , [10]Pennsylvania State University, Eberly College of Science, University Park, PA, USA, [11]University of New Haven, Henry C. Lee College of Criminal Justice and Forensic Sciences, West Haven, CT, USA, [12]Medical School REGIOMED, Coburg, Germany, [13]The National Forensic Sciences University, Gandhinagar, Gujarat, India, [14]Genos Glycoscience Research Laboratory, Zagreb, Croatia,[15]The Harry Perkins Institute for Medical Research, Centre for Diabetes Research, Perth, WA, Australia, [16]University of Melbourne, Melbourne, VIC, Australia [17]Merkur University Hospital, Department of Laboratory Medicine, Zagreb, Croatia

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

**Keywords:** Type 1 diabetes mellitus, N-glycosylation, Serum protein N-glycosylation





Presentation number: MG 43

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

**Borna Rapčan[1,2]**, Jelena Šimunović[2], Branimir Plavša[1,2], Jerko Štambuk[2], Gordan Lauc[1,2], Genadij Razdorov[2]

[1]Faculty of Pharmacy and Biochemistry, Zagreb, Croatia, [2]Genos Ltd, Glycoscience Research Laboratory, Zagreb, Croatia

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

**Keywords:** liquid handling, lab automation, glycomics, UPLC

*REGENERATIVE MEDICINE*

Presentation number: MG 44

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

**Zrinka Djukic Koroljevic[1]**, Petar Brlek[1], Vilim Molnar[1], Damir Hudetz[1,2,3], Zeljko Jelec[1,4], Eduard Rod[1], Dinko Vidovic[1,5,6], Trpimir Vrdoljak[1,2], Igor Boric[1,7,8,9], Tadija Petrovic[1,5], Fabijan Cukelj[1,5,7], Vid Matisic[1], Eduard Pavelic[1], David Karli[10], Dragan Primorac[1,3,7,8,9, 11,12,13,14,15,16]

[1]St. Catherine Specialty Hospital, Zagreb, Croatia, [2]Clinical Hospital Sveti Duh, Zagreb, Croatia, [3]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [4]Department of Nursing, University North, Varaždin, Croatia, [5]Clinic for Traumatology, University Hospital Sestre Milosrdnice, Zagreb, Croatia, [6]School of Dental Medicine, University of Zagreb, Zagreb, Croatia, [7]Medical School, University of Split, Split, Croatia, [8]Medical School, University of Rijeka, Rijeka, Croatia, [9]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina, [11]Eberly College of Science, The Pennsylvania State University, University Park, State College, PA, USA, [10]Steadman Clinic, Vail, CO USA, [12]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [13]Medical School REGIOMED, Coburg, Germany, [14]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [15]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [16]National Forensic Sciences University, Gujarat, India

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

**Keywords:** knee osteoarthritis, stromal vascular fraction, KOOS, WOMAC, VAS

Presentation number: MG 45

# THE POTENTIAL USE OF MESENCHYMAL STEM CELLS IN GYNECOLOGY

Matea Hodonj[1], Petar Brlek[2], **Ivana Erceg Ivkošić[2,3]**, Dragan Primorac[2,3,4,5,6,7,8,9,10,11]

[1]Private family practice "dr. Ivica Cvetković", Samobor, Croatia, [2]St. Catherine Specialty Hospital, Zagreb, Croatia, [3]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [4]Medical School, University of Split, Split, Croatia, [5]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA, [6]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [7]Medical School REGIOMED, Coburg, Germany, [8]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [9]Medical School, University of Rijeka, Rijeka, Croatia, [10]University of Split, University Department of Forensic Sciences, Split, Croatia, [11]National Forensic Sciences University, Gujarat, India

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

**Keywords:** mesenchymal stem cell, gynecology, regenerative medicine, infertility, Asherman syndrome





Presentation number: MG 46

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

**Marija Zekušić[1]**, Marina Bujić Mihica[1], Tamara Dolenec[1], Marija Skoko[1], Anamarija Jularić[1], Dominik Puljić[1], Ivana Vrgoč Zimić[1], Milivoje Boranić[2], Hrvoje Tomičić[3], Antun Kljenak[4], Ivanka Batarilo[5], Dinko Vidović[6], Tiha Vučemilo[1]

[1]University Hospital Center "Sestre milosrdnice", Department of Transfusion and Regenerative Medicine, Zagreb, Croatia, [2]Ruđer Bošković Institute, Zagreb, Croatia, [3]University Hospital Center "Sestre milosrdnice", Department of Burns, Zagreb, Croatia, [4]Children's Hospital Zagreb, Zagreb, Croatia, [5]Croatian Institute of Transfusion Medicine, Department of Microbiology, Zagreb, Croatia, [6]University Hospital Center "Sestre milosrdnice", Clinic for Traumatology, Zagreb, Croatia

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

**Keywords:** tissue engineering, keratinocytes, ATMP

Presentation number: MG 47

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

**Ana Maletic**[1], Ivo Dumic-Cule[2], Petar Brlek[3], Rado Zic[4], Dragan Primorac[3,5,6,7,8,9,10,11,12,13]

[1]Polyclinic Maletic, Daruvar, Croatia, [2]University North, Varazdin, Croatia, [3]St. Catharine Special Hospital, Zagreb, Croatia, [4]University Hospital Dubrava, Department of Plastic Surgery, Zagreb, Croatia, [5]University of Split, School of Medicine, Split, Croatia, [6]University of Split, University Department of Forensic Sciences, Split, Croatia, [7]University of Osijek, Faculty of Medicine, Osijek, Croatia, [8]University of Osijek, Faculty of Dental Medicine and Health, Osijek, Croatia, [9]University of Rijeka, School of Medicine, Rijeka, Croatia, [10]Pennsylvania State University, Eberly College of Science, University Park, PA, USA, [11]University of New Haven, Henry C. Lee College of Criminal Justice and Forensic Sciences, West Haven, CT, USA, [12]Medical School REGIOMED, Coburg, Germany, [13]The National Forensic Sciences University, Gandhinagar, Gujarat, India

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

**Keywords:** hair loss, vertex, autologous platelet-rich plasma





Presentation number: MG 48

# NOVEL CELL-BASED THERAPIES IN CROHN'S DISEASE

**Ana Dimova**[1], Petar Brlek[1], Branko Bogdanić[1,2], Mislav Rakić[1,3], Tihomir Grgić[1,4], Ivica Grgurević[1,3], Tomislav Bokun[1,3], Ivana Kovačić[1,5], Dragan Primorac[1,6,7,8,9,10,11,12,13,14]

[1]St. Catherine Specialty Hospital, Zagreb, Croatia, [2]University Hospital Centre Zagreb, Zagreb, Croatia, [3]University Hospital Dubrava, Zagreb, Croatia, [4]University hospital "Sveti Duh", Zagreb, Croatia, [5]General Hospital Karlovac, Karlovac, [6]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina, Croatia [7]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [8]Medical School, University of Rijeka, Rijeka, Croatia, [9]Medical School, University of Split, Split, Croatia, [10]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA, [11]University of New Haven, Henry C. Lee College of Criminal Justice and Forensic Sciences, West Haven, CT, USA, [12]Medical School REGIOMED, Coburg, Germany, [13]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [14]National Forensic Sciences University, Gujarat, India

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

**Keywords:** Stem cell, Crohn's disease, mesenchymal stem cell, TNF-**α**, adipose-derived stem cell

# *STEM CELLS*

Presentation number: MG 49

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

**Dražen Juraj Petrović**[1,2], Ana Cindrić[1], Ivan Alić[3,4], Aoife Murray[4], Dinko Mitrečić[2], Jasminka Krištić[1], Tomislav Klarić[1], Gordan Lauc[1], Dean Nižetić[4]

[1]Genos d.o.o., Laboratory for Glycobiology, Zagreb, Croatia, [2]Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Histology and Embriology, Zagreb, Croatia, [3]Faculty of Veterinary Medicine of University of Zagreb, Anatomy, Histology and Embriology, Zagreb, Croatia, [4]The Blizard Institute, Barts and The London School of Medicine, Queen Mary University of London, Genomics and Child Health, London, United Kingdom

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

**Keywords:** N-glycosylation, Down syndrome, isogenic, stem cells

*CELL THERAPY*

Presentation number: MG 50

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

**David Karli**[1], Richard Bedford[2], Joe Reyes[2], Adis Kljajic[3] Dragan Primorac[4-14]

[1]Steadman Clinic, Vail, CO USA, [2]Greyledge Technologies Lone Tree, CO USA, [3]ONQODE, Sterling Heights, Michigan, USA, [4]St. Catherine Specialty Hospital, Zagreb, Croatia, [5]Medical School, University of Zagreb, Zagreb, Croatia, [6]University of Osijek Faculty of Dental Medicine & Health, Osijek, Croatia, [7]University of Split School of Medicine, đ Split, Croatia , [8]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA, [9]The Henry C Lee College of Criminal Justice & Forensic Sciences, University of New Haven, West Haven, CT, USA, [10]The National Forensic Sciences University, Gandhinagar, Gujarat, India, [11]University of Rijeka, School of Medicine, Rijeka, Croatia, [12]Medical School REGIOMED, Coburg, Germany, [13]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [14]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina

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

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

*TRANSLATION MEDICINE*

Presentation number: MG 51

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

**Vjera Ninčević**[1,2], Tea Omanović Kolarić[1,2], Milorad Zjalić[3], Marina Čović[4], Tomislav Kizivat[5,6], Lucija Kuna[1], Robert Smolić[1], Aleksandar Včev[7], Martina Smolić[1,2], Ines Bilić Ćurčić[2,8]

[1]Department of Pharmacology and Biochemistry, Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [2]Department of Pharmacology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [3]Department of Molecular Medicine and Biotechnology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [4]Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [5]Clinical Institute of Nuclear Medicine and Radiation Protection, University Hospital Osijek, Osijek, Croatia, [6]Department for Nuclear Medicine and Oncology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, [7]Department of Pathophysiology, Physiology and Immunology, Faculty of Dental Medicine and Health Osijek, J.J. Strossmayer University of Osijek, [8]Department of Diabetes, Endocrinology and Metabolism Disorders, University Hospital Osijek, Osijek, Croatia

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

**Keywords:** diabetic nephropathy, LLC-PK1 cell culture, empagliflozin

Presentation number: MG 52

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

**Vjera Ninčević**[1,2], Tea Omanović Kolarić[1,2], Hrvoje Roguljić[2,3], Klara Ormanac[4], Dea Sabo[4], Ana Ninčević[5], Robert Smolić[1], Aleksandar Včev[6], Martina Smolić[1,2], Ines Bilić Ćurčić[2,7]

[1]Department of Pharmacology and Biochemistry, Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [2]Department of Pharmacology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [3]Department of Internal Medicine, University Hospital Osijek, Osijek, Croatia, [4]Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [5]School of Medicine, University of Zagreb, Zagreb, Croatia, [6]Department of Pathophysiology, Physiology and Immunology, Faculty of Dental Medicine and Health Osijek, J.J. Strossmayer University of Osijek, Osijek, Croatia, [7]Department of Diabetes, Endocrinology and Metabolism Disorders, University Hospital Osijek, Osijek, Croatia

Transforming growth factor-beta (TGF-**β**) has recently been associated with diabetic nephropathy (DN) development. It causes cell apoptosis induced by oxidative stress and cell proliferation and migration triggered by hyperglycemia and inflammation. Liraglutide is an antihyperglycemic agent that has a direct renoprotective effect. This study aimed to evaluate the effects of liraglutide on cell viability and TGF-**β** expression in the LLC-PK1 model of DN. Cell viability was determined by colorimetric MTT assay and erythrosine B color exclusion assay. The expression of mRNA TGF- **β**1 was measured by RT-PCR and **β**-actin was used as an internal control. LLC-PK1 cell culture was treated with different concentrations of glucose (1.5, 30 mM) and the combination of glucose and H2O2 (0.5 mM) for 24 hours. To study the renal effect of liraglutide, cells were treated for 24 hours with different combinations of glucose and liraglutide (10, 20 nM) and combinations of glucose, H2O2, and liraglutide. A significant decrease in MTT levels compared to control ($p < 0.01$; $p < 0.001$) was observed after treatment with a combination of HG30/ H2O2 and HG30 alone. Cell viability was improved by the addition of liraglutide (10 nM) to cells treated with HG30, while 20 nM had no effect. There was no significant difference in cell survival with the addition of HG30 and HG30/ H2O2 compared to control. The addition of liraglutide at both concentrations to cells treated with HG30 and HG30/H2O2 improved cell survival, although significance was only numerical, not reaching statistical significance. TGF-**β**1 expression levels were significantly increased in cells treated with HG30 ($p < 0.001$). Liraglutide inhibited TGF-**β**1 expression except in HG30/H2O2 treated cells. Our results support a protective role of liraglutide in LLC-PK1 cells, mediated by inhibition of TGF-**β**1, thus reducing oxidative stress damage.

**Keywords:** diabetic nephropathy, LLC-PK1 cell culture, liraglutide





Presentation number: MG 53

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

**Lucija Kuna**[1,2], Milorad Zjalic[3], Tomislav Kizivat[4], Hrvoje Roguljic[1,2], Vjera Nincevic[1,2], Tea Omanovic Kolaric[1,2], Ana Petrovic[1,2], Aleksandar Vcev[1,2], Catherine H. Wu[5], Martina Smolic[1,2], Robert Smolic[1,2]

[1]Department of Pharmacology and Biochemistry, Faculty of Dental Medicine and Health Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia, [2]Department of Pharmacology, Faculty of Medicine Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia, [3]Department of Medical Biology and Genetics, Faculty of Medicine, J. J. Strossmayer University of Osijek, Osijek, Croatia, [4]Department of Nuclear Medicine and Oncology, Faculty of Medicine Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia, [5]Department of Medicine, Division of Gastroenterology-Hepatology, University of Connecticut Health Center, Farmington, CT, USA

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

**Keywords:** peptic ulcer disease, garlic extracts, lansoprazole, sodium taurocholate

Presentation number: MG 54

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

**Benjamin Benzon**[1], Sandra Marijan[2], Matij Pervan[3], Angela Mastelić[2], Vedrana Čikeš Ćulić[2]

[1]University of Split, School of Medicine, Dpt. of Anatomy, Histology and Embryology, Split, Croatia, [2]University of Split, School of Medicine, Dpt. of Medical Chemistry and Biochemistry, Split, Croatia, [3]University of Split, School of Medicine, Medical doctor education program

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

**Keywords:** metastasis, breast cancer, differentiation, **ε**-PCL implant



# FORENSIC GENETICS

*FORENSIC AND COMPARATIVE GENETICS*

Presentation number: FG 1

# VALIDATION OF SERATEC HEMDIRECT ON ANIMAL BLOOD

Mia Belobrajdić[1], Dženita Vugdalić[1], Jadranko Boras[2], Damir Skok[2], Antonela Paladin[3], Livia Slišković[1], Ivan Jerković[1], **Josip Crnjac[1]**

[1]University Department of Forensic Sciences, University of Split, Split, Croatia, [2]Zagreb Zoo, Zagreb, Croatia, [3]Faculty of Science, University of Split, Split, Croatia

The validation study of the SERATEC HemDirect blood test has been performed and the test is validated for forensic use. Initial validation test animal blood but all animal blood samples used in this study were from mammalian species and animals from other systematic groups were not included. This study aimed to determine whether the SERATEC HemDirect immunochromatographic test is highly specific for forensic identification of human blood, and whether there are non-mammalian species whose blood would show a false positive result on this test. The preliminary study included 8 samples of venous blood, human and 7 different animal species. All animal blood samples were obtained from Zagreb Zoo during the regular examination of animals. We used the blood of 7 animals who are commonly involved in car accidents or illegal hunting. All blood samples were analyzed by the SERATEC HemDirect test. A dilution set (1/4, 1/8, 1/10, 1/16, 1/20, 1/24) was prepared from whole blood by adding double-distilled water. A 50 µL sample of whole blood and each dilution were placed on a white cotton cloth and left to dry at room temperature. After drying, 1/4 of the sample was cut out and extracted in HemDirect buffer for 30 minutes. After extraction, three drops of each sample were added to the test well and the results were read and recorded after 10 minutes. The SERATEC HemDirect test showed positive results in all analyzed human blood samples (whole blood and dilutions 1/4, 1/8, 1/10, 1/16, 1/20, 1/24). In the analyzed blood samples of all animal species, the test gave negative results except in one case where a diluted blood sample of Rook (Corvus frugilegus L.) showed a weak positive result. The one weak positive result is ground for extending the study to more species of birds and reptiles since those can be found as pets in homes and can be involved in forensic investigations.

**Keywords:** HemDirect, validation, forensics, blood test

Presentation number: FG 2

# GENERATING HUMAN Y-STR HAPLOTYPES FROM MEDICINAL LEECHES

**Elizabeth Knapp**, Reena Roy

The Pennsylvania State University, Schreyer Honors College and Forensic Science Program, University Park, Pennsylvania, USA

Leeches are annelids which have been used for many centuries in the field of medicine. These worms convey an anticoagulant, known as hirudin, in their salivary glands. Once they hook onto human flesh, they make a small incision, and ingest 5 mL to 15 mL of blood from one human. In this study, 420 samples from 35 leeches were analyzed using Copan microFLOQ® Direct swabs, and 4N6 FLOQSwabs® Crime Scene collection devices, as well as the Yfiler® Plus, and the PowerPlex® Y23 amplification kits. The Yfiler® Plus amplification kit is a 27-plex Y-STR system which includes seven rapidly mutating Y-STR loci which allows for discrimination among related individuals, while the PowerPlex® Y23 System includes 23 Y-STR loci. North American medicinal leeches, Macrobdella Decora, obtained from a commercial source, were fed human blood meal from a male donor, and euthanized by freezing at specific times from 0 hour to 24 hours. The first method involved collecting minute amount of blood on the tip of Copan microFLOQ® Direct swab from the midgut of these organisms and the amplification of the blood while the swab remained in the reagents during thermal cycling. In the second method, 5 µL of blood from each midgut was concentrated to approximately 0.5 µL to 2.0 µL. The microFLOQ® Direct swabs were used to collect the concentrated blood and amplified directly. Finally, the 4N6 FLOQSwabs® Crime Scene swabs were utilized to collect the blood from the midgut, extracted with the DNA Investigator Kit from Qiagen. Extracted samples were quantified with the Quantifiler Trio DNA Quantification Kit. Y-STR profiles were obtained from known blood samples (reference samples) using the same methods. The Y-STR profiles generated from the blood ingested by the medicinal leeches using the three methods were consistent with the reference profiles of the donors. The results of this research reveal that the Copan microFLOQ® Direct swab is an excellent way to generate Y-STR profiles from the blood ingested by medicinal leeches. The extracted samples using 4N6 FLOQSwabs® Crime Scene swabs also yielded consistent and concordant profiles within and between samples and thus can be utilized to extract ingested blood. The direct amplification process bypasses the time-consuming, labor-intensive extraction and quantitation steps.

**Keywords:** Copan microFLOQ® Direct swabs, Y-STR, Leech





Presentation number: FG 3

# THE ROLE OF GENETICS IN FATAL PULMONARY THROMBOEMBOLISM FORENSIC DIAGNOSIS

**Giuseppe Davide Albano**, Stefania Zerbo, Ginevra Malta, Antonina Argo

PROMISE Department, University of Palermo, Palermo, Italy

The study aims to review the most recent and best evidence provided in the literature regarding the application of genetics methodology in the forensic diagnosis of fatal pulmonary thromboembolism (PTE) cases and the medico-legal issues involved. This review was conducted by performing a systematic literature search on online resources (PubMed Central database and Google Scholar) until 31st March 2022, using the following key terms: "((Genetics) OR (Forensic genetics)) AND ((pulmonary thromboembolism) OR (fatal pulmonary thromboembolism) OR (pulmonary thromboembolism diagnosis) OR (pulmonary thromboembolism deaths))." Reviews, abstracts, animal studies, articles regarding surviving subjects, and articles in which the correlation between forensic genetics and pulmonary thromboembolism diagnosis is not discussed were excluded. Only human studies of fatal PTE with genetic analyses were included. The characteristics of the identified articles will be summarized. Most of the articles were retrospective studies on autopsy samples. Pulmonary thromboembolism was identified as the cause of death in all cases. Epidemiological data and clinical history were available in all studies. The most frequently used samples for genetic analyses were blood and postmortem tissues. Genetic testing for common prothrombotic variants included FV Leiden, FII G20210A, and methylenetetrahydrofolate reductase (MTHFR). Trauma and immobilization were the most frequent risk factors for PTE, and in most cases, the clinical and epidemiological analysis showed patients' risk conditions. However, the identified articles show the importance of thrombophilia genetic screening in PTE cases with no significant risk factors. Moreover, unique characteristics were observed among different gender, age, and ethnic groups. This study highlights an association between genetic differences in different loci and PTE risk. Clinicians must be aware of the role of genetics in PTE epidemiology to undertake the proper preventive measures. In the forensic field, genetic testing is mandatory in selected fatal PTE cases, especially in medical malpractice cases. Genetic screening in selected cases should become a routine diagnostic test for PTE prevention.

**Keywords:** forensic genetics, fatal pulmonary thromboembolism, pulmonary thromboembolism deaths, forensic diagnosis

Presentation number: FG 4

# MASTR: AN EFFECTIVE PROBABILISTIC GENOTYPING TOOL FOR INTERPRETATION OF 2, 3, AND 4-PERSON STR MIXTURES ASSOCIATED WITH DIFFERENTIALLY DEGRADED DNA

**Mitchell Holland**[1], Teresa Tiedge[2], Abigail Bender[3], Sidney Gaston-Sanchez[4], Katherine Marino[5], Sofia Canlas[6], Jennifer McElhoe[1]

[1]Pennsylvania State University, Biochemistry & Molecular Biology Department, Forensic Science Program, University Park, PA, USA, [2]Department of Population Health and Pathology, North Carolina State University, Raleigh, NC, USA [3]Texas Department of Public Safety, Austin Crime Laboratory, Austin, TX, USA [4]Armed Forces Medical Examiner System, Dover, DE, USA [5]Baptist Medical Center South, Montgomery, AL, USA [6]Massachusetts State Police Crime Laboratory, Maynard, MA, USA

The goal was to evaluate the impact of differentially degraded 2, 3, and 4-person STR mixtures on likelihood ratios (LRs) generated by the probabilistic genotyping software, MaSTR (SoftGenetics, LLC). Mixtures were prepared from donors with known Fusion 6C profiles. Ratios considered were 1:1, 1:3, 1:6, and 1:10 for 2-person (n=144), 1:1:1, 1:1:3, 1:3:6 and 1:6:6 for 3-person (n=102), and 1:1:1:1, 1:1:1:3, 1:1:3:3, 1:3:3:6, 1:3:6:6, and 1:6:6:6 for 4-person mixtures (n=52), with combinations of pristine and degraded sources of DNA rotated between major and minor contributors. Shearing of buccal DNA extracts was achieved mechanically using a Covaris S220; 150 and 250 bps to simulate severe and moderate levels of DNA degradation, respectively. Data were analyzed using GeneMarker HID (SoftGenetics, LLC). Results were imported into MaSTR and analysis performed using a model panel of Fusion 6C data run on a 3130xl Genetic Analyzer. A subset of LRs were generated using 40,000 versus 10,000 iterations per chain (eight chains total, with a burn-in of 8,000 iterations), and with or without a conditioning profile. MaSTR performed as expected. The log(LR) values for mixture samples containing high quantities of pristine sources of DNA were at optimal levels. Lower-quality mixture data associated with sources of DNA at <0.05 ngs for each contributor resulted in peak imbalance and allelic dropout which reduced the weight in support of a contributor. This was exacerbated by higher levels of degradation, with some instances resulting in log(LR) values in support of an exclusion. As expected, LRs were lower when a known contributor was not provided, especially for samples containing degraded DNA. There was no appreciable difference in LRs when comparing 10,000 and 40,000 iterations per chain for 2-person mixtures. In all cases, findings were consistent with expectations associated with CE-based profile information. Overall, MaSTR proved to be a reliable tool for the analysis of STR mixtures of differentially degraded sources of DNA. The points of view in this abstract are those of the authors and do not reflect the views of their respective agencies. In addition, this abstract in no way reflects an endorsement of products, instruments, or software.

**Keywords:** probabilistic, genotyping, STR, mixtures, degraded



*FORENSIC DNA DATABASES*

Presentation number: FG 5

# CROATIAN DNA DATABASE

**Igor Obleščuk**[1], Andrea Ledić[1], Adela Makar[1], Vedrana Škaro[2,3], Damir Marjanović[2,4], Dragan Primorac[5,6,7,8,9,10,11,12,13,14]

[1]Forensic Science Centre "Ivan Vučetić", Ministry of the Interior, Zagreb, Croatia, [2]Molecular Anthropology Laboratory, Center for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia, [3]DNA Laboratory, Genos Ltd., Zagreb, Croatia, [4]Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina, [5]St. Catherine Hospital, Zagreb, Croatia, [6]School of Medicine, University of Split, Split, Croatia, [7]University Department of Forensic Sciences, University of Split, Split, Croatia, [8]School of Medicine, University of Osijek, Osijek, Croatia, [9]Faculty of Dental Medicine and Health, University of Osijek, Osijek Croatia, [10]School of Medicine, University of Rijeka, Rijeka, Croatia, [11]Eberly College of Science, Pennsylvania State University, University Park, PA, USA, [12]Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [13]Medical School REGIOMED, Coburg, Germany, [14]The National Forensic Sciences University, Gandhinagar, Gujarat, India

The Croatian DNA database was legally formed at the beginning of the 2000s with new Police regulation. Firstly, it contains DNA profiles of persons in general and crime stains/unknown persons stored in home-made database. In 2006 database migrated to CODIS (FBI) platform, where DNA profiles of known persons were distinguished into suspects, victims, and laboratory staff. With the new system, DNA profiles originated from unknown persons also distinguished into DNA profiles originated from crime scene/stains and those originated from unidentified bodies/persons. Since the migration to CODIS, the Croatian DNA database recorded more than 2.500 matches of unsolved crime stains with suspect DNA profiles. With new regulation that came to force in late 2011 storing DNA profiles into the database, the time of retention and deletion especially DNA profiles from individuals involved in crime act was regulated more precisely. In 2018, because of Croatia's obligation to EU directives, DNA profiles were made available to EU member states through so-called Prüm decisions that enabled 400 unsolved domestic crimes to be linked to known persons. Before the start of automated exchanging DNA profiles and data, the DNA database and all stored profiles were thoroughly revised in which DNA profiles of suspects were differentiated into Suspects (general name for suspects, arrestees, and offenders not yet convicted) and Convicted Offenders according to revised legal acts. DNA profiles of crime stains also were revised in accordance with a number of loci and statistical significance. Today Croatian DNA database counts approximately 15.000 DNA profiles of which 11.000 are daily exchanging with other EU member states.

**Keywords:** DNA database, CODIS, DNA profile, crime stain, suspect

Presentation number: FG 6

## A CASE FOR INTERNATIONAL ETHICAL GOVERNANCE OF FORENSIC DNA DATABASES

**Rebecca Walter**

Northumbria University, Law School, Newcastle Upon Tyne, United Kingdom

The United Nations Educational, Scientific, and Cultural Organization has issued no fewer than three declarations on ethical conduct and genetics since 1997, but each of those declarations leave the ethical governance of forensic DNA databases to the country in which the database exists. In 2015, Kuwait attempted to create a universal DNA database, which was met with resounding international condemnation, including from the United Nations. Without international ethical governance, and the decision of the constitutional court in Kuwait, the database was not 'unethical' by the current standards. Without a cohesive, international ethical code, forensic genetics has strayed into the world of 'can we do?' often without asking the question of 'should we do?' Since 1995 when the first forensic DNA database was created, governments have amassed large databases of genetic information on a subset of their population and the use of those databases has gone largely unchecked. The international community cannot expect these databases to be used ethically if they do not provide ethical guidelines for their use, this presentation considers the creation of ethical guidelines for the creation and use of forensic DNA databases as well as a path forward for the declaration, implementation, and enforcement of those guidelines.

**Keywords:** Ethics, Forensic Genetics, International Ethics, Forensic DNA Database



# *FORENSIC DNA PHENOTYPING*

Presentation number: FG 7

# EFFECT OF OVER-THE-COUNTER DRUGS ON DNA ANALYSIS OF BLOOD INGESTED BY MEDICINAL LEECHES

**Dipesh Patel**[1], Dan Sykes[2], Reena Roy[3]

[1]Undergraduate Student, Schreyers Honors College, The Pennsylvania State University, University Park, PA, USA, [2]Chemistry Department, The Pennsylvania State University, University Park, PA, USA, [3]The Pennsylvania State University, Forensic Science Program, Department of Biochemistry and Molecular Biology, University Park, PA, USA

Painkillers, such as aspirin (acetylsalicylic acid), are used by many to alleviate the pain associated with common ailments. Although the half-life for aspirin is only 2-5 hours, it is possible that there may still be residual drug in the body post-mortem. Hence, if a medicinal leech, Macrobdella Decora, feeds on the blood of a victim or a suspect who had recently ingested an analgesic, it may be possible to simultaneously obtain a complete DNA profile and drug identification from the blood extracted from the crop (midgut) area of the leech. The potential for generating a DNA profile depends on whether or not acetylsalicylic acid inhibits amplification due to the acid having a phenol group. Human blood was spiked with 25 ppm and 50 ppm aspirin solutions followed by DNA extraction at 0, 24 and 48 hours. The extracted DNA was quantified and amplified using the PowerPlex® Fusion 6 C for autosomal STR and PowerPlex® Y-23 system for Y-STR analysis. HPLC was used to determine the presence of aspirin. These amplified products were analyzed by capillary electrophoresis and fragment analysis of these reference samples were completed using appropriate software. Blood mixed with the aspirin was fed to the leeches which were then euthanatized by freezing at 0, 12, and 24 hours after being fed. Human blood from their crop was extracted and analyzed for the presence of aspirin and for generating the DNA profiles. Since complete autosomal and Y-STR profiles were obtained from blood spiked with aspirin and ingested by the leeches, it is concluded that inhibition was not caused by acetylsalicylic acid. No inhibition was noted in the quantification data and DNA amplification resulted in complete profiles from the reference blood samples mixed with aspirin and from the blood stored in the midgut of the medicinal leeches. All profiles were consistent within and between samples, thus indicating that medicinal leeches can be a valuable source of forensic evidence.

**Keywords:** Medicinal leeches, Aspirin, Autosomal STR, Y-STR

# *GENETIC ANALYSIS OF FORENSIC NON - HUMAN MATERIAL*

Presentation number: FG 8

# ANALYSIS OF CANNABINOID PROFILES AND THCAS SEQUENCES IN SEIZED CANNABIS RECOVERED FROM GROWING FARM

**Lucija Barbarić**, Slavena Cukrov Bezbradica, Ines Gmajnički, Adela Makar, Andrea Ledić

Forensic Science Centre "Ivan Vučetić", Zagreb, Croatia

The aim of this study was to assess the genetic and chemical profiles of the seized cannabis obtained from the growing farm. According to the farmer, certified variety Santhica 27 was sown. However, police officers recovered 118 plants under the suspicion of drug production. Quantitative cannabinoid content was determined using GC-FID on Agilent 7890A GC System. Extraction of DNA from plant material was performed using DNAeasy Plant Mini kit (Qiagen) following the manufacturer's protocol. DNA quantity was determined by Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific). A portion of the tetrahydrocannabinolic acid synthase (THCAS) gene was amplified using Qiagen Multiplex PCR kit. Sequencing was performed according to the laboratory standard operating procedures using Big Dye Terminator v.3.1 Cycle Sequencing Kit on AB3500xl GA (both from Life Technologies). Data analysis was accomplished in Sequencher v.5.4.6 (Gene Codes). Sequences were aligned against the THCAS coding sequence of the drug-type Skunk deposited in NCBI (KJ469378). Based on qualitative chemotype, 61% of total samples were cannabigerol (CBG) predominant, and 39% were THC dominant. Alignment of the obtained sequences correlated with their cannabinoid content, clustering in two distinct groups. Eleven identical sequences differed from variants in hemp Finola and probably Santhica 27 that showed undetectable THCAS. Moreover, several variants were revealed including 1064A that had been previously reported to be responsible for THCAS inactivation and cannabigerolic acid (CBGA) precursor accumulation. This finding was in correlation with the average CBG content of 2%. The remaining three sequences were identical to those obtained in drug-type. The average proportion of THC in these plants was 2.5%. According to chemical and genetic results, we conclude that on the industrial hemp farm was growing THC and CBG predominant cannabis varieties under the guise of certified variety Santhica 27.

**Keywords:** Cannabis sativa L, chemotype, THCA synthase, growing farm

Presentation number: FG 9

# DNA ANALYSIS OF SCARCE FUNGAL SAMPLES INDICATES THEIR PSYCHOACTIVE ORIGIN

**Ivana Horjan Zanki**, Andrea Ledić, Adela Makar

Forensic Science Centre "Ivan Vučetić", Ministry of the Interior, Zagreb, Croatia

The majority of psychoactive fungi contain psilocybin (PY) and psilocin (PI), latter causes LSD-like psychoactive effects but lesser in intensity. PY and PI are illegal in Croatia and most of EU countries, while fungi as their source are mainly accepted. Since PY/PI are not yet synthesized in fungal spores and sclerotia ("truffles"), drug users increasingly buy them via Internet without legal consequences. Furthermore, established toxicology and morphology methods are useless on these kind of samples. Hence, main goal of this study was to present the usefulness of DNA analysis on abovementioned forensic samples in order to confirm its psychoactive origin. Study addressed sequencing of internal transcribed spacer (ITS) of nuclear DNA and large portion of 28S gene (nuclear-encoded large subunit ribosomal RNA genes, nLSU-rDNA). ITS region enables unambiguous identification of fungal species while part of LSU gene from 5' end enables distinguishing between closely related psychoactive and non-psychoactive species. DNA from total of 14 "spore-print"and „truffle" samples seized on Croatian territory (2010-2014), were isolated and ITS region and large portion of 28S gene (nLSU-rDNA) were sequenced. ITS and LSU sequences were obtained from 11 out of 14 tested samples with haplotypes congruent to GenBank database sequences of psychoactive species Psilocybe cubensis and Psilocybe mexicana. One out of 14 samples most probably represents a mixture of these two, and for the two remaining samples only ITS haplotype was obtained, matching those of P. cubensis. As previously published, P. cubensis varieties are most popular among users due to their proven potency and are easy growing. Applied DNA method can be easily implemented into routine forensic workflow. The latter legislation update, i.e. to include fungi species/body parts as the source of psychoactive substances could lead to continuing decrease of illegal fungi drug abuse.

**Keywords:** „spore print", „truffles", DNA sequencing, ITS, LSU





Presentation number: FG 10

# IDENTIFICATION OF GENETIC MATERIAL OF WATERBORNE MICROORGANISMS FROM STERNAL ASPIRATE

**Karola Petrus**, Vivien Fejes, Dominika Szűcs, Katalin Sipos, Gábor Simon, Viktor Soma Poór

University of Pécs, Medical School, Department of Forensic Medicin, Pécs, Hungary

The most widely used complementary method for the diagnosis of drowning is the detection of waterborne organisms in the organs of systemic circulation. In this study, we adopted sternal bone marrow aspiration – which in hematology a routine method – instead of femoral bone marrow opening, to prevent the possible contamination during autopsy. During autopsy, besides sternum aspirate, lung and spleen tissues, and bone marrow of the femur were also taken for test the presence of diatom shells by microscopy, and cyanobacterial DNA by PCR. From all tissue samples, 2 g were mixed with 6 ml digesting buffer (0.01 M Tris, 2 % SDS) and 20 µl Proteinase K. After 2 days of incubation at 56°C, microscopic slides were prepared for diatom test, furthermore, 400 µl aliquots were saved for cyanobacterial DNA detection. DNA extraction was carried out with Macherey-Nagel NucleoSpin Soil kit, followed by amplification of a segment of cyanobacterial 16S rRNA gene. The amplicons were separated in 2 % agarose gel and visualized with SYBR Gold intercalating dye. We were able to obtain sternal bone marrow aspirate in all the tested seven suspected drowning cases. In four of the sternal samples, both diatoms and cyanobacterial DNA were detected, while in one additional case, sternum was tested positive by PCR, but no diatom shells were identified. Femoral bone marrow was positive for diatoms only in one case, and by PCR in two cases. The rest of the cases – drowning into a bathtub, falling into a cistern -, all tissue samples were negative. According to autopsy, heart failure, and the high fall was the cause of death, respectively. Sternal bone marrow aspiration can be simply performed in the beginning of the autopsy, before the body cavity is opened, therefore minimizes the chance of contamination. Our results showed that diatom test has low sensitivity, which can be increased with PCR-based methods. Sternal bone marrow was found to be a better source for detection of waterborne organisms in our pilot study than the femoral bone marrow.

**Keywords:** Drowning, Cyanobacteria, DNA

# *GENOME - BASED APPLICATIONS IN FORENSIC SCIENCE*

Presentation number: FG 11

# DETECTION OF 13 HYPERVARIABLE REGION 1 (HV1) SNPS USING SINGLE-BASE EXTENSION (SBE) PRIMERS IN PARALLEL WITH SANGER SEQUENCING

**Rijad Konjhodžić**[1], Lana Salihefendić[1,2], Ivana Čeko[1,2], Enis Kandić[1], Adna Ašić[2], Milovan Kubat[3]

[1]Alea Genetic Center, Sarajevo, Bosnia and Herzegovina, [2]International Burch University, Department of Genetics and Bioengineering, Sarajevo, Bosnia and Herzegovina, [3]University of Zagreb, Faculty of Medicine, Institute for Forensic Medicine, Zagreb, Croatia

The aim of this study was to determine whether single-base extension (SBE) chemistry can be applied to the forensic practice of testing the target single nucleotide polymorphisms (SNPs) of the mitochondrial DNA (mtDNA) Hypervariable Region 1 (HV1). Despite its rather weak discrimination power compared to the short tandem repeat (STR) markers, high copy number of mtDNA per cell and its stability against degradation still guarantee mtDNA testing a place in modern forensic genetics. Buccal swab samples were obtained from 294 unrelated individuals from Bosnia and Herzegovina, following signing of the informed consent form by all participants. After DNA isolation, full sequencing of HV1 was done using chain-termination Sanger sequencing method. SBE reactions were then performed by targeting 13 SNPs that were identified to be the most frequent in the study population. Uniplex SBE reactions for each individual SNP, as well as two multiplex reactions were prepared for both pure and mixed samples, and results thus obtained were compared with those obtained by Sanger sequencing. The results showed complete agreement of the Sanger sequencing results with SBE reactions for both uniplex and multiplex reactions. No significant differences in signal intensity between reactions with forward and reverse SBE primers were observed. The results obtained with SBE were encouraging in regard to multiplexing and processing of the mixed samples, since the allele of minor contributor to the sample was observed in SBE electropherogram in all prepared mixtures. SBE method is limited by the fact that only target SNPs of interest will be analyzed, meaning that they must be carefully selected and curated for each population. However, typing with SBE protocol is accurate, as compared to the golden standard of Sanger sequencing, but was more time- and labor-efficient and simpler to analyze.

**Keywords:** hypervariable region 1 (HV1), mtDNA, mtDNA control region, single-base extension (SBE), single nucleotide polymorphism (SNP)

Presentation number: FG 12

# SINGLE CELL TRANSCRIPTOME AND GENOME SEQUENCING FOR GENETICALLY SEPARATING, CHARACTERIZING AND IDENTIFYING INDIVIDUALS FROM BIOLOGICAL MIXTURES

**Lucie Kulhankova**[1], Diego Montiel Gonzales[1], Eric Bindels[2], Daniel Kling[4], Eskeatnaf Mulugeta[1,3], Manfred Kayser[1]

[1]Erasmus University Medical Center, Department of Genetic Identification, Rotterdam, The Netherlands, [2]Erasmus University Medical Center, Department of Hematology, Rotterdam, The Netherlands, [3]Erasmus University Medical Center, Department of Cell Biology, Rotterdam, The Netherlands, [4]National Board of Forensic Medicine, Department of Forensic Genetics and Toxicology, Linkoping, Sweden

Separating individuals who contributed to biological mixtures and their genetic identification are crucial in forensic investigations where mixed crime scene traces are often encountered, but remains largely unsolved despite several attempts. Here, we present a single cell transcriptome sequencing approach with a novel bioinformatics pipeline aiming to solve this long-standing, societally relevant problem. Our pipeline extracts different sets of single nucleotide polymorphisms (SNPs) from single cell RNA sequencing (scRNA-seq) data we obtained from biological mixtures and uses them for the different purposes. Our approach also allows determining the tissue(s) of origin of the cells present in the mixture. We validated our approach using de novo generated scRNA-seq datasets from multi-person blood mixtures and in-silico mixtures generated from individual scRNA-seq datasets involving different numbers and bio-geographic ancestries of contributors and different ratios. For all up to 9-person balanced and imbalanced mixtures with ratios up to 1:60, we achieved a clear single cell cluster separation. Sex, bio-geographic ancestry of the maternal, paternal and bi-parental sides as well as individual identification were genetically determined correctly for all separated contributors. To further increase the number of captured SNPs, thereby increasing the ability of deconvoluting more complex mixtures including minor contributions based on less cells, we additionally investigated single cell genome sequencing by applying the single cell chromatin accessibility assay (scATAC-seq) to various biological mixtures. Our single cell omics approach has the potential to solve forensic mixture deconvolution for genetically separating, characterizing, and individually identifying perpetrators from multi-person biological mixtures found at crime scenes and can also be applied for detecting and resolving contamination in cell cultures or to separate cancer cells from normal ones.

**Keywords:** mixtures, single cell, transcriptomics, SNPs





Presentation number: FG 13

# EVALUATING THE USE OF ANCIENT DNA LABORATORY PROTOCOLS IN THE DOWNSTREAM DNA IDENTIFICATION OF BURNED FORENSICALLY-DERIVED SAMPLES

Cody Parker[1], Mathew V. Emery[1,2,3], Katelyn L. Bolhofner[4,5], Suhail Ghafoor[2], Erin Rawls[6], Stevie Winingear[1], Robert Oldt[6], Sreetharan Kanthaswamy[4], Jane E. Buikstra[1,5], Giovanna Vidoli[7], Joanne Devlin[7], Laura C. Fulginiti[8], **Anne C. Stone**[1,2,5]

[1]School of Human Evolution and Social Change, Arizona State University, Tempe, AZ, USA, [2]Center for Evolution and Medicine, ASU, Tempe, AZ, USA, [3]Department of Anthropology, Binghamton University, Binghamton, NY, USA, [4]School of Math and Natural Sciences, ASU, Glendale, AZ, USA, [5]Center for Bioarchaeological Research, ASU, Tempe, AZ, USA, [6]School of Life Sciences, ASU, Tempe, AZ, USA, [7]Department of Anthropology UT, Knoxville, TN, USA, [8]Maricopa County Office of the Medical Examiner, Phoenix, AZ, USA

DNA analysis is a pivotal tool in the identification of human remains recovered from forensic contexts. Under ideal conditions, DNA is sufficiently preserved for DNA identification, including short tandem repeat (STR) profiling. However, natural exogenous factors can limit the amount of recoverable DNA from skeletal material. Where skeletal tissues are exposed to more severe insults, such as fire, obtaining adequate quantities of DNA for downstream analysis has proven challenging. As ancient DNA (aDNA) research is already heavily invested in optimizing the recovery of DNA from challenging samples under similar contexts (e.g., low yields of highly fragmented/degraded DNA), we evaluate DNA yield, quality, usability in STR analyses, NGS library preparation, mitochondrial DNA (mtDNA) recovery, and targeted single nucleotide polymorphism (SNP) assays using protocols adapted from aDNA and forensic analyses. Initial STR and NGS analyses showed that the aDNA extraction protocol recovered higher quantities of shorter DNA fragments at temperatures >350°C. Additionally, our results suggest that there may be an acute point of DNA degradation at temperatures >350°C resulting in a drop in observed STR allele recovery, mtDNA genome read counts and depth of coverage, and SNP calling efficiency. We continue this research using samples obtained via controlled burning of ~10 donor cadavers to evaluate DNA recovery and useability across multiple skeletal elements by comparing methodologies in samples from the same sampling locations across individuals. Preliminary results from this second study show that aDNA extraction protocols appear to provide adequate DNA yields more consistently for initial STR analyses, especially at higher levels of thermal exposure. Our results demonstrate that by optimizing our laboratory protocols to recover DNA efficiently from highly degraded bone samples, we greatly enhance individual identification possibilities in such challenging forensic contexts.

**Keywords:** forensic DNA, burned bone, genomes

Presentation number: FG 14

# EFFECT OF OZONE DISINFECTION ON FORENSIC SAMPLES

**Vivien Fejes**, Dominika Szűcs, Katalin Sipos, Gábor Simon, Viktor Soma Poór

University of Pécs, Medical School, Department of Forensic Medicine, Pécs, Hungary

In certain situations, disinfection of forensic case sample may be required, e.g., in the early stages of COVID-19 pandemic, or in case of objects which might have been related to biological warfare or terrorism. Our aim was to test the applicability of ozone disinfection technique on forensic samples. We examined the effect of ozone on mock casework samples. We collected samples from worn surgical masks. We investigated blood, saliva, and semen stains. Immunoassays were applied to detect special antigens in blood, saliva and semen samples. As supplementary test, luminol, Phadebas test and microscopy was applied, respectively, in addition to examination of body fluid specific RNA markers. In surgical masks, the detected allele counts between control and ozone-treated samples showed no significant difference ($p = 0.513$). However, the two tested sampling sites, the elastic earloop and the middle part of the nosepiece, showed significant difference in detected allele numbers ($p = 0.011$). Comparison revealed a statistically significant difference between the contributors ($p = 0.001$). Immunoassays were able to identify the sample type after the ozone-treatment. Phadebas showed that ozone-treated samples showed no or only very low enzyme activity. By RT-PCR, we could detect the specific markers in both ozone-treated and control samples. The STR profiles from the masks showed that sampling site and contributor had greater effects on profiling that the ozone treatment itself. Ozone does not damage the structure of hemoglobin, amylase and prostate specific antigen; however, it decreases the enzymatic function of salivary amylase. Detection with luminol was successful. Microscopic observation of sperm cells also showed no alteration between the ozone-treated and the control samples. RT-PCR was successful in all cases; therefore this disinfection method does not hamper the RNA-based biological fluid identification. According to our pilot study, ozone treatment does not encumber the routine forensic sample processing, so ozone treatment could become an accepted method to disinfect crime scene samples.

**Keywords:** STR, ozone, biological fluid, RNA biomarkers





Presentation number: FG 15

# AN ASSESSMENT OF PROBABILISTIC APPROACHES TO MTDNA MIXTURE INTERPRETATION

**Jennifer McElhoe**[1], Alyssa Addesso[1], Brian Young[2], Jeff Smith[2], Mitchell Holland[1]

[1]The Pennsylvania State University, Forensic Science Program, Department of Biochemistry and Molecular Biology, University Park, PA, USA, [2]NicheVision, LLC, Akron, OH, USA

Mitochondrial (mt) DNA plays an important role in the fields of forensic and clinical genetics, molecular anthropology, and population genetics, with mixture interpretation being of particular interest in medical and forensic genetics. In forensics, mixture deconvolution generally relies on genotyping of STRs, but this approach struggles to resolve samples with similar contributor proportions and degraded samples. The high copy number, haploid state (single haplotype contributed per individual), high mutation rate, and well-known phylogeny of mtDNA, makes it an attractive marker for mixture deconvolution in damaged and low quantity samples of all types. Given the desire to deconvolute mtDNA mixtures, the goals of this study are to 1) combine and assess two existing software tools, MixtureAce™ and Mixemt (1), to deconvolute mtDNA mixtures 2) create a dataset of in-silico MPS mixtures from whole mitogenome haplotypes representing a diverse set of population groups, and consisting of two and three contributors at different dilution ratios to test the combined tools, and 3) since amplicon targeted sequencing is desirable, and is a commonly used approach is forensic laboratories, create biological mixture data associated with two amplification kits: PowerSeq™ Whole Genome Mito (Promega™) and Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific by AB™) to further validate the software for use in forensic laboratories. Findings will include qualitative measures and statistical evaluation of forensic evidence and will significantly enhance the value of mtDNA testing in forensic laboratories through the assessment of software tools and best practices regarding the deconvolution of mtDNA mixtures. Overall, exact contributors were detected in 17.7% (42/237) of 2 and 3-person in-silico mixtures of small amplicon targeted MPS data, and increased to 70% when closely related haplogroups were included. Spurious haplogroups, most likely due to private mutations, were detected in addition to the contributing haplogroups in approximately 30% of the mixtures. Inclusion of a randomly selected, known haplotype in the analysis reduced the number of samples with spurious haplogroups from 32 (56.1%) to 15 (26.4%). Biological samples were also considered. 1. Samuel H. Vohr, et al. 2017. FSIG 30; 93-105.

**Keywords:** mtDNA, mixtures, MPS

# ANTHROPOLOGICAL GENETICS

*ANCIENT DNA*

Presentation number: AG 1

# MULTIDIMENSIONAL APPROACH IN THE APPLICATION OF ETHICAL STANDARDS IN ANCIENT DNA RESEARCH

**Ante Lozina**, Željana Bašić, Ivana Kružić

University of Split, University Department of Forensic Sciences, Split, Croatia

Goal was to determine the extent of (normative) uniformity on international level regarding application of ethical standards in contemporary research of ancient DNA (aDNA), and to assess to which degree this uniformity contributes to the easier implementation of scientific research on aDNA. We conducted a literature scoping review of the online databases Scopus, Web of Science as well as gray literature. The combination of keywords: ethics, ancient DNA, aDNA without any limitations was used. Afterwards the publications were analyzed in reference manager (duplicates and non-relevant literature excluded). Total of 94 publications were found. After the analysis of the results 16 of the publications were excluded as duplicates and 59 as non-relevant publications. The remaining 19 publications were analyzed in detail. The analysis indicated that a majority of relevant publications on the aDNA ethical standards were published in the last 5 years. 12 publications discuss ethical dilemmas on aDNA research regardless of specific population, while 7 publications discuss ethical dilemmas considering specific populations (4 of them deal with the topic of native populations of Americas, 2 of them with native populations of Africa, and 1 of them with remains from ancient Egypt). Information extracted from relevant publications indicate the importance of DNA analysis in the array of anthropological techniques but also emphasize the significance of conducting the research methods on aDNA in accordance with ethical standards. The research community faces the challenge of defining global and/or supranational ethical guidelines, which would define minimum of ethical standards acceptable to different countries, cultures, religions, social groups, and other stakeholders. Recent history indicates to the possibility of misuse of DNA research, in promoting theories aimed at the dominance of certain social groups over others. Results of our research emphasize the importance of unification of ethical principles, application of uniform ethical standards and promotion of a healthy balance between scientific interest in aDNA and respect for cultural, social, religious, and other differences among different stakeholders.

**Keywords**: ancient DNA, ethical guidelines, stakeholders, cultural differences, religion

Presentation number: AG 2

# EVALUATION OF MITOCHONDRIAL DNA OF SKELETAL REMAINS FROM PERIOD OF MEDIEVAL BOSNIA

**Merima Miralem**, Belma Jusić, Mirela Džehverović, Abdurahim Kalajdžić, Amela Pilav, Jasmina Čakar

University of Sarajevo - Institute for genetic engineering and biotechnology, Sarajevo, Bosnia and Herzegovina

Mitochondrial DNA (mtDNA) haplotype analysis is a valuable tool to study human migrations and evolutionary history as well as intrapopulation and interpopulation diversity of human populations. Therefore, the aim of our study was to determine mitochondrial DNA haplotype frequencies of skeletal remains from Medieval Bosnia and compare obtained results with the results for the modern Bosnian-Herzegovinian population. Samples of twenty-six skeletal remains, excavated from different medieval necropolis in Bosnia and Herzegovina, were washed and ground to a fine powder, followed by decalcification with 0.5M EDTA. Extraction of DNA was done using optimized phenol-chlorophrom-isoamyl alcohol method with additional purification using filter columns. Sequencing of hypervariable segment I (HVSI) of the control region (CR) as well as analysis of restriction fragment length polymorphism (RFLP) of isolated mtDNAs was used for mitochondrial DNA haplogroup prediction. Sequences were aligned and analyzed in Bioedit 7.2 software, while for haplogroup prediction Mitomaster was used. This tool utilizes Haplogrep2 with Phylotree 17. For all analyzed samples H haplogroup was predicted which is also a predominant haplogroup in modern Bosnian-Herzegovinian population (more than 50%). Among all samples, two were determined as H5 and the others as H2a sub-haplogroup. Our results indicate the prevalence of H mtDNA haplogroup among the inhabitants of Medieval Bosnia which can be considered as important information in regards to the genetic structure of medieval Bosnian population.

**Keywords:** mtDNA, ancient DNA, medieval Bosnia, haplogroups, RFLP, HVSI





Presentation number: AG 3

# DNA ANALYSIS REVEALED KINSHIP BETWEEN PEOPLE FROM A SMALL COMMUNITY LIVING IN MEDIEVAL BOSNIA

**Belma Jusic**[1], Mirela Dzehverovic[1], Edin Bujak[2], Amela Pilav[1], Jasmina Cakar[1]

[1]University of Sarajevo-Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina, [2]Department of Archeology, Faculty of Philosophy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Skeletal remains of 11 individuals, stored in Travnik Homeland Museum, originated from Travnik municipality (localites Klisa-Guca Gora, Alihodze and Glavica-Han Bila) were sampled for DNA extraction. Well preserved teeth were grounded to a fine powder, after washing and subjected to decalcification with 0.5 M EDTA solution during seven day. Ancient DNA was extracted according to an optimized phenol-chloroform-isoamyl alcohol extraction technique, and then purified using filter columns. PowerPlex® Fusion System and Investigator® 24plex QS Kit were used for amplification of selected STR markers. Additionally, PowerPlex® Y23 System was used to generate Y-STR haplotypes for male individuals. In general, autosomal STR profiles were successfully generated for all samples. Male sex was determined in eight samples. Comparative analysis of two male aDNA profiles showed matching at 18 out of 22 analyzed autosomal STR loci. Statistical analysis confirmed siblingship with calculated kinship probability (KP) of 99.99996%. Furthermore, statistical analysis of two compared male profiles revealed probability of 97.77112% that they are in half brother-half brother, grandfather-grandchild or uncle-nephew relationship. For other samples, statistical analysis revealed probability of first cousins relationships with KP in the range of 59.48719 to 99.32699%. Additional Y-STR analysis showed that all eight male individuals share the same Y-haplotype confirming kinship through paternal line. Determined Y-haplotypes were found to belong to the J2a haplogroup.

**Keywords:** ancient DNA, skeletal remains, STR markers, Y-haplogroups, archaeology

*MIGRATION HISTORY*

Presentation number: AG 4

# A NEW FINDING WITHIN THE MITOCHONDRIAL X HAPLOGROUP IN CROATIAN ISLAND ISOLATES CONFIRMED BY MASSIVELY PARALLEL SEQUENCING

**Dubravka Havaš Auguštin**[1], Marina Korolija[2], Jelena Šarac[1], Kristian Vlahoviček[3], Natalija Novokmet[1], Saša Missoni[1,4], Andrea Ledić[2], Adela Makar[2]

[1]Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia, [2]Forensic Science Centre "Ivan Vučetić", Ministry of the Interior, Zagreb, Croatia, [3]Bioinformatics Group, Faculty of Science, University of Zagreb, Zagreb, Croatia, [4]School of Medicine, "J. J. Strossmayer" University of Osijek, Osijek, Croatia

Mitochondrial haplogroup (hg) X was estimated to originate in the Near East cca 30 ka years ago. Its approximate prevalence in the general European population is 2%, with most sublineages belonging to the X2 clade. The X2 clade is far less common in Croatian insular populations according to our previous findings - 6 out of 13 detected X samples belong to a new, local variant based on specific mutations in both the control and coding region of the mitochondrial genome. The aim of this study was to fully explore mentioned 6 mitochondrial haplotypes found in islands of Rab and Cres. Complete sequencing of one Rab and one Cres mitochondrial genome was performed using both Sanger sequencing and massively parallel sequencing with the Illumina® Human mtDNA Genome assay on MiSeq FGx™ instrument. Sequenced samples indicate a novel lineage within the global mitochondrial X hg phylogeny according to both PhyloTree and Mitomap. The lineage is connected with the hg X3 by a common polymorphism G3531A in the coding region of the mitochondrial genome, but it lacks all other X3 defining mutations. Mutations specific for this lineage are T195C, C338T, A7518G, G7853A, A10113G, C10673T, C10920T, A11380G, A13614G, C13950T, A15903G, G15927A, T16136C and A16289G. Analysis of non-phylogenetic variants exposed a rare, A7518G mutation in the tRNA gene, which is predicted as possibly pathogenic by MitoTIP. Findings of different heteroplasmic mutation patterns between samples indicate a more distant kinship within the same mitochondrial lineage. Our finding indicates a recent local microdifferentiation process within hg X. Described lineage could possibly be marked as a new, island-specific X twig formed within the Croatian population. The extension of present research, including genealogical and clinical data, is needed to confirm further enrichment of existing mtDNA phylogeny and to establish possible functional manifestations of locally specific variants.

**Keywords:** X haplogroup, mtDNA, massively parallel sequencing

Presentation number: AG 5

# GENETIC SUB-STRUCTURING OF CROATIAN ISLAND POPULATIONS IN A WIDER SOUTHEAST-EUROPEAN CONTEXT- A META-ANALYSIS

**Natalija Novokmet**[1], Ana Galov[2], Vedrana Škaro[1,3], Petar Projić[1,3], Jelena Šarac[1], Dubravka Havaš Auguštin[1], Saša Missoni[1,4], Pavao Rudan[5], Dragan Primorac[4,6,7,8,9,10,11,12,13,14], Damir Marjanović[1,15]

[1]Laboratory for Molecular Anthropology, Center for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia, [2]Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia, [3]DNA Laboratory, Genos Ltd., Zagreb, Croatia, [4]School of Medicine, University of Osijek, Osijek, Croatia, [5]Scientific Council for Anthropological Research, Croatian Academy of Sciences and Arts, Zagreb, Croatia, [6]St. Catherine Hospital, Zagreb, Croatia, [7]School of Medicine, University of Split, Split, Croatia, [8]University Department of Forensic Sciences, University of Split, Split, Croatia, [9]Faculty of Dental Medicine and Health, University of Osijek, Osijek Croatia, [10]University of Rijeka, School of Medicine, Rijeka, Croatia, [11]Eberly College of Science, Pennsylvania State University, University Park, PA, USA, [12]Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [13]Medical School REGIOMED, Coburg, Germany, [14]The National Forensic Sciences University, Gandhinagar, Gujarat, India, [15]Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina

Goal was to determine the influence of island population isolation on the sub structuring of the Croatian population, and the influence of regional population groups on the sub structuring of Southeast Europe with regards to basic population genetic statistical parameters calculated using STR locus analysis. Bio-statistical analyzes were performed for a total sample of 2877 unrelated participants of both sexes from the area of Southeastern Europe was analyzed. Nine autosomal STR loci (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S82) were analyzed using standard F-statistics and population structure analysis (program STRUCTURE). The total coefficient of genetic differentiation of Croatian subpopulations calculated by the FST method is higher at the level of the Croatian population (0.005) than at the level of Southeast Europe (0.002). In the population of Croatia, the subpopulation of the island of Vis shows the most pronounced separation, and in the population of Southeast Europe the population of Albanians from Kosovo, then the populations of Croatia, Bosnia and Herzegovina and Hungary. The established higher structure of Croatian subpopulations in relation to the populations of Southeast Europe suggests the existence of a certain degree of genetic isolation, most likely due to the influence of endogamy within rural island populations. The total genetic differentiation coefficient of Croatian subpopulations calculated by the FST method is higher at the level of the Croatian population (0.005) than at the level of Southeast Europe (0.002). The established higher structure of Croatian subpopulations in relation to Southeast Europe suggest the existence of a certain degree of genetic isolation, most likely due to the influence of endogamy within rural island populations.

**Keywords:** STRs, genetic sub-structuring, Croatian island populations, Southeast Europe



# *GENETIC ADAPTATION*

Presentation number: AG 6

# GENETIC DIVERSITY OF MALE POPULATION IN SIX MUNICIPALITY OF THE NORTH-EASTERN MONTENEGRO

**Azra Licina Sinanovic**[1], Fehim Licina[2]

[1]Association for Nutrition and Dietetics "Food for Health", Tuzla, Bosnia and Herzegovina, [2]Association of the family Licina, Podgorica, Montenegro

Commercial genetic tests become more popular in recent years, especially for genetic genealogy research. The most popular marker in genetic genealogy that combines genetic data and family history is the Y chromosome. This popularity is based on its haploid character and its close association with the patrilineage and paternal inherited surname. The aim of this paper is to collect, summarize and analyze data on the genetic diversity of the male population of the six municipality at the North-eastern Montenegro, with the majority population of the Islamic religion: Berane, Petnjica, Plav, Gusinje, Rozaje and Bijelo Polje through the Y chromosome. The paper presents the results of data were obtained by testing 267 samples of DNA materials taken from the male inhabitants of six municipality at the North-eastern Montenegro, of which 205 samples of male inhabitants' Islamic denomination (group 1) and 62 samples of male inhabitants' Orthodox denomination (group 2). The results of research point there are thirteen haplogroups: R1b, I2, E, J2, I1, G2, J1, G, R1a, N, C, T1, Q1. The most common haplogroups are I2 and R1b, both identified in 23.97% of samples, followed by E (22.47%), J2 (11.61%), I1 (6.74%), G2 (3.75%), R1a (3.37%), I1 (1.12%), G (1.12%), N (0.75%), C (0.37%), T1 (0.37%) and Q1 (0.37%). Related to the ethnic denomination, the most common haplogroup for group 1 is R1b (30.24%), followed by I2 (22.44%), E (20.98%), J2 (12.68%), I1 (4.39%), G2 (4.39%), R1a (3.41%), C (0.49%), T1 (0.49%), and Q1 (0.49%), while I1, G and N are not identified. For the group 2 the most common haplogroup is I2 (29.03%), followed by E (27.42%), I1 (14.52%), J2 (8.06%), J1 (4.84%), G (4.84%), R1a (3.23%), R1b (3.23%), N (3.23%), G2 (1.61%), while C, T1 and Q1 are not identified. These results will contribute to bringing together ethnically different confessions by understanding that they are all a mixture of different genetic markers.

**Keywords:** Haplogroup, Y chromosome, Montenegro

# INTERDISCIPLINARY POSTER SESSION

Presentation number: IPS 1

# CHALLENGES ASSOCIATED WITH MEDICAL TRAVEL FOR CANCER PATIENTS IN THE ARAB WORLD: A SYSTEMATIC REVIEW

**Wafa K. Alnakhi**[1,2], Faryal Iqba[3], Waleed Al Nadabi[4], Amal Al Balushi[5]

[1]Dubai Health Authority, Health Research & Survey Section, Data Analysis, Research & Studies Department Strategy & Corporate Development, Dubai, United Arab Emirates, [2]Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates, [3]Burjeel Medical City, Innovation and Research Center - Burjeel Cancer Institute, Abu Dhabi, United Arab Emirates, [4]Ministry of Health, Directorate General of planning and studies, Al Khuwair, Sultanate of Oman, [5]Ministry of Health, Oman College of Health Sciences, Nursing Program, Al Khuwair, Sultanate of Oman

Each year, millions of patients around the world seek medical care abroad. Medical travel is becoming very common in the Gulf Cooperation Council Countries (GCC) due to many motivational factors. Cancer seems to be one of the top medical conditions for patients from the GCC to seek healthcare overseas. There are many factors associated with cancer patients seeking treatment overseas. However, unfortunately, there are very few studies that discuss the risks and challenges associated with the medical travel experience for those patients. We conducted a systematic review to summarize the evidence related to the complications and challenges associated with the medical travel experience for oncology patients in the Arab world. This systematic review was guided by PRISMA. PubMed was used as a search database by using a combination of medical travel, complications, and cancer keywords for publications which yielded 76 articles. Four coders independently determined eligibility based on PICOS and then extracted information from 14 articles. The resulting articles are based on three main categories, i.e., primary, and secondary data collection, and review articles. Of the total 76 articles, only 14 were included because they met the criteria. 62 articles were excluded because of irrelevance of the title, abstract, and insufficient data. Although this systematic review aimed to look at the medical complications that may arise from the medical travel experience for oncology patients, other challenges were found. The challenges reported can be grouped into the following themes: a) financial and economic aspects, b) medical care aspects, c) social and cultural aspects. Overall, more research studies are required in the Arab world for cancer patients treated overseas. The existence of such information around this topic will help in improving policies and strategies related to medical travel for the different stakeholders involved in the medical travel market. Moreover, these studies will not only aid in improving the quality of care for cancer patients who are engaging in medical travel, but they will also help in overcoming the challenges associated with medical travel experience for cancer patients at the different stages of the experience.

**Keywords:** medical travel, treatment overseas, outsourced patients, treatment destinations, oncology

Presentation number: IPS 2

# PRECISION MEDICINE AND CARDIOVASCULAR DISEASES: WHICH BIO JURIDICAL ISSUES MUST BE CONSIDERED?

Antonina Argo, **Giuseppe Davide Albano**, Giulio Perrone, Clio Bilotta, Ginevra Malta, Stefania Zerbo

University of Palermo, Department Pro.Mi.SE, Medico legal Unit, Palermo, Italy

The area of cardio-vascular diseases - in translation from the animal to the human model - needs to test and validate many of the biomarkers identified, by demonstrating the presence/absence of a marker (be it a cDNA, a miRNA, a protein, a metabolite or a metabolomic profile) in healthy and affected tissue, that can be implemented in common clinical practice. Genetic variants were identified that modify the response to some relevant cardiovascular drugs, including beta-blockers (ADRB1, ADRB2, GRK5, GRK4); angiotensin converting enzyme inhibitors (ACE, AGTR1); diuretics (ADD1, NPPA, NEDD4L); and calcium channel blockers (CACNB2, CACNA1C). To date, however, genetic testing has not been employed routinely to guide selection of these drugs, and many genetic variants require confirmation in larger studies. The highlight topics can support the translational clinical application, with collaboration between knowledge that is the basis of precision medicine, in add taking evidence of unresolved bio juridical issues. The authors analyze some questions underlying precision medicine: equity in access preventive and therapeutic treatments based on pharmacogenetic principles; a guaranteed access to diagnoses and personalized treatments; the use of precision medicine diagnostics in the context of gender differences and its non-discriminatory use; use of precision medicine approach in prevention and its use in the workplace, with guarantees of the freedom and dignity of the person; integrity in the use of information located in biobanks. The guidelines proposed by scientific societies and National Institution of health are considered; the aspects worthy of discussion and bio-juridical implementation are emphasized. However fascinating, the area of interest of precision medicine, especially in the broad field of cardiovascular pathologies, also highlights some aspects that require careful evaluation and investigative criticism; therefore, requiring further regulatory interventions.

**Keywords:** Precision Medicine, cardiovascular, genetic variants, biojuridical





Presentation number: IPS 3

# AWARENESS REGARDING ORAL HEALTH AMONG ORTHODONTIC PATIENTS AND NON-ORTHODONTIC PATIENTS

**Ingrid Kovačević**[1], Katarina Major[1], Ivana Zovak[1], Sanja Šuper-Kirsch[1], Dora Dragičević[1], Dorotea Petrović[1], Marija Čandrlić[1], Allesia Cerin[1], Katarina Judnić[1], Marta Furdi[1], Marko Babić[1], Marko Matijević[2]

[1]Faculty of Dental Medicine and Health Osijek, Department of Dental Medicine, J.J. Strossmayer University of Osijek, Osijek, Croatia, [2]Health Center Osijek, Osijek, Croatia

The aim of the study was to examine oral health awareness among orthodontically treated and orthodontically untreated patients, to examine their attitude about the importance of oral hygiene, their familiarity with toothbrushing techniques and familiarity and source of information on the use of toothbrushes and other toothpastes. Also, the aim was to examine their opinion on the importance of brushing teeth and their interest in expanding their own knowledge about oral hygiene. The study is organized as a cross-sectional study. Study included 98 patients of dental and orthodontic surgeries of the Health Center in Osijek. The examination was conducted during April 2020. An anonymous survey questionnaire was used to conduct the research. 98 patients participated in the study. Orthodontically treated patients use a toothbrush three times a day, far more than orthodontically untreated patients. They use a soft toothbrush and interproximal (interdental) toothbrushes significantly more than orthodontically untreated patients. They no longer use mouthwash, compared to orthodontically untreated patiens, but they go to regular check-ups much more than orthodontically untreated patients. Also, the orthodontist / dentist far more explains to them the importance of brushing teeth, which is not the case with orthodontically untreated patients. This study has shown that orthodontically treated patients have significantly more awareness regarding oral health and the importance of oral hygiene protocols, than orthodontically untreated patients.

**Keywords:** oral hygiene, teeth, orthodontically patients, knowledge, attitudes

Presentation number: IPS 4

# CONTRADICTORY ARGUMENT OF HYPERTENSION AMONG YOUTHFUL AGES

**Agbaje Olatunde Faliud**

The Vision for Teenagers Adolescents and Youths Wellbeing Initiative

Hypertension is one of the major community health challenges among elderly people on the risk of cardiovascular disease, is now a disease that is found among people who fall within the age brackets between 20 and 30 years. Our community outreaches reveal this at Ikorodu and Lagos Mainland Local Governments in particular. A community cross-sectional research was carried out and it was shocking that people of the aforementioned age brackets were found hypertensive during one of our Community Medical Outreaches at the aforementioned Local Government Areas. Our discovery was terrific that the prevalence of hypertension was found among males than females during the BP test to found results as 125.27 $\pm$ 17.08mmHg and 93.55 $\pm$ 9.48mmHg, respectively. TV-TAY Wellbeing Initiative has a pre-conclusive agreement to present this that the odds of being hypertensive in the community is majorly the problem of challenges such as economic instability the situation, social unrest, and political disenchantment in the country which bring about the unnecessary taking of uncontrollable alcoholic drink, tobacco, illiteracy unawareness of the danger with continuous indulging and engagement because of unawareness risk of it based on our findings.

**Keywords:** uncontrollable alcoholic drink, tobacco, illiteracy unawareness, hypertensive





Presentation number: IPS 5

# IRRITABLE BOWEL SYNDROME (IBS) MICROBIOME DIVERSITY

**Jasmin Ramić**[1], Irina Milovac[2], Zoran Mavija[3], Naida Lojo-Kadrić[1], Maida Hadzić[1], Stojko Vidović[2], Beate Niesler[4], Nikolas Dovrolis[5], Maria Gazouli[6], Naris Pojskić[1], Lejla Pojskić[1]

[1]Institute for genetic engineering and biotechnology, Sarajevo, BiH, [2]Medical Faculty, University of Banja Luka, Banja Luka, Bosnia and Herzegovina, [3]Department of Gastroenterology, Clinical Center of Republic of Srpska, Banja Luka, Bosnia and Herzegovina, [4]Institute of Human Genetics, Department of Human Molecular Genetics, University of Heidelberg, Germany, [5]Laboratory of Medical Biology, Department of Medicine, Democritus University of Thrace, Alexandroupolis, Greece, [6]Laboratory of Biology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

(Dys)functional gut brain gastrointestinal (GI) disorder known as irritable bowel syndrome (IBS) have a rather high prevalence in working population. 10-15% of members of this group have problems with constipation or diarrhea followed by abdominal pain, all of which can usually be associated with IBS (10, 1 % according to Rome III and 4, 1% according to Rome IV, Enck P. et al 2016, Sperber A.D. et al 2020, Black C.J. et al 2020). Frequency of these symptoms is a major obstacle to the functional physical and social wellbeing of patient diagnosed with IBS. One of potential pathogenic mechanisms responsible for this condition include microbiome dysbiosis in patients with IBS. Intestinal microbiome is a ecosystem of localized microorganisms and inner gastrointestinal (GI) tract lining cells with all kind of genetic, biochemical and ecological interactions between them. Diversity of microbiome is age, sex, habit and physiologically (pregnancy or disease) related. In persons with IBS diagnosis these diversities can be seriously impaired, and aim of this study was to determine if we can establish statistically significant connection between IBS condition and microbial taxa present in feces of examinees with IBS and control group. We are also looking forward to see differences in relative abundance of microbial composition in these two groups. Fecal microbiota profiling in search for eventual change of composition and abundance in nine clinically confirmed IBS patients and six corresponding healthy controls is profiled based on their species specific 16s RNA gene using Illumina NGS mini seq platform. Initial raw data were analyzed using Illumina 16s Metagenomics application, and further analyses were performed on the MicrobiomeAnalyst online platform. Total Sum Scaling (TSS) was applied to the data. Alpha-diversity was calculated using the CHAO1 index and ANOVA on the OTU level. Beta-diversity was calculated and visualized using non-metric multidimensional scaling (NMDS) and Principal Coordinate Analysis (PCoA) on the OTU level. Correlation analysis was carried out using Spearman's correlation algorithm. Mann-Whitney U test was used to compare differences between IBS and healthy controls and variance in taxonomic distribution between clinical subtypes (Med Calc v.19.0.4).

**Keywords:** IBS, fecal microbiome, 16s rRNA gene, dysbiosis

Presentation number: IPS 6

# ASSOCIATION BETWEEN POLYGENIC RISK SCORES FOR PLASMA PROTEIN N-GLYCOSYLATION TRAITS AND 273 ICD-10 DISEASES

**Olga O. Zaytseva**[1], Arina V. Nostaeva[2], Sodbo Zh. Sharapov[2], Elizaveta E. Elgaeva[2], Gordan Lauc[1], Yurii S. Aulchenko[2], Yakov A. Tsepilov[2]

[1]Genos Glycoscience Research Laboratory, Zagreb, Croatia, [2]Institute of Cytology and Genetics, Novosibirsk, Russia

N-glycosylation is a post-translational modification of proteins by covalent attachment of a carbohydrate structure to an asparagine residue. N-glycans physical properties and biological activity of the serum proteins. The composition of the N-glycome of blood plasma proteins is changing in ageing and diseases, therefore, N-glycans are often regarded as promising biomarkers of various physiological and pathologic states. Recently, genome-wide association studies (GWAS) identified a number of loci that are involved in regulation of plasma protein N-glycosylation. Some of these loci are also associated with inflammatory, autoimmune, cancer and other diseases. Elucidating the degree of genetic architecture overlap and causal relationships between plasma N-glycome and diseases, remains one of the important tasks of the glycobiology field. To answer this question, we have used polygenic risk score (PRS) analysis. We have calculated PRSs for 117 plasma N-glycosylation traits using summary statistics of a GWAS performed in a cohort of European descent (N = 7541), where 31 loci were found to be associated with N-glycosylation. As a next step we are planning to perform an association study of the PRS for the 117 plasma glycan traits and 273 diseases included in the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) list, available through the UK BioBank (UKBB) database, with prevalence > 0.05 and < 0.95. For the pairs of glycan trait and disease that show a statistically significant association we plan to perform Two-sample Mendelian randomization (MR) analysis in both directions to answer the question of causal relationships between the said traits. As exposure and outcome for the Two-sample MR we are going to use summary statistics from GWAS for plasma N-glycosylation traits performed in a cohort of European descent (N = 7541) and those available from UKBB for the diseases. We expect our research project to shed light on the connection between plasma protein N-glycome and various pathologies, on the genetic basis of protein N-glycosylation, to improve the existing glycan biomarkers and potentially to discover new ones. Acknowledgments: This work was supported by a grant from the Russian Science Foundation (RSF) No. 19-15-00115.

**Keywords:** N-glycosylation, plasma proteins, polygenic risk scores, Mendelian randomisation





Presentation number: IPS 7

# SEX DIFFERENCES IN RISK OF BLOODSTREAM AND SURGICAL SITE INFECTIONS

**Stefania Zerbo**, Giulio Perrone, Clio Bilotta, Ginevra Malta, Giuseppe Davide Albano, Antonina Argo

University of Palermo, Department Pro.Mi.SE, Medico legal Unit, Palermo, Italy

Due to the exponential increase in the incidence of nosocomial infections and the increasing rates of antibiotic resistance, the identification of nosocomial infections' risk factors remains a current topic. Sex is one of the risk factors currently under study. The role of sex in the context of urinary tract infections (UTI) is well known. Conversely, the link between sex and bloodstream (BSI) and surgical site (SSI) infections is less well known and more uncertain. the author analyzed the literature data about the role of sex in the BSI and SSI development. significantly higher BSI and SSI incidence rates were observed in the male population. It has been hypothesized that it may be due both to genetic factors related to the thicker and coarser hair in men, and to the shaving of the hair. This hypothesis is further supported by the absence of sex differences with regard to the incidence of BSI and SSI in the pediatric population. Conversely, in the adolescent population the girls had significantly lower odds of community-associated BSI and lower odds of SSI. Biological differences between men's and women's skin could constitute another possible reason for gender differences in the case of BSI and SSI incidence. A greater bacterial colonization of the skin surrounding the surgical insertion site of the central venous catheter was observed in men compared to women. Furthermore, sex would affect the immune system's response via the hormonal system. In fact, the levels of estrogen promote the response of the immune system, playing a protective role; differently androgens suppress it. The increased rates of infection in the female population observed during menopause would confirm these observations. It has also been hypothesized that women may be at greater risk for SSI in the case of cardiac surgery due to the small size of the arteries and the increased tension of the thoracic incisions due to the pendulous breasts. New studies are expected regarding cultures of the bacterial colonization of the skin surrounding a central venous catheter at the insertion site or a surgical wound to clarify the role of genetics and sex in the development and incidence of BSI and SSI in order to customize the preventive and therapeutic strategies of BSI and SSI and reduce their mortality.

**Keywords:** sex, risk infection, surgical site infection, bloodstream infection

# ABSTRACTS OF THE INTERDISCIPLINARY SESSION OF AMERICAN ACADEMY OF FORENSIC SCIENCES AND ISABS 2022

**Presentation number: AAFS 1**

# FENTANYL: HISTORY, ABUSE, DANGER

**Peter Ausili**

American Academy of Forensic Science, Colorado Springs, CO, USA

Illegal drug use and trafficking in the United States is at an all-time high. In addition to traditional drugs of abuse (Heroin, Cocaine, Methamphetamine and Marijuana) there has been a huge increase in the abuse of Novel Psychoactive Substances (NPS) such as Fentanyl during the last 15 years. Illicit abuse of Fentanyl and Fentanyl-related synthetic opioids began in the mid 1990's of in the United States. This was the result regulations, policies and medical practice that focused on opioid medications (i.e. Oxycodone) as the primary treatment for pain. Hospital management and administration encouraged doctors to reduce patient pain to a self-described level of zero. Pharmaceutical manufacturers were eager to provide the products. As patients became addicted they had difficulty getting prescriptions legally renewed and then sought other sources for similar drugs. Gradually, other supply networks developed to provide Oxycodone and look-alike clandestinely manufactured synthetic opioids. Eventually, US drug control regulations improved, which led to entirely new substances of abuse. Fentanyl became a popular drug of choice. Precursor chemicals originate in China that are then shipped to Mexico for synthesizing into Fentanyl and Fentanyl analogues. These products are commonly mixed with Heroin, Cocaine, Methamphetamine, Marijuana and also compressed into look-alike tablets such as XANAX. It should be noted that the illicit drug user and the drug trafficker frequently don't know the content or the concentration/potency of the drugs. There are many literature references that refer to the abuse of Fentanyl as like playing "Russian Roulette'. Fentanyl is 50 times more potent than Morphine and 100 times more potent than Heroin. Fentanyl analogues are even more potent. In 2021 there were 100,000 Fentanyl related drug overdose DEATHS in the United States. What about the future?? It might get worse before it gets better. Has anyone heard about Isotonitazine compounds?

**Keywords:** Fentanyl, Synthetic Opioids, Drug Abuse

Presentation number: AAFS 2

# COLLABORATION TO EFFECT IDENTIFICATION FOR UNKNOWN INDIVIDUALS RECOVERED IN A FORENSIC CONTEXT

**Laura C. Fulginiti**

American Academy of Forensic Sciences, Colorado Springs, CO, USA

In 2015 Arizona State University and the Maricopa County Office of the Medical Examiner agreed to collaborate to advance identification efforts for badly degraded and burned decedents. The concept was to apply techniques derived for extracting and amplifying ancient DNA samples to these individuals to assess the efficacy for obtaining useful profiles. In Arizona, as in many arid, hot environments, individuals who die in the desert and are not found for a protracted time are subjected to extremes of temperature, solar exposure and animal predation. These combine to remove the organic matrix of the bone and the most likely sources of usable DNA. Current analytical kits have failed to produce enough DNA for scientific identification. Similarly, in badly burned bodies there is a threshold beyond which standard techniques cannot extract enough DNA to create a strong profile. One mandate of a medical examiner or coroner is to make an identification of decedents within their jurisdiction. When standard techniques are unsuccessful, the decedent remains unidentified. Practitioners are constantly reviewing these cases in the context of scientific advances and thinking outside of the box to effect opportunities for identification. The proposal to use aDNA methodologies for these cases provides a new avenue for success. NIJ funding has allowed testing several methods in order to determine which is the best for yielding enough DNA for identification. The protocols have had differing levels of success ranging from very good to no results. Predictably the cremated and most degraded samples are the ones with minimal yields. DNA profiling is a boon to forensic science in many contexts. Identification of unknown decedents is a small portion, but it plays a critical role. Without identification families are left in limbo, legal processes stop, and law enforcement investigations are derailed. In the United States there are tens of thousands of unidentified decedents languishing in medical examiner and coroner offices, or in pauper cemeteries. Advancing methods for assessing these individuals creates hope that more will move from the unidentified column into the identified column. This research hopes to play a large role in that transition.





Presentation number: AAFS 3

# THE PAST, CURRENT, AND FUTURE OF FORENSICS IN THE US. CRIME SCENE TRAINING

**Henry Lee**[1,2,3]

[1] Henry C. Lee Institute of Forensic Science, West Haven, CT, USA, [2]College of Criminal Justice and Forensic Sciences, University of New Haven, USA, [3]Singapore Home Team Science & Technology Agency (HTX) (TSAP), Singapore

Contemporary law enforcement has expanded its ability to solve crimes by the adoption of standardized forensic testing procedures. Today, crimes can only be solved by the combinations detailed examination of the crime scene and analysis of forensic evidence, the utilization of the open and close sources of big data bases and the theory of Cloud data mining and crime scene reconstruction. Knowledge of forensic evidence is not only crucial in criminal investigations and prosecutions, but also vital in civil litigations, major man-made and natural disasters, and the investigation of global crimes. However, there was not much consensus on what and how to be training or educate future forensic scientists in crime scene investigation. In the past, this type training is done by localized apprenticeship. The current training program is focused on the standardized procedure for crime scene technician. This type of inadequate training was unfortunately overlooked and created many issues and problems for forensic field. The future crime scene training program should be conducted with a global effort in the ability of observation, and recognition, the systematic and logical analysis, the correct interpretation of test results, and reconstruction of the scene. This includes not only the first responding officer, detective, the crime scene technicians, investigators, forensic scientists but also the judges and attorneys. The training of advanced investigative skills (such as GPS positioning, Cell phone tracking, Social network, E-evidence, Video image analysis, Big Date Analytics, Artificial intelligence, Theory formation, Forensic DNA Genealogy, Trace Evidence Analysis, Pattern Evidence Recognition and Crime Reconstruction) will be discussed, and the ability to observe and analysis of crime scene and Forensic Evidence will also be covered, Famous cases will be utilized to illustrate the importance of new concepts in forensic crime scene training.

Presentation number: AAFS 4

# FORENSIC SCIENCE STANDARDIZATION EFFORTS BY THE AMERICAN ACADEMY OF FORENSIC SCIENCES (AAFS) ACADEMY STANDARDS BOARD (ASB)

**Carl R. McClary**

Department of Justice, Bureau of Alcohol, Tobacco, Firearms, and Explosives, Atlanta, Georgia, USA

The goal of this presentation is to educate an international audience on the history and progress and also the motivation behind the American Academy of Forensic Sciences, AAFS, Academy Standards Board, ASB. The presenter is the past president of the Academy with extensive experience with standards development organizations. The AAFS' contribution to forensic science standards has been significant since first forming its standards development organization beginning in 2015. In that same year it received American National Standards Institute, ANSI, accreditation for the American Standards Board, the ASB. Material and Methods – Academy Standards Board, ASB, statistics on standards, guidelines, and technical reports were utilized to present a historical account of ASB progress. When the ASB was created, consensus bodies were formed representing numerous forensic science disciplines. Within the consensus bodies, the interest categories, developed and approved by ANSI, represent various stakeholders who provide various perspectives resulting in standards that meet necessary objectives by the end user. A public balloting process, an ANSI requirement, ensures a free and open process. Results – The presentation will include types of interest categories, stakeholders, ASB procedural requirements, and up to date progress and goals of the ASB consensus bodies. Since the date of inception, the ASB has gone from an idea to a fully functioning development program with volunteers from thirteen (13) forensic disciplines creating over 76 standards, guidelines, or technical records. Conclusion – The ASB 's progress in publishing standards, guidelines, and technical reports is impressive given the relatively short period of time since they were formally established and accredited by ANSI. That progress, along with the history of the program, will be provided in detail in this presentation.

**Keywords:** AAFS, ASB, forensic standards





Presentation number: AAFS 5

# CORRECTING MISCARRIAGES OF JUSTICE – INNOCENCE PROJECT OF CROATIA

**Damir Primorac[1,2], Lucija Sokanovic[3], Andrej Bozhinovski[4]**

[1]Faculty of Law, University of Mostar, Mostar, Bosnia and Herzegovina, [2]University Department of Forensic Sciences at University of Split, Split, Croatia, [3]Faculty of Law, University of Split, Split, Croatia, [4]Department of Criminal Law, Faculty of Law, University of Zagreb, Zagreb, Croatia

The presentation focuses on the issue of wrongful convictions and subsequent DNA testing as a method for proving innocence at persons deprived of liberty, through the perspective of the newly established and experimental "Innocence Project in Croatia". The presenters will talk about the project's main aim, specific objectives, as well as obtained initial results from the field research. Furthermore, the presenters will present the positive influence of the American innocence projects of the Croatian criminal justice system, through the perspective of the Croatian legal practice and the position of the criminal justice system as a whole concerning the ramification, obtaining and storage of DNA evidence from severe form of criminal acts, including the re-opening of the criminal procedure due to new DNA evidence as a cause wrongful conviction. The presentation will conclude with the encouragement of establishing a national database providing detailed information on previous cases of wrongful convictions and the positive experiences from the U.S. National Registry of Exonerations and the establishment of conviction integrity units as positive examples from which Croatia and other legal jurisdictions in the region should learn.

Presentation number: AAFS 6

# ARREST-RELATED DEATH BY PRONE RESTRAINT CARDIAC ARREST

**Victor W. Weedn**[1,2], Alon Steinberg[3]; Pete Speth[4]

[1]District of Columbia Office of the Chief Medical Examiner, SW, Washington, DC, USA, [2]Department of Forensic Sciences, George Washington University, NW, Washington, DC, USA, [3]Cardiology Associates Medical Group, Ventura, CA, USA [4]Retired forensic pathologist/ medical examiner; Consultant, Wenonah, NJ, USA

Several causes of death have been postulated for agitated subjects who have succumbed after a police encounter, particularly when they have been restrained in a prone position. Much of the literature has focused on excited delirium or restraint asphyxia as the cause of these deaths; often stress with underlying cardiac issues has been imputed. A recent review of the literature declares that restraint is the common factor.[1] More recently, Steinberg ascribed acute severe metabolic acidosis with an inability to compensate with respiratory alkalosis as the cause of these deaths in the setting of prone restraint and proffered the term, "Prone Restraint Cardiac Arrest" (PRCA).[2] Pulseless electrical activity (PEA) and asystole are consistent with this concept. We present two cases that demonstrate the danger of prone restraint and, we believe, serve as examples of PRCA. The first case involved an obese 51-year-old male subject, high on PCP and cocaine and hallucinating. His hands were cuffed behind his back and his legs shackled by police while supine on the ground. Emergency medical personnel placed him prone and strapped on a manual stretcher, which was then placed on a wheeled stretcher and further straps placed. He struggled against the restraints, but then became unconscious as he was loaded on the ambulance. He was repositioned supine in the ambulance. He had been prone for 10 minutes. His initial EKG rhythm was bradycardia, but subsequently no carotid pulse was felt and then the electrical activity was lost (asystole). He arrived in the emergency room in a state of pulseless electrical activity (PEA), but he was successfully resuscitated. His end title $CO_2$ was found to be markedly elevated. Upon his hospital admission, an arterial blood gas revealed severe acidosis (pH of 7.015) and a significantly elevated $pCO_2$ (70.5 mmHg). An echocardiogram in the hospital showed a normal heart function. He died of brain death 3 ½ days after his arrest. Autopsy revealed an enlarged and dilated heart with no gross atherosclerosis and evidence of reperfusion injury. The second case involved an obese 41-year-old male, high on amphetamine and methamphetamine, seen by police stepping in and out of a busy roadway, shouting and talking to an imaginary, threatening person. He sat, sweating and breathing heavily, as requested by the police and his hands were cuffed behind his back. He suddenly got up and began to run when three officers took him to the ground where he thrashed about and kicked his legs. Police rolled him prone and leg shackles were applied. At one point, he uttered, "I can't breathe!" Emergency medical personnel arrived and placed a rigid board on his back and an officer was asked to sit on top. After a couple of minutes, he was turned supine and found to be unresponsive. He had been prone for 15 minutes. ACLS was initiated. The EKG revealed PEA which deteriorated to asystole. Venous blood gases revealed a profound acidosis (pH 6.64) and a markedly elevated $pCO_2$ (157 mmHg). He was pronounced dead shortly after arrival in the emergency room. An autopsy disclosed bruising on his back, a moderately enlarged heart with focal severe coronary artery stenosis.





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Presentation number: AAFS 7

# OPEN WATER AQUATIC SCENE PROCESSING FOR DEATH INVESTIGATIONS

**Andrea Zaferes**

Forensic Aquatic Consulting & Training, LLC., Shokan, New York, USA

Learning Overview: After attending this presentation, attendees will understand the critical need of crime scene investigators (CSI) and other investigators to identify, document, and preserve potential evidence on open water scenes. Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the unique challenges presented by bodies reportedly found in open water scenes, the detection and documentation of evidence in open water scenes, and the recognition and investigation of crimes that involve open water scenes. This presentation will present novel solutions, tools, and protocols to utilize CSIs, marine patrol officers, public safety dive team personnel, and other first responders, to identify and collect often-missed evidence. Bodies reportedly found in open water (BRFIOW) may represent natural, accident, suicide, and homicide manners of death. In the latter case the death may have occurred on land with a postmortem body disposal, may have initiated on land with resulting incapacitation followed by submersion with one of the causes of death being drowning, or with the homicide solely involving submersion and drowning. Asphyxiation, whether criminal, accidental, or suicidal, is a process that can be fatal or non-fatal. Asphyxiation directly impedes respiration or blood flow to the brain through various methods, such as strangulation (1), drowning (2), and inert gas (3). Criminal asphyxiation occurs in domestic violence (4), child/elder abuse (5), sex crimes (6), state and non-state tortur (5), and human trafficking (7). The diagnosis of fatal asphyxia, criminal or benign, requires an informed and accurate scene investigation, and a careful exclusion of underlying injury or disease processes (8). Despite the adoption of widespread legislation(x) specifically targeting asphyxiation violence, evidence collection is sparse and convictions are rare (9). This is partly because criminal asphyxiation often presents with nonspecific findings (10) and minimal or no obvious external injury, can be a diagnosis of exclusion, and lends itself to crime scene staging (11). Therefore, these cases require the preservation and collection of often circumstantial evidence present at the initial scene. Capturing this evidence is especially critical when victims and witnesses may be unable to participate beyond the initial scene for fear of retaliation and safety. As such, crime scene investigators (CSIs) are in a unique position to provide a variety of solutions. It is the job of CSIs to document and process a scene (location, victim, suspect) in its totality as it appears upon arrival. In criminal asphyxiation cases, CSIs are the most apt to capture often missed evidence, including fingernail scrapings and injuries present on suspects. CSIs may also be the only practitioners who can forensically document initial and follow-up injury presentation. For example, in non-sexual asphyxiation cases, victims are unlikely to receive forensic nurse examinations. CSIs' interactions with victims and suspects can also provide unique opportunities to identify and document signs and symptoms of asphyxia and indicators of abuse. For equivocal fatal cases, CSIs can provide the information necessary for investigators to detect staged scenes and for medical examiners to accurately determine cause and manner of death. This novel use of CSIs can be achieved by developing protocols, using investigative forms, and implementing training. Protocols are needed to dispatch CSIs to possible criminal asphyxiation scenes and to provide guidance for evidence recognition, documentation, and processing. CSIs must also be properly equipped with knowledge and evidence-based forms in order to effectively work these often-challenging cases. A current obstacle, as will be demonstrated in this presentation, is insufficient CSI training on criminal asphyxiation in the US as shown by a lack of its mention in CSI textbooks and training syllabi. Overcoming this requires a forensic community response. As a first step in this response, this presentation will introduce tools for CSIs, including investigative forms and protocols for possible asphyxiation scenes. This presentation illustrates the challenges presented by criminal asphyxiation, provides solutions that CSIs can provide, and CSI tools for investigating strangulation cases. With standardized agency protocols, tools, and specialized criminal asphyxiation training, CSIs can help identify, document, and preserve critical evidence in these cases.

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Interdisciplinary session of AAFS and ISABS 2022

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# ABSTRACTS OF 8TH CROATIAN HUMAN GENETICS CONFERENCE & 1ST CROATIAN PERSONALIZED AND PRECISION MEDICINE CONFERENCE

Presentation number: CSHG 1

# ACETABULAR PROTRUSION - UNDERESTIMATED BUT FREQUENT DEFORMITY IN PATIENTS WITH OSTEOGENESIS IMPERFECTA

**Darko Antičević**[1,2], Željko Jeleč[1,2], Dragan Primorac[1,2,3,4,5,6,7,8,9,10]

[1]School of Medicine, University of Osijek, Osijek, Croatia, [2]St. Catherine Hospital, Zagreb, Croatia, [3]School of Medicine, University of Split, Split, Croatia, [4]University Department of Forensic Sciences, University of Split, Split, Croatia, [5]Faculty of Dental Medicine and Health, University of Osijek, Osijek Croatia, [6]University of Rijeka, School of Medicine, Rijeka, Croatia, [7]Eberly College of Science, Pennsylvania State University, University Park, PA, USA, [8]Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [9]Medical School REGIOMED, Coburg, Germany, [10]The National Forensic Sciences University, Gandhinagar, Gujarat, India

Acetabular protrusion (AP) is common deformity in people with osteogenesis imperfecta (OI) or brittle bone disease, a genetic disease of the connective tissues caused mainly by mutations in collagen type I. Clinical features of OI are brittle bones, low-energy fractures, skeletal deformities, joint laxity, blue sclerae, dentinogenesis imperfecta, cardiovascular and respiratory problems and hearing loss. Research on AP in OI is limited. However, AP is found in about 50% of people with OI, with even increase to 70% in people with OI type III. The purpose of this text is to draw attention to this frequently unrecognized deformity. We reviewed recently published articles in orthopaedic literature and compared data from literature with data on patients with OI treated in our hospital in the last five years (2016. – 2021.). Radiographs of hips and pelvis in our 17 patients (10 females and 7 males) were reviewed. Literature review has shown that AP is predominantly responsible for gastrointestinal problems and for increased risk for proximal femoral fractures and particularly for femoral neck fractures in OI population. We found one patient with OI type I, thirteen patients with OI type III; two patients with OI type IV and one patient with X-linked type OI. In our cohort, 65% of our patients with OI type III had AP of some degree. Three adult patients had significant AP as measured according to Kohler line. Two of them had obstruction constipation as gastro-intestinal complication of AP and this was resolved with dietary adjustments. No patients had femoral neck fracture, so far. This could be explained that majority of our patients were children and had limited ambulation prior long bone corrective surgery with expandable intra-medullary implants. There are single case reports of technical surgical problems in urologic prostate surgery due to AP in adult patient with OI type I, as well as carcinoma of left colon presenting as mechanical obstruction in a patient with osteogenesis imperfecta type III. In multidisciplinary management approach for patients with OI one should not underestimated deformity of pelvic bones, AP in particular, as there are significant potential problems that can affect quality of life in this population.

**Keywords:** osteogenesis imperfecta, acetabular protrusion, treatment, prevention

Presentation number: CSHG 2

# THE ROLE OF PHARMACOGENOMICS IN EVALUATING THE EFFICACY AND SAFETY OF DRUGS

**Lidija Bach-Rojecky**[1], Elizabeta Topić[2], Mario Štefanović[3], Dragan Primorac[4,5,6,7,8,9,10]

[1]Department of Pharmacology, University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia, [2]Croatian Society of Medical Biochemists, Zagreb, Croatia, [3]University Department of Chemistry, Sestre Milosrdnice University Hospital, Zagreb, Croatia, [4]St. Catherine Specialty Hospital, Zagreb and Zabok, Croatia, [5] School of Medicine,University of Split, Split, Croatia· [6]Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [7]School of Medicine, University of Rijeka, Rijeka, Croatia, [8]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [9]Eberly College of Science, State College, Penn State University, PA, USA, [10]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [11]Medical School REGIOMED, Coburg, Germany

To achieve a pharmacological activity, drugs should undergo passage through cell membranes from the site of application, transport to cell compartments, biotransformation to active or inactive metabolites, elimination from the body, and finally bind to specific biological macromolecules (molecular targets). The biological molecules involved are metabolic enzymes and transporters, membrane/cell receptors, and other intra- or extracellular proteins. The polymorphisms of the genes encoding these proteins can lead to changes in the drug's pharmacological effect, resulting in therapeutic failure and/or the occurrence of adverse drug reactions. Variations in genes involved in drug absorption, distribution, metabolism, and elimination (ADME) can alter its pharmacokinetic profile, influencing systemic exposure and concentration at the site of action. Additionally, variations associated with a drug's target molecules can directly affect its pharmacodynamic effect. In the last twenty years, pharmacogenomics has attracted attention as a discipline that can contribute to the quality of patient health care and present an essential part of the personalized medicine concept. The use of genetic information would help predict the response to the drugs to enable safer, more effective, and cost-effective treatment to each patient.





Presentation number: CSHG 3

# INFLUENCE OF GENETIC SUB-STRUCTURING OF STATISTICAL FORENSIC PARAMETERS ON GENETIC STR MARKERS IN THE POPULATIONS OF SOUTHEASTERN EUROPE

**Natalija Novokmet**[1], Marijana Peričić Salihović[1], Vedrana Škaro[1,2], Petar Projić[1,2], Jelena Šarac[1], Dubravka Havaš Auguštin[1], Saša Missoni[1,3], Pavao Rudan[4], Dragan Primorac[3,5,6,7,8,9,10,11,12,13], Damir Marjanović[1,14]

[1]Laboratory for Molecular Anthropology, Center for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia, [2]DNA Laboratory, Genos Ltd., Zagreb, Croatia, [3]School of Medicine, University of Osijek, Osijek, Croatia, [4]Scientific Council for Anthropological Research, Croatian Academy of Sciences and Arts, Zagreb, Croatia, [5]St. Catherine Hospital, Zagreb, Croatia, [6]School of Medicine, University of Split, Split, Croatia, [7]University Department of Forensic Sciences, University of Split, Split, Croatia, [8]Faculty of Dental Medicine and Health, University of Osijek, Osijek Croatia, [9]University of Rijeka, School of Medicine, Rijeka, Croatia, [10]Eberly College of Science, Pennsylvania State University, University Park, PA, USA, [11]Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [12]Medical School REGIOMED, Coburg, Germany, [13]The National Forensic Sciences University, Gandhinagar, Gujarat, India, [14]Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina

The goal was to perform a meta-analysis of synthesized data and investigate the influence of specific intrapopulation genetic structures on interpopulation relationships. Special focus was the influence of island population isolation on the substructuring of the Croatian population, and the influence of regional population groups on the substructuring of Southeast Europe. A long-term goal is to develop a model of appropriate sampling of the total population when creating a database of genetic STR markers that would properly reflect all the characteristics of included subpopulations. Autosomal STR loci were analyzed using four forensic parameters (match probability, power of discrimination, power of exclusion and the degree of polymorphism) on a sample of 2877 unrelated participants of both sexes. The analysis was performed using the statistical package PowerStats v1.2. The comparison of forensic parameters between different subpopulations of Croatia and Southeast Europe indicates that the isolation of individual Croatian subpopulations and certain rare alleles in their gene pool affect the values of forensic parameters. Specific features of (sub)populations should be taken into account for appropriate sampling of the total population when creating a DNA database of STR markers.

**Keywords:** STRs, genetic sub-structuring, forensic parameters, Croatia, Southeast Europe

Presentation number: CSHG 4

# EXOME SEQUENCING AND AUTISM SPECTRUM DISORDER-DIAGNOSTIC CHALLENGES AND FUTURE DIRECTIONS

**Ljubica Odak**, Katarina Vulin, Ana-Marija Meašić, Adriana Bobinec, Ivona Sansović,

Children's Hospital Zagreb, Centre of Excellence for Reproductive and Regenerative Medicine, Medical School University of Zagreb, Zagreb, Croatia

The genetic basis of autism spectrum disorder (ASD) is still poorly understood in many patients. Next-generation sequencing enables simultaneous detection of pathogenic variants in hundreds of disease-causing genes. The goal of this study is to define diagnostic utility of clinical exome sequencing in elucidating autism spectrum disorder (ASD) etiology and create future diagnostic and therapeutic directions. Material and Methods For this study, we analyzed 55 ASD patients that were diagnosed and treated at the Department of Medical Genetics and Reproductive Health in Children's Hospital Zagreb. All of them had a confirmed ASD diagnosis and were underwent to detailed clinical geneticist's evaluation. Chromosomal disorders and fragile X syndrome have been previously excluded in all patients. Clinical exome sequencing has been performed using Illumina TruSight One Kit. In 14 out of 55 patients (25.4%) pathogenic variants were identified (14/55) and involved genes: CAMTA1, DEAF1, EP300, DICER1, MED13, CHD7, DYRK1A, FOXG1, SOS1, MED12, EHMT1, CHD8, TCF4, NFIX, PAK3. Variants of unknown significance (VUS) were present in 16.4 % of patients (9/55), in genes: AUTS2, DLG3, IHIF1, DEAF1, BICRA, CREBBP, CTNNB1, RELN. The remaining patients had negative test results (32/55; 58.2%). Clinical exome sequencing elucidated genetic basis of autism in 25 % of ASD patients, mostly attributable to genes involved in fundamental genetic processes; transcription (CAMTA1, DEAF1, EP300, MED13, FOXG1, MED12, TCF4, NFIX), chromatin remodeling (CHD7, CHD8, EHMT1), ribonuclease activity (DICER1) and activity of various kinases (DYRK1A, SOS1, PAK3). High percentage of negative and inconclusive (VUS) results (74.6%) requires additional genetic (whole exome/genome), epigenetic and environmental risk factor analysis. Establishing autism genetic basis is the prerequisite for the development of new therapeutic strategies and personalized treatment for the ASD patients. Acknowledgment: This work was supported by Scientific Center of Excellence for Reproductive and Regenerative Medicine and by the EU through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project „Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials"

**Keywords:** autism, exome sequencing, personalized management





Presentation number: CSHG 5

# SEX-RELATED IMMUNOPHENOTYPE DIFFERENCES IN HUMAN STROMAL VASCULAR FRACTION FROM LIPOASPIRATE AND MICROFRAGMENTED LIPOASPIRATE REVEALED BY POLYCHROMATIC FLOW CYTOMETRY

**Denis Polancec**[1], Lucija Zenic[1], Damir Hudetz[2,3,4], Zeljko Jelec[2,5], Eduard Rod[2], Dinko Vidovic[2,6,7], Mario Staresinic[8,9], Srecko Sabalic[6,10], Trpimir Vrdoljak[2,3], Tadija Petrovic[6], Fabijan Cukelj[6,10], Vilim Molnar[2,4], Martin Cemerin[2,9], Vid Matisic[2], Petar Brlek[2], Zrinka Djukic Koroljevic[2], Igor Boric[2,11,12,13], Gordan Lauc[14,15], Dragan Primorac[2,4,10,11,12,16,17,18,19,20,21]

[1]Srebrnjak Children's Hospital, Department for Translational Medicine, Zagreb, Croatia, [2]St. Catherine Specialty Hospital, Zagreb, Croatia, [3]Clinical Hospital Sveti Duh, Zagreb, Croatia, [4]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [5]Department of Nursing, University North, Varaždin, Croatia, [6]University Hospital Sestre Milosrdnice, Clinic for Traumatology, Zagreb, Croatia, [7]School of Dental Medicine, University of Zagreb, Zagreb, Croatia, [8]Department of Traumatology, Medical University Merkur Hospital, Zagreb, Croatia, [9]Medical School, University of Zagreb, Zagreb, Croatia, [10]Medical School, University of Split, Split, Croatia, [11]Medical School, University of Rijeka, Rijeka, Croatia, [12]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina, [13]Department of Health Studies, University of Split, Split, Croatia, [14]Genos Glycoscience Research Laboratory, Zagreb, Croatia, [15]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia, [16]Eberly College of Science, The Pennsylvania State University, University Park, State College, PA, USA, [17]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA, [18]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia, [19]Medical School REGIOMED, Coburg, Germany, [20]The National Forensic Sciences University, Gandhinagar, Gujarat, India, [21]University of Split, University Department of Forensic Sciences, Split, Croatia

Mesenchymal stem/stromal cells or from recently referred to as medicinal signaling cells (MSC) hold tremendous therapeutic potential in regenerative medicine. Although successfully used for treating knee osteoarthritis (OA), a broader application of MSC in the field requires a better understanding of functional and cellular heterogeneity. In order to gain insight into the human MSC from adipose tissue applied for autologous OA treatment, we performed extensive comparative immunophenotyping of stromal vascular fraction (SVF) from lipoaspirate or microfragmented lipoaspirates by polychromatic flow cytometry. Sixteen OA patients (eight females and eight males) were enrolled in the study. For each of the patient SVF was obtained from lipoaspirate and microfragmented lipoaspirate counterpart. Isolated SVF cells were stained with fluorochrome-labelled antibodies specific for the CD31, CD34, CD45, CD73, CD90, CD105, CD146 cell surface markers and analyzed by flow cytometry. We found an enrichment of the endothelial progenitor cells in the clinically applied microfragmented stromal vascular fraction. Sex-related differences were observed in the MSC marker expression and the ratio of the progenitor cells from fresh lipoaspirate; in female patients it contained a higher expression of CD90 on the three progenitor cell types including pericytes, a higher expression of CD105 and CD146 on CD31highCD34high endothelial progenitors as well as of CD73 on supraadventitial-adipose stromal cells. Some of these MSC-expression differences were present after microfragmentation, which indicates

a diverse phenotype pattern of the applied MSC in female and male patients. Adding to the understanding of the perplexed heterogeneity of the adipose MSC subpopulations serving as OA therapeutics, our results tackle on the sex-related molecular signatures in women and men that might contribute to a personalized approach of regenerative medicine.

**Keywords:** osteoarthritis, mesenchymal stem/stromal cells, medicinal signaling cells, stromal vascular fraction, immunophenotyping





Presentation number: CSHG 6

# CROATIAN GENETIC HERITAGE: RENEWED Y CHROMOSOME STORY TWO DECADES LATER

Dragan Primorac[1,2,3,4,5,6,7,8,9,10], Vedrana Škaro[11,12], Petar Projić[11,12], Saša Missoni[11], Ivana Horjan Zanki[13], Siniša Merkaš[13], Jelena Šarac[11], Natalija Novokmet[11], Andrea Ledić[13], Adela Makar[13], Gordan Lauc[12,14], Šimun Anđelinović[15], Željana Bašić[3], Ivana Kružić[3], Marijana Neuberg[16], Martina Smolić[4,5], Robert Smolić[4,5], Irena Hrstić[6,17], Dragan Trivanović[6,17], Rijad Konjhodžić[18], Lana Salihefendić[18], Naida Babić Jordamović[19], **Damir Marjanović[11,19]**

[1]St. Catherine Hospital, Zagreb, Croatia, [2]University of Split, School of Medicine, Split, Croatia, [3]University of Split, University Department of Forensic Sciences, Split, Croatia, [4]University of Osijek, Faculty of Medicine, Osijek, Croatia, [5]University of Osijek, Faculty of Dental Medicine and Health, Osijek, Croatia, [6]University of Rijeka, School of Medicine, Rijeka, Croatia , [7]Pennsylvania State University, Eberly College of Science, University Park, PA, USA, [8]University of New Haven, Henry C. Lee College of Criminal Justice and Forensic Sciences, West Haven, CT, USA, [9]Medical School REGIOMED, Coburg, Germany, [10]The National Forensic Sciences University, Gandhinagar, Gujarat, India, [11]Institute for Anthropological Research, Center for Applied Bioanthropology, Molecular Anthropology Laboratory, Zagreb, Croatia, [12]Genos Ltd., DNA Laboratory, Zagreb, Croatia, [13]Forensic Science Centre „Ivan Vučetić", Zagreb, Croatia,[14]University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Pharmacology, Zagreb, Croatia, [15]University Hospital Center, Split, Croatia, [16]University North, Varaždin, Croatia , [17]General Hospital Pula, Pula, Croatia, [18]Alea Genetic Center, Sarajevo, Bosnia and Herzegovina, [19]International Burch University, Department of Genetics and Bioengineering, Sarajevo, Bosnia and Herzegovina

The aim of the study was to analyze additional set of Y-Chromosome genetic markers to acquire a more detailed insight into the diversity of the Croatian population. The total number of 518 Yfiler™ Plus profiles was genotyped. Allele, haplotype frequencies and haplotype diversity, were calculated using the STRAF software package v2.0.4. Genetic distances were quantified by Rst using AMOVA online tool from the YHRD. The evolutionary history was inferred using the neighbor-joining method of phylogenetic tree construction in MEGAX software. Whit Athey's Haplogroup Predictor v5 was used for additional comparison with selected European populations. The total of 507 haplotypes were used for genetic STR analysis. The interpopulation comparison with the original 27 Y-STR markers shows the lowest genetic diversity between Croatian and Serbian population, and the highest between Croatian and Spanish population. Interpopulation study on 17 Y-STR markers shows the lowest genetic diversity between Croatian and Bosnian-Herzegovinian population, and the highest between Croatian and Irish population. Total of 518 haplotypes were used in the determination of haplogroup diversity. Haplogroup I with its sublineage I2a expressed the highest prevalence. Haplogroup R, with its major sublineage R1a, is the second most abundant in the studied Croatian population, except for the subpopulation of Hvar, where E1b1b is the second most abundant haplogroup. Rare haplogroups also confirmed in this study are L, T and Q. G1 is detected for the very first time in Croatian population. New insight into differences between examined subpopulations of Croatia and their possible (dis)similarities with neighboring abroad populations was notified.

**Keywords:** Y chromosome, haplogroup, Croatian population, STR, genetic heritage

Presentation number: CSHG 7

## CHROMOSOMAL MICROARRAY IN CLINICAL DIAGNOSIS OF CEREBRAL PALSY

**Katarina Vulin**, Ljubica Odak, Leona Morožin Pohovski, Ivana Đaković, Ana Tripalo Batoš, Ana-Maria Meašić, Mijana Kero, Ivona Sansović, Adriana Bobinec, Ingeborg Barišić

Children's Hospital Zagreb, Centre of Excellence for Reproductive and Regenerative Medicine, Medical School University of Zagreb, Zagreb, Croatia

Cerebral palsy (CP) is a group of non-progressive disorders of posture, tone, and/or movement. It is caused by a non-progressive lesion of the developing brain or brain malformation. Recent studies have implicated that genetic factors could contribute to diagnosis of CP or even cause CP. Potentially deleterious genomic copy number variants (CNVs) have been found in several CP cohorts, but estimates varied considerably depending on study criteria. The goal of this study is to define diagnostic utility of chromosomal microarray in children with CP, with well-defined phenotype according to Surveillance of cerebral palsy in Europe (SCPE) criteria. This study included 79 patients with CP, referred to the Department of Medical Genetics and Reproductive Health, Children's Hospital Zagreb. All of them had confirmed CP diagnosis by the criteria of SCPE. The analysis was conducted using Agilent 60K oligonucleotide array-based comparative genomic hybridization. Clinically relevant variants were detected in 7 of 79 patients (8,86%): deletions 14q32.31q32.33, 1q21.1q21.2, 15q11.2, 17p13.3 and 22q11.2; duplications 1q21.1q21.2 and Xq28 (in male patient). Variants of unknown significance (VOUS) were present in 5 patients (6,33%): duplications 15q11.2, 3p26.3p26.2, 18p11.31 and Xq28 (in female patient) and deletion 17p12. Remaining patients had negative test results. Among the patients with clinically important variants, three had brain MRI classified as maldevelopment, three as predominant white matter injury, and one patient with 22q11.2 deletion as predominant grey matter injury. The genomic architecture of CP is highly complex, similar to other neurodevelopmental disorders. Continued analysis and reporting of CNV findings alongside massively parallel SNV analyses are needed to expand our knowledge of CP. Better understanding of all the possible genes involved in CP etiology is the cornerstone for understanding neurobiology of CP. This work was supported by Scientific Center of Excellence for Reproductive and Regenerative Medicine and by the EU through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project „Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials".

**Keywords:** cerebral palsy, chromosomal microarray, copy number variants



# ABSTRACTS OF THE ANTHROPOLOGICAL SESSION "BIOANTHROPOLOGY AND GLOBAL HEALTH IN THE TIMES OF CRISIS"

Presentation number: ANTR 1

# INTEGRATIVE APPROACHES TO GLOBAL HEALTH

**Luka Bočkor**

Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia

Modern bioanthropology, although always interdisciplinary, nowadays is breaking down barriers in science ever more efficiently. Multiple factors – genetics, environment and lifestyle – influence disease development. The main problem of multiple branches of science is their usual monochromatic perspective, where every problem is viewed from its exclusive point of reference. Our genetics is a result of natural selection, and although we are as fit as can be, we are the result of all the adaptations that human species acquired during the past, not only as our species, but also as all our ancestral species. Furthermore, with the advancement of science and technology (reflecting in every aspect of our everyday life), human lifestyle has dramatically changed in a brief amount of time on a historical timescale. Unfortunately, adaptive changes of the genome are not able to compensate and, as a result, multiple non-communicable diseases (e. g. diabetes, cardiovascular diseases, tumours) are emerging within human populations worldwide with increasing costs for the health systems on a global scale. In order to decrease the incidence of diseases and consequently their financial and social burden, a comprehensive approach taking into account social, behavioural, psychological, and biological factors is necessary. By applying methods from analytical chemistry, molecular biology and biochemistry combined with different types of questioners and interviews and data from populations from the past, we can better understand causes of diseases, both on organism and molecular level. Importantly, taking into account environment (toxicological burden) and ecological systems inhabiting our organisms (microbiota) bioanthropology can strongly contribute to finding the proper treatment of diseases in personalized manner and, more importantly, help in their prevention.

Presentation number: ANTR 2

# TOXICOLOGY IN THE CONTEXT OF GLOBAL HEALTH

**Aleksandra Buha Djordjevic**

Department of Toxicology „Akademik Danilo Soldatović", Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Human health has been immensely influenced by environmental factors. Various reports have shown that nearly 25% of global deaths and about a third of the world's burden of disease in children can be attributed to such factors. Although some environmental contaminants are difficult to avoid, amelioration or even elimination of these factors is possible and the diseases arising because of these factors are preventable. Accordingly, this attributable disease burden is often 10-fold higher in poor countries than in developed ones. This disparity is mainly caused by a lack of modern technology, weak laws and regulation in the field, a lack of awareness among the population, and general poverty in these countries. Furthermore, residents of wealthy countries are also affected by many environmental factors such as air pollution, lead poisoning, etc. Hence, the role of environmental health in global health is of great concern. The most potent environmental causes of worldwide illness are unclean water, air pollution, and exposure to various industrial chemicals. Concerning chemical exposure, it is important to stress that of the more than 30,000 chemicals commonly used today, scientists have studied only fewer than 1% in detail, while our understanding of the effects of simultaneous exposure to low levels of hundreds or thousands of chemicals we face on daily basis is rudimentary, at best. Cohesive and comprehensive policies that would protect both people's health and planet health require strengthening of the institutional capacity and social actions that encompass raising public awareness and public health measures in the field.





Presentation number: ANTR 3

# CHILD GROWTH AND ARMED CONFLICT

**Noël Cameron**

School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom

The widely publicised invasion of the Ukraine by Russia has highlighted yet again the devastation caused by armed conflict at individual, community, and national levels. Human society in the 20th and 21st Centuries has never been free of armed conflict either within or between nations. Whilst the first half of the 20th century was dominated by the 'World Wars' of 1914-18 and 1939-45, the Uppsala Conflict Data Program records that never less than 40 countries worldwide were involved in armed conflicts between 1989 and 2018. In their Lancet review of 2021, Bendavid et al (2021) emphasise that whilst "…every conflict-affected region, every conflict, and every affected community is different from all others…" common features are shared and yet the evidence that supports health consequences is "weak" and "limited", and in the case of adolescents almost "non-existent". The status of human growth and development over time has been used globally to determine the health and wellbeing of children. Given the ubiquitous nature of armed conflict, it is not surprising that research in human growth and development has provided several significant studies highlighting the effects of armed conflict on children and adolescents in war zones. Data reflecting the effect of the 1st and 2nd World wars on young people in England, the Netherlands, Germany, and Japan, demonstrates the power of armed conflict to interrupt the positive secular trends characteristics of improved physical growth and development at national levels in both those experiencing conflict first hand, and the long-term consequences to those experiencing it through maternal exposure during pregnancy. Studies of the growth outcomes of armed conflict are, of course, complicated by the inability to plan prospective studies. Most studies examine proximal outcomes of conflict such as mortality, malnutrition, injury, disability, and disease. However, the existence of repeated cross-sectional surveys provides unequivocal retrospective evidence for the reduction of growth rates across the age range from infancy to adulthood and the differential effects of armed conflict in children and adolescents.

Presentation number: ANTR 4

# UNRAVELLING DATA FOR RAPID EVIDENCE-BASED RESPONSE TO COVID-19 – INANTRO EXPERIENCE FROM THE UNCOVER PROJECT

**Miran Čoklo**[1], Ivan Dolanc[1], Antonija Jonjić[1], Jelena Šarac[1], Luka Bočkor[1], Natalija Novokmet[1], Marko Tarle[2], Sanda Mustapić[2], Marta Kmet[2], Biserka Orehovec[2], Ivica Lukšić[2], Saša Missoni[1]

[1]Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia, [2]Dubrava University Hospital, Zagreb, Croatia

"unCoVer-Unravelling data for rapid evidence-based response to COVID-19" is a Horizon 2020-funded network of 29 partners from 18 countries. Formed with the aim to collect and use real-world data (RWD) resulting from response and provision of care to COVID-19 patients by health systems across Europe and elsewhere, unCoVer exploits the full potential of this information to rapidly address clinical and epidemiological research questions arising from the COVID-19 pandemic. These heterogeneous datasets comprise from information on over 22 000 hospitalized patients, as well as registry data on over 1 900 000 COVID-19 cases across Europe, with continuous updates. So far, the datasets have been described, harmonized and integrated into a multi-user data repository operated through Opal-DataSHIELD, an interoperable open-source server application. Federated data analyses, without sharing or disclosing any individual-level data, is being performed to resolve various research questions emerging from dealing with the COVID-19 pandemic. Institute for Anthropological Research, Zagreb, Croatia, is one of the partners on the unCoVer project, together with our related third-party – Dubrava University Hospital, Zagreb, Croatia, providing clinical data on COVID-19 patients. Special emphasis will be given to our specific experience and lessons learned from participating in this project (data acquisition, ethical and GDPR issues, data analyses, influence of restrictions related to COVID-19 pandemic).





Presentation number: ANTR 5

# DOMESTICATION OF CATTLE: CURRENT STATUS AND ANCIENT DNA PERSPECTIVES

**Vlatka Čubrić-Čurik**

University of Zagreb, Faculty of Agriculture, Zagreb, Croatia

The domestication of animals is considered one of the most influential processes that have shaped the development and growth of human civilization. Cattle, which are divided into Taurus cattle (Bos taurus taurus) and Zebu cattle (Bos taurus indicus), are capable of converting large quantities of roughage into high-quality food, and the other benefits are also considerable. Therefore, cattle are often considered one of the most important domestic animals. Here, I first provide a historical overview of bovine domestication from the perspective of mitogenomes, Y chromosomes, and nuclear genomic markers. Second, I present the results of two recent genomic analyses of ancient cattle. Third, I show the results of our large-scale meta-analyses of the complete bovine mitogenome. Next, I present the archaeogenetics laboratory established this summer at the University of Zagreb - Faculty of Zagreb. Finally, I present our future perspectives in research on ancient genetics/genomics and domestication of animals.

Presentation number: ANTR 6

# RUNS OF HOMOZYGOSITY AS AN IMPORTAN CONCEPT IN POPULATION GENOMICS

**Ino Čurik**

University of Zagreb, Faculty of Agriculture, Zagreb, Croatia

Runs of homozygosity (ROH) are contiguous long stretches of homozygous genomic regions used to estimate realised autozygosity from high-throughput marker information. I will explain how ROH can be used to infer a number of important genomic population parameters. For example, ROH are used to estimate recent and distant inbreeding at the individual and population level. In addition, ROH are used to estimate inbreeding depression and to map genomic regions with detrimental effects. Genomic regions with above average occurrence of ROH or extreme ROH islands are used to identify genomic regions that exhibit patterns of positive selection. Finally, I will explain how ROH are used to estimate current and historical effective population size. All applications and examples presented are related to animals and humans.





Presentation number: ANTR 7

# IMPLICATING EFFECTOR GENES AT COVID-19 GWAS LOCI IN DISEASE-RELEVANT IMMUNE CELL TYPES

**Struan F. A. Grant**

Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA

SARS-CoV-2 infection results in a broad spectrum of COVID-19 disease, from mild or no symptoms to hospitalization and death. COVID-19 disease severity has been associated with some pre-existing conditions and the magnitude of the adaptive immune response to SARS-CoV-2, and a recent genome-wide association study (GWAS) of the risk of critical illness revealed a significant genetic component. To gain insight into how human genetic variation attenuates or exacerbates disease following SARS-CoV-2 infection, we implicated putatively functional COVID risk variants in the cis-regulatory landscapes of human immune cell types with established roles in disease severity and used high-resolution chromatin conformation capture to map these disease-associated elements to their effector genes. This functional genomic approach implicates 16 genes involved in viral replication, the interferon response, and inflammation. Several of these genes (PAXBP1, IFNAR2, OAS1, OAS3, TNFAIP8L1, GART) were differentially expressed in immune cells from patients with severe vs. moderate COVID-19 disease, and we demonstrate a previously unappreciated role for GART in T cell-dependent antibody-producing B cell differentiation in a human tonsillar organoid model. This study offers immunogenetic insight into the basis of COVID-19 disease severity and implicates new targets for therapeutics that limit SARS-CoV-2 infection and its resultant life-threatening inflammation.

Presentation number: ANTR 8

# GENDER DIFFERENCES AND GENETIC GEOGRAPHICAL VARIATION IN THE MEDITERRANEAN AREA OF COVID-19 DISEASE IN RELATION WITH INFERTILITY – OUTPUT OF EUROPEAN MEDIGENE PROGRAM

**Florin Grigorescu**

Institut de Convergences Migration – Collège de France, Paris, France

Human populations were faced to COVID-19 pandemic due to emerging SARS-CoV-2 coronavirus from Wuhan (China) and with dramatic Public Health consequences. Although scientific community demonstrated an incredible innovation potential producing in one-year efficient vaccines, researchers are intrigued about ethnic and geographic diversity of endocrine or metabolic manifestations of COVID-19, including gender differences and effects on fertility. Man displays 1.5- and 2.5-fold higher Covid-19 mortality and SARS-CoV-2 infection, partially explained by differential expression of ACE2 (angiotensin-converting enzyme 2) receptor and TMPRSS2 (serine protease transmembrane protease serine2) located on Chr21q22.3. ACE2 is located on Chr-X, males being hemizygous while females expressing a mosaic pattern and potentially heterozygous with lower sensitivity to infection. Sex hormones regulate ACE2 expression and activity shifting the activity of Renin Angiotensin Aldosterone System (RAAS). TMPRSS2 gene has no sex-dimorphism but contains an androgen-responsive enhancer located on specific haplotypes more frequent in Europeans (e.g. Italians) and totally absent in East Asians. Gender disparity for Covid-19 infection also concerns the immune response, females being in general protected. Androgens have deleterious effects on thrombo-inflammation, raising the hypothesis on potential role of hyperandrogenism in women with polycystic ovary syndrome (PCOS) and infertility. In PCOS, there is indeed a 50% higher risk of Covid-19 infection, but vulnerability of patients may be explained by multiple factors such as chronic pro-inflammatory state (increase in TNFa and IL-6), ethnic background (e.g. expression of Androgen Receptors in Black, Asian and Minority Ethnic groups) or low vitamin D levels. Recent GWAS studies indicated several susceptibility loci for Covid-19 infection with high ethnic disparities and structurally related to ancestral components of the human genome. All these epidemiological variations prompt scientists to further investigate susceptibility genes and long-term complications of Covid-19 infection.





Presentation number: ANTR 9

# SARS-COV-2 AMONG SARAJEVO INHABITANTS - THE PRE-VACCINATION PERIOD

**Jasminka Prguda-Mujic**[1], Osman Hasanic[2], Larisa Besic[2], Adna Asic[2], Sabina Halilovic[2], Aida Kulo Cesic[3], Neira Ljevakovic[2], Fildesa Muminovic[2], Sukrija Huseinovic[2], Daria Ler[2], Lana Salihefendic[4], Rijad Konjhodzic[4], Dragan Primorac[5,6,7,8,9,10,11,12], Damir Marjanovic[2,13]

[1]Eurofarm Molecular Diagnostics Laboratory, Eurofarm Centre, Sarajevo, Bosnia and Herzegovina, [2]Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina, [3]Department of Pharmacology, Clinical Pharmacology and Toxicology, Medical Faculty, University of Sarajevo, Bosnia and Herzegovina, [4]Alea Genetic Centre, Health Institute Alea Dr. Kandić, Sarajevo, Bosnia and Herzegovina, [5]St. Catherine Specialty Hospital, Zagreb, Croatia, [6]Eberly College of Science, The Pennsylvania State University, University Park, State College, Pennsylvania, United States of America, [7]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of NewHaven, West Haven, Connecticut, United States of America, [8]Medical School, University of Split, Split, Croatia, [9]School of Medicine, Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University Osijek, Osijek, Croatia, [10]School of Medicine, Josip Juraj Strossmayer University Osijek, Osijek, Croatia, [11]Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [12]Medical School REGIOMED, Coburg, Germany, [13]Institute for Anthropological Research, University of Zagreb, Zagreb, Croatia

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing a novel COVID-19 infection. While the standard diagnostic test is molecular analysis using qRT-PCR, serological testing for COVID-19 is becoming increasingly important in research community and immunity surveillance efforts. Serological testing is performed on whole blood, serum, or plasma samples in a relatively simple and rapid procedure, requiring less expertise and simpler laboratory settings compared to molecular methods. Tests are designed to detect either total immunoglobulins (Ig) or to differentiate between immunoglobulins G (IgG) and immunoglobulins M (IgM) fractions. Preliminary study completed in 2020 offered the very first report on serological testing for SARS-CoV-2 in Bosnia and Herzegovina. Compared to the period April–July 2020, when anti-SARS-CoV-2 antibodies were detected in 3.77% of samples, one year later (May 2021) the estimated percentage within the same population of the urban Canton Sarajevo was 29.9% (5,406/18,066). Of all anti-SARS-CoV-2 Ig-positive individuals, 53.27% were men, and 69.00% were of 50 years of age or younger. Also, the current update found the individuals 50 years of age or younger to be more frequently anti-SARS-CoV-2 Ig positive compared to older individuals. On the other hand, higher median anti-SARS-CoV-2 Ig levels were found in individuals > 50 years old than in younger individuals, as well as in men compared to women. Seropositivity gradually increased from September 2020 to May 2021, with the lowest frequency of positive cases (3.5%) observed in September 2020, and the highest frequency (77.7%) in January 2021. Our results published within two separated publications in 2020 and 2022, provided important seroprevalence data that could help in planning restrictive local public health measures to protect the population of Sarajevo Canton, especially considering that at the time of the study the vaccines were virtually inaccessible to the general population not belonging to any of the high-priority groups for vaccination.

Presentation number: ANTR 10

# ANTHROPOLOGICAL RESEARCH ON CROATIAN ISLANDS – CRIBS BIRTH COHORT

**Saša Missoni**[1,2]

[1]Institute for Anthropological Research, Zagreb, Croatia, [2]School of Medicine, "J. J. Strossmayer" University, Osijek, Croatia

The "Croatian Islands Birth Cohort Study (CRIBS)" is the first birth cohort study ever conducted in Croatia, designed to prospectively follow a sample of 500 pregnant women and their children up to two years of age in populations from Croatian Dalmatian islands (Hvar and Brač) and mainland population (city of Split with its surroundings). Data collected within the CRIBS cohort include data on social factors (age, marital status, education, employment, income, religious views, number of children), psychosocial factors (quality of life, health beliefs and attitudes, postnatal depression), lifestyle (smoking, alcohol consumption, dietary habits, physical activity), medical data (maternal obstetric data and medical history, potential pre-, peri- and post-natal complications, information on child's growth and development, feeding habits, allergies, vaccination, other medical issues etc.) and biological samples (blood samples from the mother and cord blood samples from the newborns). The results of the CRIBS study already showed that certain environmental and biological variables (e.g., maternal age, smoking, parity, lipid profile and fasting blood glucose level) are risk factors for the metabolic syndrome (MetS) and adverse pregnancy outcomes in this Croatian birth cohort. Especially pre-pregnancy BMI has been observed as an indicator of adverse maternal health during pregnancy (deviated biochemical parameters, high blood pressure), as well as an indicator of negative pregnancy outcomes. The advantage of the CRIBS cohort is the wealth of collected data (including biological samples of mother-child dyads), enabling future investigation of biological and environmental risk factors for the development of MetS and associated complex diseases.





Presentation number: ANTR 11

# MEDITERRANEAN DIET ADHERENCE AND ITS ASSOCIATION WITH BIOLOGICAL MARKERS AND HEALTH IN DALMATIA

**Jelena Šarac**[1,2], Dubravka Havaš Auguštin[1,2], Mario Lovrić[1,3,4], Natalija Novokmet[2], Saša Missoni[2,5]

[1]Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia, [2]Institute for Anthropological Research, Zagreb, Croatia, [3]Know-Center, Graz, Austria, [4]Institute of Interactive Systems and Data Science, Graz University of Technology, Graz, Austria, [5]School of Medicine, "J. J. Strossmayer" University, Osijek, Croatia

Although the Mediterranean diet has beneficial effects on health, recent studies have shown low adherence in Europe. Rapid changes in the traditional way of life and the "westernization" of the diet in Mediterranean populations, especially in younger generations, has led to progressive abandonment of healthy dietary patterns. Adherence to the Mediterranean diet in Dalmatia, Croatia was assessed through the Mediterranean Diet Serving Score (MDSS) (Monteagudo et al. 2015) in two Dalmatian populations – pregnant women from Split and islands Hvar and Brač, and the adult population from the island of Hvar. Adherence to the Mediterranean diet was low to moderate among pregnant women, with no significant mainland–island differences. The highest adherence was observed among wealthier women with generally healthier lifestyle choices. The most significant mainland–island differences were observed for maternal lifestyle and socioeconomic factors (income, education, physical activity). Namely, adverse socioeconomic and lifestyle conditions were more pronounced in the island population, which, together with the observed non-Mediterranean dietary pattern, calls for more effective pre- and perinatal intervention strategies. When compared to the adult population from the island of Hvar, the young, reproductively active generation (pregnant women) in Dalmatia, Croatia, although having a higher education and socioeconomic status, exhibits a more adverse eating behaviour (lower adherence to the Mediterranean diet) and lifestyle (excessive smoking in pregnancy) than the older population from the same region. MDSS scores across aggregated age groups in both cohorts also showed significant association with age, blood lipid levels and smoking frequency.

Presentation number: ANTR 12

# THE GENETIC LEGACY OF ARCHAIC HOMININ ADMIXTURE

**Serena Tucci**

Department of Anthropology, Yale University, New Haven, CT, USA

Modern humans overlapped for most of their history with other hominin groups, and an enduring question is whether or not our ancestors admixed with these, now extinct, hominin groups. DNA retrieved from Neandertal and Denisovan fossils revealed that our ancestors did admix with archaic hominin contemporaries, and remnants of Neandertal and Denisovan genomes still survive in present-day individuals. While the landscape of Neandertal and Denisovan ancestry in present-day populations has been outlined, the identification of archaic variation inherited from other hominin groups, such as H. erectus and H. floresiensis, is hampered by the absence of archaic reference genomes retrieved from their fossils. To overcome this limitation, we developed a "fossil free" statistical framework to detect putative "unknown" archaic sequences without relying on archaic genomes. We applied this method to genomic data we have generated from a contemporary pygmy population living on Flores Island (Indonesia), near the same cave where remains of the enigmatic small-bodied hominin species, H. floresiensis, were found. These data provide new insights into the interaction of archaic hominins in Island Southeast Asia and shed new light on how the unique biogeographical setting of the Flores pygmy population shaped their history and mechanisms of evolutionary change.





Presentation number: ANTR 13

# OBESITY IN CHILDHOOD RELATED TO MATERNAL FACTORS DURING PREGNANCY IN THE ECHO-FGS COHORT STUDY

**John E. Vena**

Medical University of South Carolina, Charleston, SC, USA

The prevalence of obesity in US children has more than tripled in the past 40 years; hence, it is critical to identify potentially modifiable factors that may mitigate the risk. To examine the association between maternal pre-pregnancy body mass index (BMI), gestational weight gain (GWG) and child adiposity as measured by BMI, waist circumference and percent body fat in a racial-ethnically diverse cohort. In a prospective cohort study of healthy women without chronic disease, we examined the association between pre-pregnancy BMI, GWG and child adiposity. Children ages 4–8 years (n = 816) in the Environmental Influences on Child Health. Outcomes-NICHD Fetal Growth Studies were assessed. Trained study staff ascertained maternal pre-pregnancy BMI, GWG and child adiposity. The odds of child obesity (≥95th BMI percentile) increased independently for each unit increase in maternal pre-pregnancy BMI [OR = 1.12 (95% CI: 1.08, 1.17)] and for each 5-kg increase in GWG [OR = 1.25 (95% CI: 1.07, 1.47)]. The odds of child waist circumference (≥85th percentile) also increased independently for pre-pregnancy BMI [OR = 1.09 (95% CI: 1.05, 1.12)] and GWG [OR = 1.18 (95% CI: 1.04, 1.34)]. Maternal pre-pregnancy BMI and GWG were each independently and positively associated with child obesity and high child waist circumference.

Presentation number: ANTR 14

## REGIONAL SENSITIVITY BAROMETER: STUDYING THE EFFECTS OF THE PANDEMIC ON DEVELOPMENT

**Maria Zafiropolou**

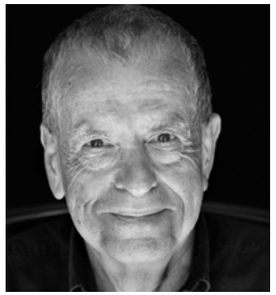
European Commission, Expert in the Healthcare and Social Sector, Patras, Greece

The regional sensitivity barometer evaluates the regional sensitivity of European regions and the regional needs amid the COVID-19 health and financial crisis. In this paper qualitative and quantitative indicators were combined in European level and then an overall sensitivity index was calculated for each average European region. Demographic, economic, health, tourism-related and COVID-19 pandemic-related data are taken into account, using differentiated weighting factors. The statistical data used was lastly evaluated on December 31st, 2020 in Eurostat database and processed in order to create the regional sensitivity for every country. The total index highlighted the final ranking between the European regions and formed the basis for the depiction of sensitivity on the European map. A series of conclusions came up; namely the existence of three groups of European Union (EU) member states in terms of regional resilience amid the COVID-19 crisis, as well as the extreme sensitivity of regions that later faced significant difficulty in handing the health and financial crisis, such as Portugal, Greece, Italy and the United Kingdom. In addition, Slovak Regions have shown preparedness to health threats. Comparing barometer results becomes clear that the sensitivity of a region is inextricably linked to its course over time, the chronic systemic pathogens and the readiness that has already developed in emergencies.

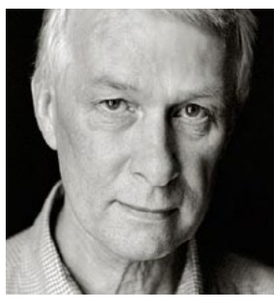


# ISABS 2022

# ABOUT NOBEL LAUREATES



**Aaron Ciechanover, M.D., PhD**, was born in Haifa, Israel in 1947. He is currently a Distinguished Research Professor in the Faculty of medicine at the Technion - Israel Institute of Technology in Haifa, Israel. He received his M.Sc. (1971) and M.D. (1973) from the Hebrew University in Jerusalem. He then completed his national service (1973-1976) as military physician, and continued his studies to obtain a doctorate in biological sciences in the Faculty of Medicine in the Technion (D.Sc.; 1982). There, as a graduate student with Dr. Avram Hershko and in collaboration with Dr. Irwin A. Rose from the Fox Chase Cancer Center in Philadelphia, USA, they discovered that covalent attachment of ubiquitin to a target protein signals it for degradation. They deciphered the mechanism of conjugation, described the general proteolytic functions of the system, and proposed a model according to which this modification serves as a recognition signal for a specific downstream protease. As a post- doctoral fellow with Dr. Harvey Lodish at the M.I.T., he continued his studies on the ubiquitin system and made additional important discoveries. Along the years it has become clear that ubiquitin-mediated proteolysis plays major roles in numerous cellular processes, and aberrations in the system underlie the pathogenetic mechanisms of many diseases, among them certain malignancies and neurodegenerative disorders. Consequently, the system has become an important platform for drug development. Among the numerous prizes Ciechanover received are the 2000 Albert Lasker Award, the 2002 EMET Prize, the 2003 Israel Prize, and the 2004 Nobel Prize (Chemistry; shared with Drs. Hershko and Rose). Among many academies, Ciechanover is member of the Israeli National Academy of Sciences and Humanities, The European Molecular Biology Organization (EMBO), the American Academy of Arts and Sciences (Foreign Fellow), the American Philosophical Society, the National Academies of Sciences (NAS) and Medicine (NAM) of the USA (Foreign Associate), the Pontifical Academy of Sciences at the Vatican, the Chinese Academy of Sciences (CAS; Foreign Member), the Russian Academy of Sciences (Foreign Member), and the German Academy of Sciences (Leopoldina).

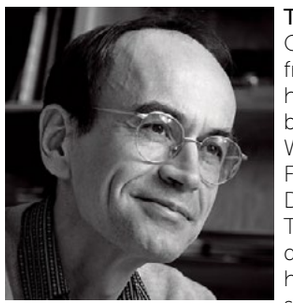


**Richard J. Roberts, PhD,** is the Chief Scientific Officer at New England Biolabs. He was educated in England, attending St. Stephen's School and the City of Bath Boys' School in Bath before moving to the University of Sheffield where he obtained a B.Sc. in Chemistry in 1965 and a Ph.D. in Organic Chemistry in 1968. His postdoctoral research was carried out in Professor J.L. Strominger's laboratory at Harvard, where he studied the tRNAs that are involved in the biosynthesis of bacterial cell walls. From 1972 to 1992, he worked at Cold Spring Harbor Laboratory, reaching the position of Assistant Director for Research under Dr. J.D. Watson. He began work on the newly discovered Type II restriction enzymes in 1972 and in the next few years more than 100 such enzymes were discovered and characterized in Dr. Roberts' laboratory. His laboratory has cloned the genes for several restriction enzymes and their cognate methylases and studies of these enzymes have been a major research theme. Dr. Roberts has also been involved in studies of Adenovirus-2 beginning with studies of transcription that led to the discovery of split genes and mRNA splicing in 1977. This was followed by efforts to deduce the DNA sequence of the Adenovirus-2 genome and a complete sequence of 35,937 nucleotides was obtained. This latter project required the extensive use of computer methods, both for the assembly of the sequence and its subsequent analysis. His laboratory pioneered the application of computers in this area and the further development





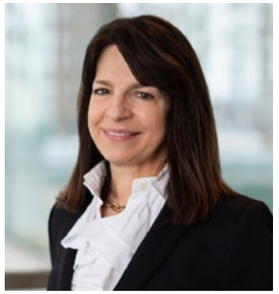
of computer methods of protein and nucleic acid sequence analysis continues to be a major research focus. The field of DNA methyltransferases is also an area of active research interest and crystal structures for the HhaI methyltransferase both alone and in complex with DNA have been obtained in collaboration with Dr. X. Cheng. The latter complex is quite remarkable as the protein causes the target cytosine base to flip completely out of the helix so that it is accessible for chemical reaction. This extreme, but elegant, distortion of the double helix had not been seen previously. A major interest at present is the semi-automatic identification of restriction enzyme and methylase genes within the GenBank database and the development of rapid methods to assay function. Already several new specificities have been found and it is clear that there are many more restriction enzyme genes in Nature than had been previously suspected. Most recently, Sir Roberts is one of the leaders of the COMBREX project that is concerned with the functional annotation of prokaryotic genomes.



**Thomas Christian Südhof, M.D., PhD**, was born in Göttingen, Germany in 1955 and obtained his M.D. and doctoral degrees from the University of Göttingen in 1982. He performed his doctoral thesis work at the Max-Planck-Institut für biophysikalische Chemie in Göttingen with Prof. Victor P. Whittaker on the biophysical structure of secretory granules. From 1983 to 1986, Südhof trained as a postdoctoral fellow with Drs. Mike Brown and Joe Goldstein at UT Southwestern in Dallas, TX, and elucidated the structure, expression and cholesterol-dependent regulation of the LDL receptor gene. Südhof began his independent career in 1986 at UT Southwestern, where he stayed until 2008 and, among others, was the founding chair of the Department of Neuroscience. In 2008, Südhof moved to Stanford, and became the Avram Goldstein Professor in the School of Medicine at Stanford University. In addition, Südhof has been an Investigator of the Howard Hughes Medical Institute since 1986. Prior to becoming a neuroscientist, Südhof was trained in the biophysics of subcellular organelles at the Max-Planck-Institut für biophysikalische Chemie and in cholesterol metabolism at UT Southwestern. When Südhof started his laboratory, he decided to switch to neuroscience to study synapses because of their central, as yet incompletely understood role in brain function. Südhof's work initially focused on the mechanism of neurotransmitter release, which is the first step in synaptic transmission that accounts for the speed and precision of information transfer in the brain. It was for this work that Südhof was awarded in 2013 the Albert Lasker Basic Medical Research Award (with Richard Scheller) and the Nobel Prize in Physiology or Medicine (with James Rothman and Randy Schekman). In the last decade, Südhof's research emphasis has switched to focus on a different unsolved problem in neuroscience that regards synapses, namely how synapses are established specifically between defined pre- and postsynaptic neurons, and how such connections are endowed with specific properties by these neurons. Addressing this fundamental question is essential for understanding how circuits are wired and how they process information, but the basic rules that govern synapse formation and specification are only now beginning to emerge. Elucidating these rules is the goal of Südhof's present work.

# ISABS 2022

# ABOUT INVITED SPEAKERS

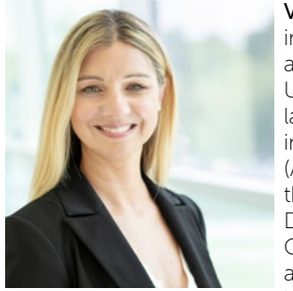


**Julie Allickson, PhD**, is the Michael S. and Mary Sue Shannon director of Mayo Clinic's Center for Regenerative Medicine and the Otto Bremer Trust director, Biomanufacturing and Product Development, Center for Regenerative Medicine and Associate Professor of Regenerative Medicine. Dr. Allickson joined Mayo Clinic from Wake Forest Institute for Regenerative Medicine at Wake Forest School of Medicine, where she was the chief manufacturing development center officer. Dr. Allickson is leading the next phase of development of the Center for Regenerative Medicine as it delivers on innovations that Cure, Connect and Transform patient care in alignment with Mayo Clinic's 2030 vision. She directs the enterprise-wide biomanufacturing strategy that aspires to introduce new regenerative therapeutics into the practice and establish Mayo Clinic as a category of one in regenerative medicine for rare and complex conditions. Dr. Allickson provides strategic leadership for all center activities and operations across Mayo Clinic. The Center for Regenerative Medicine has over 200 clinical trials and projects underway and has filed more than 150 regenerative patents. The center performs over 100,000 regenerative procedures annually. With more than 25 years of experience in clinical translation of cellular therapies and regenerative medicine products, Dr. Allickson has expertise in business management, regulatory affairs, strategic planning, project management and team-building. She has served as an executive officer of a publicly traded company that builds services for cellular banking, including licensure of technology with international affiliates.

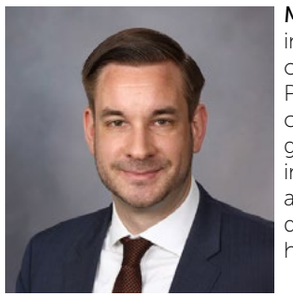
**Jack Ballantyne, PhD**, is a Professor of Chemistry at the University of Central Florida (UCF) and an Associate Director of the National Center for Forensic Science in Orlando, Florida. He possesses a B.Sc. (with Honors) in Biochemistry from the University of Glasgow, Scotland, a M.Sc. in Forensic Science from the University of Strathclyde, Scotland and a PhD in Genetics from the State University of New York at Stony Brook, NY. His current duties include teaching and conducting research in forensic molecular genetics. Prior to entering academia, he was a casework forensic scientist in Scotland, Hong Kong and New York where he proffered expert testimony in the criminal courts of these jurisdictions. He was the full time DNA technical leader in Suffolk County, New York and then served as a part-time consultant DNA technical leader for the States of Mississippi and Delaware, the City of Dallas and Sedgwick County, Kansas. He served as the the Chair of the New York State DNA Sub-committee, is a regular visiting guest at the FBI's Scientific Working Group on DNA Analysis Methods (SWGDAM) and was a member of the NIST Mixture Resource Committee and the Y-STR Interpretation Commission of the International Society of Forensic Genetics. His research interests can be classified as "getting blood from a stone: more and more probative information from less and less genetic material". Specifically, his current projects include the efficient use of Y chromosome markers for sexual assault investigations, RNA profiling and genotyping for body fluid and tissue identification and association with a DNA profile, and single-cell/low copy number analysis including DNA mixture deconvolution.



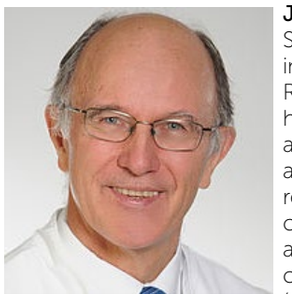




**Veronique Belzil PhD**, graduated with a Bachelor's degree in Psychology from McGill University, Canada, in 2003, and a Master's degree in Psychology from Walden University, USA, in 2007. She then received her graduate training in the laboratory of Dr. Guy A. Rouleau—one of the foremost leaders in human medical genetics and amyotrophic lateral sclerosis (ALS) research—and received her Ph.D. in Neuroscience from the University of Montreal, Canada, in 2012. After graduating, Dr. Belzil started her postdoctoral research training at the Mayo Clinic in Florida under the supervision of Leonard Petrucelli, a world leader in neurodegeneration research, where she initiated and developed the genetic/epigenetic program she is now independently leading. Her laboratory has the mission of developing patient-centered approaches to treating amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD)-related dementias (ADRDs), including the most common ADRD, frontotemporal dementia (FTD). Her group uses human biospecimens to systematically identify disease-specific variants in biofluids, bulk tissues, sorted cell populations, and at single-cell resolution.



**Moritz Binder M.D., M.P.H**, is an Assistant Professor of Medicine in Mayo Clinic's Division of Hematology and Assistant Director of Mayo Clinic's Center for Individualized Medicine Epigenomics Program. His work involves the integration and analysis of (single-cell) multi-omics data generated from cancer transcriptomes, genomes, and epigenomes. His focus in computational biology includes the application of different modeling strategies such as statistical learning approaches to improve therapeutic target discovery, early disease detection, and risk stratification in hematologic malignancies.



**Johannes Brachmann, M.D., PhD**, graduated in 1979 at Medical School, University of Heidelberg in Germany. Dr Brachmann in 1980 received a research fellowship of the DFG (German Research Foundatio) at the University of Oklahoma, USA where he studied on arrhythmia mechanisms in myocardial infarction and proarrhythmia in collaboration with Prof. Ralph Lazzara and Prof. Benjamin Scherlag. From 1981 to 1988 he did his residency and fellowship in cardiology at the Department of cardiology at University of Heidelberg. Since 1985, he has been active participant and principal investigator of more than 50 clinical studies mostly in the area of cardiac electrophysiology (antiarrhythmics, ICD, PM) and interventional cardiology (stents, atherectomy). Since 1998, he is working as Chief of Cardiology in Klinikum Coburg in Germany and from 2003 as Professor of Medicine at University of Würzburg. Furthermore, from 2016, Dr Johannes Brachmann is a Director of REGIOMED Centrum for Cardiology and Angiology and Leader of the Medical Boards REGIOMED-KLINIKEN and Chief physician II at Clinic cardiology, angiology, pneumology and also works as full professor at University of Split. He published more than 400 peer-reviewed articles, numerous book chapters, published abstracts, editorships. He is member of various leadership positions and committees of the German cardiac society, European society of cardiology, and Heart Rhythm Society.

**Joachim Burger PhD**, is Professor of Anthropology at the Johannes Gutenberg University in Mainz and heads the "Palaeogenetics Group" (https://palaeogenetics-mainz.de). Burger is a pioneer in the field of prehistoric human population genetics. Burger's team demonstrated that the first farmers were not the descendants of the hunter-gatherer population that previously lived in Europe (Bramanti et al. 2009). His team established ancient Aegeans as a robust genomic proxy for the ancestors of early European farmers (Hofmanová et al. 2016). In the same year, his team published the first Neolithic genome from the Near East providing evidence that the earliest agriculturists of Iran were not direct ancestors of Neolithic humans of Anatolia and Europe. In 2022, he and Swiss colleagues presented a demographic model for the origins of the Neolithic humans in Anatolia and Europe, showing that the earliest Neolithic people only emerged through the mixing of different Ice Age forager groups. In addition to his work on human demography, Burger has also conducted pioneering research on episodes of Darwinian selection in prehistoric humans (Burger et al. 2020) and on animal domestication during the Neolithic period in the Near East and Europe.

**Angel Carracedo, M.D., PhD**, is a Professor of Legal Medicine (University of Santiago de Compostela), Director of the Galician Foundation of Genomic Medicine (Galician Service of Health), Director of the Genomics and System Biology Group (CIMUS and IDIS), Director of the Genomic Medicine Group (USC), Director of Translational Medicine group and member board of CIBERER (Network Center for Rare disease research). President of Kaertor Foundation, President of INGADA Foundation, Ex-director of the Institute of Forensic Science, Past-President of the International Society for Forensic Genetics and the President of the International Academy of Legal Medicine. His group leads scientific production in the SCI area of legal and forensic science worldwide (http://sciencewatch.com/ana/fea/11julaugFea). Director of the Institute of Legal Medicine (USC) from 1995 to 2015. From 2002 he has been working also in the clinical genetics area where he has set up the Galician Foundation of Genomic Medicine (current director). This center is carrying out most of the molecular genetics and cytogenetic analysis requested by the Galician system of Health, covering a population of 3.5 million inhabitants and being one of the most important public genetic services in Spain. Most of AC recent research is now mainly concentrated in the genetics of mendelian and complex traits and personalized medicine and he is coordinating the IMPACT-Genomics project (the Spanish National Initiative for personalized medicine) and the mirror group of the 1+M Genome Initiative and he has participated in more than 20 international projects (coordinating 7 of them. AC has published 8 books and more than 800 papers in SCI journals (HI 100, and around 50,000 cites). Former president of different scientific societies on Cancer, Pharmacogenomics and Forensic Science. Current president of the International Academy of Legal Medicine. Member of the Forensic advisory board of the ICRC and the International Criminal Court.





**John "Al" Copland M.D., PhD**, is Professor of Cancer Biology in the Department of Cancer Biology at Mayo Clinic in Florida. Dr. Copland received his Ph.D. in Physiology and Endocrinology from the Medical College of Georgia. He completed his post-doctoral fellowship at the University of Texas Medical Branch (UTMB) and became a faculty member in the Endocrine Division of Internal Medicine Department at UTMB. He was recruited to Mayo Clinic, Jacksonville FL in 2003 as one of the founding members of the Cancer Biology Department. His research interests revolve around discovery of novel cancer genes and signaling pathways involved in cancer tumorigenesis and progression using genomic and functional genomic technologies that interrogate patient tumor tissues and preclinical models. The Copland lab has developed over 80 patient tumor-derived cell lines and 120 patient derived xenograft (PDX) mouse models.2, 3 PDX models are highly predictive of patient response to therapy and a gold standard to date for testing novel therapies. The major goal is to understand the genetic and molecular underpinnings of the biology of cancer in order to develop targeted lifesaving therapies. Additionally, Dr. Copland is a member of the Developmental Therapeutics Group in the NCI Designated Mayo Clinic Comprehensive Cancer Center. In collaboration with Dr. Tamas Ordog, he has been working towards an understanding of an intriguing discovery related to FOXO3, acting as an oncogenic transcriptional factor (TF) in anaplastic thyroid cancer (ATC), the deadliest of cancers.5 FOXO3 is well-known for its tumor suppressor activity in regulating genes that antagonize tumor growth and survival.

**Julie M. Cunningham, PhD,** was born in England, moving to Australia after first grade. Since 2004 she is a collaborative scientist and a director of the Genomic Analysis Core facility at the Mayo Clinic in Rochester, MN, USA. A fascinating job really, while pursuing her research interests of genomic aspects of cancer, as a core lab director she works with a wide variety of investigators whose disciplines further her knowledge, surely a good thing for a scientist.

**Henry Erlich, PhD,** is currently Emeritus at the Benioff UCSF Children's Hospital Oakland Research Institute. His laboratory has been involved for over 35 years in the development and application of molecular genetic technology such as PCR and DNA-based HLA typing for the analysis of infectious diseases and autoimmune diseases. He has also focused on the development of diagnostic tests for monogenic disease as well as the application of PCR in forensics genetics, performing the first forensic DNA case in the US in 1986 (Penn. Vs. Pestinikas) and the first post-conviction review. His lab has applied HLA NGS systems to the analysis of cell free DNA in plasma for detecting donor DNA in recipient plasma for early detection of graft rejection. The lab has applied probe capture and NGS to analyze forensics mixtures with mitochondrial DNA, SNP, and STR markers and for analyzing fetal DNA in the maternal plasma for developing a non-invasive prenatal test for the hemoglobinopathies.

**William A. Faubion, M.D., PhD,** is Professor of Internal Medicine, Pediatrics, and Immunology at Mayo Clinic. He has served as Chairs for Research of the Division of Gastroenterology and the Dept of Medicine prior to his most recent role as the Associate Medical Director of the Center of Regenerative Medicine. His interests are mucosal immunology and Inflammatory Bowel Disease.

**Mark A. Frye, M.D., PhD,** is a Consultant in the Department of Psychiatry & Psychology at Mayo Clinic. He is the past Chair of the Department of Psychiatry & Psychology (2010-2020) and is recognized with the distinction of the Stephen and Shelley Jackson Family Professorship in Individualized Medicine. Dr. Frye received his M.D. from the University of Minnesota and completed his psychiatric training at the Semel Institute for Neuroscience and Human Behavior at the David Geffen School of Medicine at UCLA. He subsequently completed a fellowship at the National Institute of Mental Health in Bethesda, Maryland with a research focus on the neurobiology of treatment resistant depression and bipolar disorder. Dr. Frye's current research centers on biomarkers of mood disorders and alcoholism complementing his international clinical expertise in mood disorders and addiction. Dr. Frye and his team established the Mayo Clinic Individualized Medicine Biobank for Bipolar Disorder to identify the underpinning mechanisms of bipolar disorder through genomic studies. An active clinical investigator, he has received research support from NIMH, NIAAA, Mayo Foundation, Brain & Behavior Research Foundation, and industry partners, and has published more than 400 peer-reviewed papers. Dr. Frye is the President of the National Network of Depression Centers (NNDC), a network of 23 academic centers of excellence collaborating in clinical innovation, research, and education, and the Scientific Advisory Board Vice-Chair of the Depression Bipolar Support Alliance (DBSA), a leading national organization mission driven to improve the lives of people who live with mood disorders.

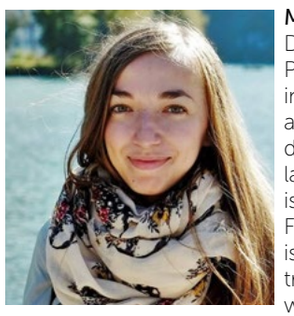
**Alexandre Gaspar Maia**, **PhD,** leads Mayo Clinic's Functional Epigenomics Laboratory, where he combines advanced sequencing techniques to profile the epigenomic landscape at a single-cell level, mostly focusing on enhancer elements, or enhancers. Dr. Maia has worked extensively in epigenetic regulation of stem cells, cellular reprogramming and cancer, and is now particularly interested in using epigenomic profiling of enhancer elements to identify biomarkers of sensitivity to poly (ADP ribose) polymerase (PARP)-inhibitors in the context of ovarian and breast cancers. Dr. Maia's long-term goal is to understand how enhancers drive resistance to drug treatment in cancer and to dissect the mechanisms by which those enhancers are activated and silenced during cancer transformation. The identification of biomarkers for drug resistance to determine which patients will be responsive to which treatments will be a great step forward in cancer treatment. Dr. Maia aims to develop the technology to detect such biomarkers using liquid biopsy, which will greatly increase applications in the clinical setting.





**Struan F. A. Grant, PhD,** has been conducting human genomics research for over 20 years. The highlights of my career are the discovery of the polymorphic Sp1 site in the COL1A1 gene and its association with osteoporosis, the identification of variation in the TCF7L2 gene playing a key role in conferring type 2 diabetes risk and providing leadership in an international genetics effort to characterize genes influencing birth weight and common childhood obesity risk. I have also previously played a role in uncovering genes involved in other traits, including cleft lip with or without palate, scoliosis, inflammatory bowel disease, autism, ADHD, head circumference, intracranial volume, myocardial infarction, pediatric eosinophilic esophagitis, type 1 diabetes, asthma, multiple sclerosis and neuroblastoma. As a Director of the Center for Spatial and Functional Genomics at the Children's Hospital of Philadelphia, my current work primarily involves investigating disease genomics with a specific focus on pediatrics. Utilizing high-throughput genotyping and sequencing technologies, combined with statistical and bioinformatic approaches, my goals include unraveling genomic puzzles related to childhood obesity, pediatric bone strength determination, early onset diabetes and cancer.

After graduating on 'Ancient DNA from Europe's first farmers' in 2006, **Wolfgang Haak PhD,** spent his Postdoc years working on National Geographic's 'The Genographic Project' (2007-2011) and as 'Ancient human DNA' Group leader at the Australian Centre for Ancient DNA in Adelaide, Australia (2010-2015). Since 2015 he is leading the 'Molecular Anthropology' group at the Max-Planck-Institute for the Science of Human History in Jena, Germany. His work is positioned at the interface of human populations genetics, archaeology, anthropology, medical sciences, and linguistics. The main aim of his group is to investigate and evaluate ancient human genome-wide data in the light of contextual information from neighboring disciplines, such as archaeology and anthropology, in order to generate a detailed and comprehensive portrait of human prehistory over the last 20,000 years. The project portfolio ranges from global outlooks on population affinities, past migrations and demography to intra-group relationships and kinship-reconstruction, and also encompasses the interaction with, and response to, changing environmental factors, such as climate, diet and diseases.



**Mateja Hajdinjak, PhD,** is a molecular biologist using ancient DNA to study human evolutionary history. She completed her PhD at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, under supervision of Dr. Matthias Meyer and Prof. Svante Pääbo, recovering and analysing genome-wide data and piecing together population history of some of the last Neandertals and earliest modern humans in Eurasia. She is currently a Marie Skłodowska Curie Individual Fellow at the Francis Crick Institute in London, United Kingdom, where she is continuing her work on human evolutionary genomics and tracing origins of modern human ancestry using ancient DNA, with a special focus on ancient hunter gatherer groups in Africa and west Eurasia.

**Mitchell Holland, PhD,** is a Fellow in the American Academy of Forensic Sciences, and has served as an associate professorial lecturer and adjunct faculty member at various colleges and universities. Dr. Holland has been on the Editorial Board of the Journal of Forensic Sciences and a member of the Advisory Board of the International Journal of Legal Medicine. Prior to being asked in early 2005 to help develop the Forensic Science Program at Penn State, Dr. Holland was the Senior Vice President of Operations and Laboratory Director of The Bode Technology Group. At Bode, Dr. Holland led the efforts to produce DNA profiles from victim remains recovered from Ground Zero (World Trade Centers) following the terrorist attacks of 9/11. His group is currently leveraging the power of massively parallel sequencing (MPS) to measure rates of mtDNA heteroplasmy in different population groups; evaluate the transmission of heteroplasmic variants between maternal relatives and tissue types; assess the impact of damage on the interpretation of low-level heteroplasmic variants; and develop best practices for the application of MPS approaches in forensic casework. In addition, members of Dr. Holland's group are exploring ways to extract small fragments of DNA from highly degraded skeletal material for STR and SNP analysis on an MPS platform.

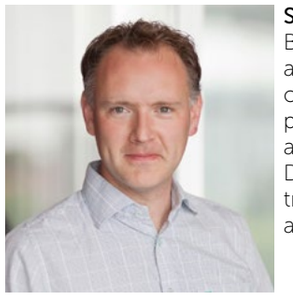
**Haojie Huang, PhD,** is the Gordon H. and Violet Bartels Professor of Cellular Biology and Professor of Biochemistry and Molecular Biology and Urology at Mayo Clinic. He is also the Director for Urology Basic Sciences Research and the Associate Director of the Epigenomics Program at the Center for Individualized Medicine (CIM) at Mayo Clinic. Dr. Huang's research has been focusing on genetic alteration, transcription regulation, epigenetic reprogramming, cell lineage plasticity, and therapy resistance in cancers, especially in prostate cancer. Dr. Huang have published over 140 peer-reviewed papers, some of which are in high profile journals such as Science, Nature Medicine, Cancer Cell, Nature Cell Biology, Molecular Cell, etc. Currently, Dr. Huang is the President of the Society for Basic Urologic Research (SBUR) and serves on the Editorial Board of the AACR flag journal Cancer Research and as an Associate Editor for Cancer Research Communications and an Editorial Advisor for Asian Journal of Urology.

**Jodi Irwin** joined the FBI Laboratory as a Research Scientist in 2012. She supports the lab's DNA testing operations by developing and validating methods for near-term implementation into forensic casework. Her focus is primarily on optimizing and streamlining mitochondrial DNA workflows and developing methods to advance the FBI Laboratory's NGS initiatives. Prior to joining the FBI, she spent 14 years at the Armed Forces DNA Identification Laboratory where she developed software applications for database searching and kinship statistics and explored novel technologies to expand the AFDIL's DNA testing capabilities. Her early career was spent in a number of academic laboratories focused on evolutionary genetics. She has a bachelor's degree from the University of Notre Dame, a master's from San Francisco State University and a doctorate from the University of Innsbruck.

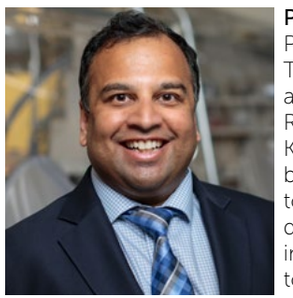




**Mattias Jakobsson, PhD,** has a broad interest in population genetics and human evolution. His lab uses computational approaches for deciphering complex patterns of large-scale human genomic variation from both modern-day and ancient humans in order to understand human evolutionary history. The lab focus on interrogating long-standing questions in human evolution, including the colonization and migration in Stone Age Eurasia and the population history of sub-Saharan Africans.



**Steven A. Johnsen, PhD,** is the Scientific Director of the Robert Bosch Center for Tumor Diseases in Stuttgart, Germany. Before assuming this position in February 2022, he was a Full Professor of Medicine and Pharmacology at the Mayo Clinic. He also previous held faculty positions at the University of Göttingen and the University Medical Center Hamburg- Eppendorf. Dr. Johnsen's group studies the molecular epigenetic and transcriptional mechanisms controlling cell fate determination and adaptive resistance.



**Purna Kashyap, M.D., PhD,** is Professor of Medicine and Physiology, Co- Director of the Microbiome and High-Definition Therapeutics program in the Center for Individualized Medicine and Director of the germ-free mouse facility at Mayo Clinic, Rochester, MN. The Gut Microbiome laboratory led by Dr. Kashyap is interested in understanding the complex interactions between diet, gut microbiome and host physiology and strives to move the field beyond associations of microbiome with different diseases to defining the functional role of gut microbes in regulating host physiology. The overall goal of the program is to develop novel microbiota-targeted therapeutic agents such as genetically engineered microbes that will restore altered microbial functions in diseases such as Irritable Bowel Syndrome. His research is driven primarily by his clinical practice which is focused on patients with gastrointestinal motility disorders. Dr. Kashyap has published over 80 peer reviewed articles including journals like Cell, Cell Host Microbe, Science Translational Medicine, Nature Communications, and Gastroenterology. He was inducted to American Society of Clinical Investigation in 2021. He has previously served on the scientific advisory board of American Gastroenterology Association Gut Microbiome Center, and on the council of American Neurogastroenterology and Motility Society. He now serves on the council and the research committee of American Gastroenterology Association, in editorial roles for Gut Microbes and Neurogastroenterology and Motility journal and as an ad-hoc reviewer on NIH study sections.

Born in Baku, Azerbaijan, in the Soviet Union in 1963, **Garry Kasparov** came to international fame at the age of 22 as the youngest world chess champion in history in 1985. Kasparov's famous matches against the IBM super-computer Deep Blue in 1996-97 were key to bringing artificial intelligence, and chess, into the mainstream. They also sparked his interest in AI and the human relationship with our increasingly intelligent machines. He retired from professional chess in 2005 to form the pro-democracy opposition in Russia. In 2012, Kasparov was named chairman of the New York-based Human Rights Foundation. In 2016, he was named a Security Ambassador by Avast Software, where he discusses cybersecurity and the digital future, and to the executive board of the Foundation for Responsible Robotics. In 2017, he founded the Renew Democracy Initiative, dedicated to advocating the principles of the free world. The US-based Kasparov Chess Foundation non-profit promotes the teaching of chess in education systems around the world. Since 1990, Kasparov has been a regular contributor to many major publications, including The Wall Street Journal, The Washington Post, CNN, and The New York Daily News. He speaks frequently to business audiences around the world on strategy, decision-making, politics, and artificial intelligence. Kasparov's book How Life Imitates Chess on strategy and decision-making is available in over 20 languages. His prescient book, Winter Is Coming: Why Vladimir Putin and the Enemies of the Free World Must Be Stopped, was released in 2015. His latest book on artificial intelligence and the human-machine relationship is Deep Thinking: Where Machine Intelligence Ends and Human Creativity Begins (2017).

**Adrijana Kekic, PhD, PharmD, BCACP** (Board Certified Ambulatory Care Pharmacist), is a Pharmacogenomics Clinical Pharmacy Specialist, Assistant Program Director (APD) for Community-Based Pharmacy Residency, and APD for Outpatient Pharmacy Education at Mayo Clinic in Arizona (MCA). As a pharmacogenomics clinician, researcher, and educator she leads implementation of pharmacogenomics services across the Mayo Clinic. She is the current Secretary for Pharmacogenomics (PGx) Task Force and Co-chair for Pharmacy Research Council. Her research work includes pharmacogenomics studies in anesthesia, transplant, oncology, palliative care, cardiology, and large-scale whole exome sequencing study for pre-emptive medication monitoring. Currently pursuing Master of Healthcare Innovation degree with focus on precision medicine, Dr.Kekic is especially interested in developing multiomics clinical decision platforms, digital tools for greater health equality, and building interactive platforms for science communication. She lectures extensively on pharmacogenomics with niche in psychopharmacology. With almost two decades of pharmacy leadership and clinical expertise in individualized medication therapy management, Adrijana continues to advance pharmacy practice at the intersect of precision medicine and digital health. She is a founder of a networking platform dedicated to high impact professional women in healthcare.





**Manolis Kellis, PhD,** is a Professor of Computer Science at MIT, an Institute Member of the Broad Institute of MIT and Harvard, a member of the Computer Science and Artificial Intelligence Lab at MIT, and head of the MIT Computational Biology Group (compbio.mit.edu). His research spans an unusually broad spectrum of areas, including disease genetics, epigenomics, gene circuitry, non-coding RNAs, comparative genomics, and phylogenetics. He has helped direct several large-scale genomics projects, including the Roadmap Epigenomics project, the ENCODE project, the Roadmap Epigenomics Project, the Genotype Tissue-Expression (GTEx) project, and comparative genomics projects in mammals, flies, and yeast. He received the US Presidential Early Career Award in Science and Engineering (PECASE) by US President Barack Obama, the NSF CAREER award, the Alfred P. Sloan Fellowship, the Technology Review TR35 recognition, the AIT Niki Award, and the Sprowls award for the best Ph.D. thesis in computer science at MIT. He has authored more than 150 journal publications, which have been cited more than 47,000 times. He lived in Greece and France before moving to the US, and he studied and conducted research at MIT, the Xerox Palo Alto Research Center, and the Cold Spring Harbor Lab.

**Janet Kelso, PhD,** leads the Bioinformatics research group at the Max-Planck Institute for Evolutionary Anthropology in Leipzig, Germany. Her research focusses on the analysis of ancient genomes, particularly the genomes of archaic humans. In particular, her group is interested in the development of novel computational approaches for the analysis of ancient DNA, and in using these approaches to gain insights into genome evolution. Janet received her PhD in bioinformatics from the South African National Bioinformatics Institute at the University of the Western Cape under the supervision of Professor Winston Hide. She is the co-Editor-in-chief of the journal Bioinformatics together with Alfonso Valencia, as well as an Executive Editor of the journal Database. For many years Janet has been an active member of the Board of the International Society of Computational Biology, and she was named a Fellow of the Society in 2015.

**Johannes Krause, PhD,** earned his Ph.D. in Genetics at Leipzig University. He was appointed junior professor for Paleogenetics at the University of Tübingen in 2010, and subsequently full professor for Archaeo- and Paleogenetics at the same university in 2013. In 2014, he became founding director of the Max Planck Institute for the Science of Human History in Jena, heading the Department of Archaeogenetics. In 2018 he became full professor at the Friedrich Schiller University Jena. He is one of the founding directors of the Max Planck-Harvard Research Center for the Archaeoscience of the Ancient Mediterranean (MHAAM), established in 2017. In 2020 he was reappointed to the Max Planck Institute for Evolutionary Anthropology and his department moved to Leipzig. Prof. Dr. Krause focuses on the analysis of ancient DNA to investigate such topics as pathogens from historic and prehistoric epidemics, human genetic history and human evolution. He contributed substantially to deciphering the Neanderthal genome and the shared genetic heritage of Neanderthals and modern humans. In 2010, while working at the Max Planck Institute for Evolutionary Anthropology

in Leipzig, he discovered the first genetic evidence of the Denisovans, an extinct hominin discovered in Siberia. His recent work includes revealing the genetic heritage of ancient Egyptians, reconstructing the first Pleistocene African genomes, uncovering the source of the epidemic plague bacteria that periodically caused historic and prehistoric epidemics in Europe, or clarifying the complex history of Europe's prehistoric mass migrations.

**Greger Larson, PhD,** received his bachelor's degree in 1996 from Claremont McKenna College, a small liberal arts college in California. He read just about everything Stephen J Gould ever wrote over the following three years while he wandered the deserts of Turkmenistan and worked for an environmental consultancy in Azerbaijan. Deciding that evolution was cooler than oil, Greger studied at Oxford and the University of Colorado before receiving his PhD in Zoology in 2006. He then spent two years in Uppsala, Sweden on an EMBO postdoctoral fellowship before starting a job in the department of archaeology at Durham University. Greger then moved to Oxford University to become the Director of the Palaeogenomics & Bio-Archaeology Research Network Greger where he is continuing his focus on the use of ancient DNA to study the pattern and process of domestication. He rarely wonders what his salary would be had he stuck to oil.

**Gordan Lauc, PhD,** is the Professor of Biochemistry and Molecular Biology at the University of Zagreb, Director of the National Centre of Scientific Excellence in Personalised Healthcare, and founder and CEO of Genos Glycoscience Research Laboratory. He is also honorary professor at the University of Edinburgh and the Kings College London, member of the Johns Hopkins Society of Scholars and co-Director of the Human Glycome Project. His research team is pioneering high throughput glycomic analysis and the application of glycan biomarkers in the field of precision medicine. By combining glycomic data with extensive genetic, epigenetic, biochemical and physiological data in a systems biology approach they are trying to understand the role of glycans in normal physiology and disease. Professor Lauc co-authoredover 200 research articles that are cited over 3,500 times. He participated in two NIH, two FP6, seven FP7, six H2020, and two ESI Funds projects and coordinated three of them. In 2007 he founded Genos, a biotech company that is currently global leader in high-throughput glycomics.

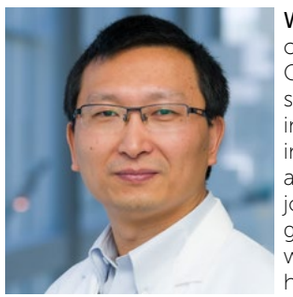
**Konstantinos N. Lazaridis, M.D.,** serves as the Carlson and Nelson Endowed Executive Director, Mayo Clinic Center for Individualized Medicine. Dr. Lazaridis is a Professor of Medicine, Mayo Clinic College of Medicine and Science and a Consultant in the Division of Gastroenterology and Hepatology, Department of Internal Medicine at Mayo Clinic, with a joint appointment in the Department of Clinical Genomics. He joined the staff of Mayo Clinic in 2000. He received his medical degree at the University of Ioannina in Greece. He completed his internal medicine and gastroenterology fellowship training at Mayo Clinic and was a Mayo Foundation Scholar in Genomics in the laboratory of Dr. Francis Collins, director of the National Human





Genome Research Institute at the National Institutes of Health. Dr. Lazaridis is considered an international leader in the area of chronic cholestatic liver diseases, namely, primary sclerosing cholangitis and primary biliary cholangitis. Since 2003, he has established and is the principal investigator of the two NIH funded, national consortia for studying patients afflicted with these diseases. His research group applies the latest genomic and exposomic approaches to better understand the pathogenesis and outcomes of patients with chronic cholestatic liver diseases as well as improve their therapy. He was elected to the American Society of Clinical Investigation in 2015.

**Zhenkun Lou, PhD,** is a Professor of Pharmacology at the Mayo Clinic College of Medicine and Science, Rochester, MN, co-leader of the Experimental Therapeutics Program at the Mayo Clinic Cancer Center, Rochester, MN and a consultant at the Division of Oncology Research, Department of Oncology, Mayo Clinic, Rochester, MN Professor of Pharmacology, MN. Since 2022 he is also the chair of the Division of Oncology Research. His lab focuses on signaling pathways that are activated by DNA damage-inducing radiation and chemotherapy DNA damage activates a signaling cascade called the DNA damage response (DDR) pathway that initiates DNA repair and cell cycle checkpoint activation. Understanding this pathway will elucidate the cause of genomic instability, a driving force of tumorigenesis. Several genetic syndromes have also been linked to mutations of genes in this pathway, such as ATM mutation in Ataxia-Telangiectasia (AT) syndrome. My lab has been studying the DNA repair pathway for 20 years and have made unique contributions to the field.



**Weibo Luo, PhD,** is an Assistant Professor in the Departments of Pathology and Pharmacology at UT Southwestern Medical Center (UTSW) since December 2014. He received his Ph.D. summa cum laude from the University of Magdeburg, Germany in 2007, and completed his postdoctoral fellowship and instructorship with 2019 Nobel Laureate Dr. Gregg Semenza at the Johns Hopkins University School of Medicine before joining UTSW. Dr. Luo studies oxygen homeostasis in tumor growth and metastasis at the molecular and cellular levels, with a particular focus on the master regulators of oxygen homeostasis named hypoxia-inducible factors (HIFs). He utilizes the multidisciplinary approaches to study crosstalk of HIF, epigenetics, and metabolism and their roles in tumor growth and metastasis. His overall goals are to identify the novel hypoxia-dependent therapeutic vulnerabilities and ultimately to translate knowledge to cancer therapy.

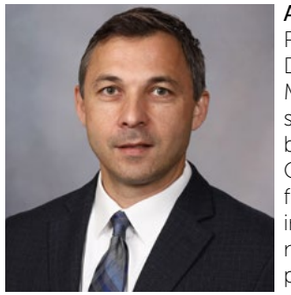
**Tomislav Maricic, PhD,** is a staff scientist in the genetics department of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. He was involved in developing methods for the generation of the first Neandertal genome sequence. Using the Neandertal genome scientists were able to define all positions in the human genome where all modern humans are different from Neandertals and apes. Those positions could be called a genetic recipe for being a modern human, and their function is largely unknown. Tomislav is interested in characterizing those positions and to achieve that, he introduces Neandertal mutations in human pluripotent stem cells using CRISPR editing.

**Ulrika Marklund, PhD,** is a principal investigator at the Department for Medical Biochemistry and Biophysics at Karolinska Institutet in Stockholm, Sweden. Her research is focused on neural cell diversity in the Enteric Nervous System (ENS) of the gastrointestinal (GI) tract. Ulrika gained her Master Degree in Molecular Biology from Umeå University in 2002, and then moved to Stockholm for the PhD work at Karolinska Institutet. Her thesis focused on the molecular control of cell identity and neurogenesis in the developing central nervous system, with most noticeable achievements on dopamine neuron specification. She thereafter switched field to the peripheral nervous system and gained expertise in ENS development as a postdoctoral fellow (2009/2010) in the lab of Prof. Pachnis at National Institute for Medical Research, London, UK. Ulrika established an independent team at Karolinska Institutet in 2013, and has since then used transcriptome techniques (single cell RNA-sequencing, microarrays) to classify enteric neural subtypes and determine developmental principles for their diversification. She has recently broadened the scope of research to enteric neural networks and their role in gut disorders. An ultimate goal is to recapitulate cell fate determination and circuit formation in the purpose of disease modeling and cell-based therapy of GI neuropathology.

**Charla Marshall, PhD,** is Chief of the Emerging Technologies Section at the Armed Forces Medical Examiner System's Armed Forces DNA Identification Laboratory (AFMES-AFDIL). She is a molecular anthropologist by training and has a background in ancient DNA. Dr. Marshall's group develops and validates new MPS/NGS methods to improve DNA-assisted identification of missing US service members. Current efforts are focused on SNP-based extended kinship analyses and mitochondrial genome sequencing, both for degraded DNA and reference quality samples. Other active areas of research are DNA extraction and library preparation for improved DNA recovery. Aside from her work at the AFMES-AFDIL, Dr. Marshall is a member of the FBI's SWGDAM NGS Committee, an editorial board member of FSI:Genetics, and an adjunct professor at the Pennsylvania State University.







**Aleksey Matveyenko, PhD,** is a Professor in the Department of Physiology and Biomedical Engineering and Endocrinology and Diabetes at the Mayo Clinic College of Medicine in Rochester Minnesota, USA. Dr. Matveyenko completed his doctoral studies at the University of Southern California, (USC), followed by postdoctoral fellowship in islet biology at the University of California, Los Angeles, (UCLA). He subsequently served on faculty at UCLA followed by transition to Mayo Clinic. The work in Matveyenko laboratory has long been focused on identifying molecular and physiological mechanisms underlying loss of pancreatic beta cell function and mass in diabetes, with an emphasis on gene environment interactions. Specifically, recent research focus is on the role of circadian clocks and circadian system as novel regulators of beta-cell secretory function, survival and regeneration. To accomplish these research goals the laboratory uses an integrative approach ranging from cellular, molecular, and biochemical studies to in vivo physiological models.

**Ian Maze, PhD,** completed his B.S. in Microbiology at The Ohio State University and his Ph.D. in Neuroscience at the Mount Sinai School of Medicine under the mentorship of Dr. Eric Nestler, M.D., Ph.D. He then completed his Postdoctoral Studies at the Rockefeller University under the mentorship of Dr. C. David Allis, Ph.D. before joining the faculty at the Icahn School of Medicine at Mount Sinai (ISMMS) as a Tenure-Track Assistant Professor in the Department of Pharmacology and Systems Therapeutics in 2014. Dr. Maze is currently a Howard Hughes Medical Institute Investigator and Professor of Neuroscience and Pharmacological Sciences at the ISMMS and is Director of Mount Sinai's Center for Neural Epigenome Engineering. Dr. Maze's research program is focused on investigations of chromatin regulatory mechanisms controlling neurodevelopment and disease, with an extended focus on novel roles for monoamine neurotransmitters in the direct regulation of gene expression and synaptic signaling in brain.

**Mait Metspalu, PhD,** studied geography and molecular biology and evolution at the University of Tartu where he also defended his PhD on phylogeography of human mtDNA in South Asia in 2006. In 2012-2013 Mait was a visiting research fellow in UC Berkeley. His research concentrates on using and developing population genetics approaches to understand the genesis of the genetic diversity patterns of humans through reconstructions of past population movements, splits and admixtures as well as adaptations to local environments (both natural and manmade). During the few past years Mait has also started a dedicated ancient DNA program aiming mostly at reconstructing population changes in the East European Plain since the Paleolithic. He became the vice-director (2010) and subsequently the director (2014) of the Estonian Biocentre, the leading research institute in Estonia in the interdisciplinary and interconnected fields of evolutionary genomics, population genetics and archaeogenetics. In 2018 EBC merged with Estonian Genome Center, which houses the population based Estonian Biobank containing genetic and health data for a cohort of 200 000. Mait became the director of the new Institute of Genomics, University of Tartu in 2018. As the head of

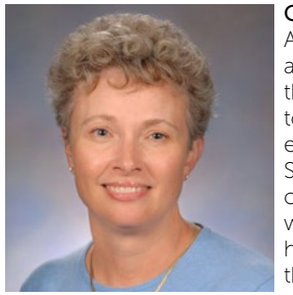
IGUT Mait is involved in facilitating the transfer of knowledge of genetic risks for diseases into the medical system in Estonia.

**Matthias Meyer, PhD,** is a biochemist, head of the 'Advanced DNA sequencing techniques' group at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, and co-head of the Ancient DNA Core Unit. His research mainly focuses on the development of methods that improve the recovery of trace amounts of DNA from ancient biological material and their application to questions surrounding human evolution and prehistory. Methods developed in his group have led to the reconstruction of the first high-quality genome from ancient DNA, which was retrieved from the finger bone of a Denisovan individual, a member of an extinct archaic human group. They also enabled the recovery of DNA sequences from the ~430,000 year-old Sima de los Huesos hominins, the oldest human DNA sequences known to date, and the isolation of trace amounts of ancient human DNA from cave sediments. His methods have also found applications in biomedical research, for example in the analysis of DNA from formalin-fixed biopsy samples or cell-free DNA.

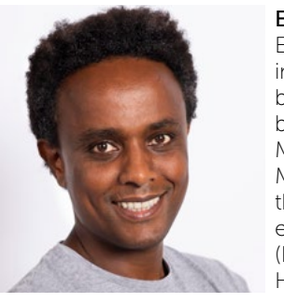
**Eva Morava-Kozicz, M.D., PhD,** did her specialty trainings in Europe and also in the US, as pediatrician, geneticist and metabolic specialist. Her major clinical expertise is inborn errors of metabolism (IEM). She has decades of experience in the diagnostics, follow-up and treatment in IEM, especially in congenital disorders of glycosylation (CDG) and mitochondrial disorders. She is on the Minnesota newborn screening committee. She is council member of the Society for the Study on Inborn Errors of Metabolism (SSIEM). She is actively involved in developing novel therapies in genetic disorders. Currently she focusses on clinical trials in IEM. She is also the main PI of the U54 FCDGC consortium studying congenital disorders of glycosylation. CDG consists of more than 140 different genetic disorders, some of these counting only 4-10 patients in the world. A strong patient association, committed clinicians and a devoted group of scientists have formed the FCDGC consortium to improve patient outcomes. On this foundation, Dr Morava and her team established a nationwide network of regional centers to develop treatment in these individually ultra-rare conditions, and meet currently unmet patient needs. FCDGC's mission is to improve clinical symptoms as well as improve quality of life and life expectancy of individuals with congenital disorders of glycosylation through advancing and sharing knowledge, developing and validating new diagnostic tools, and develop therapies to restore appropriate glycosylation.



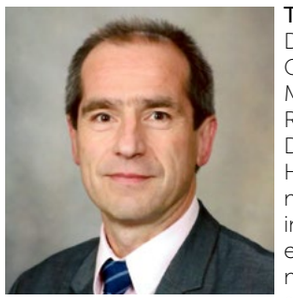




**Connie Mulligan, PhD,** is a Professor in the Department of Anthropology and faculty member of the Genetics Institute at the University of Florida. She is interested in understanding the genetic and psychosocial factors that influence response to stress, and is particularly focused on stress-associated epigenetic changes that may appear future generations. Specifically, Dr. Mulligan and her team investigate how stress or trauma that is experienced during pregnancy may associate with epigenetic changes in the mother and future offspring. She has two ongoing projects: 1) new mothers and their infants in the Democratic Republic of Congo and 2) three-generation refugee families from Syria with different war exposures.



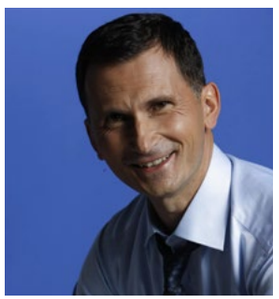
**Eskeatnaf Mulugeta, BPharm, PhD,** is an assistant professor at Erasmus University Medical Center Rotterdam (Erasmus MC) in The Netherlands. He has a multi-disciplinary educational background with a Bachelor's degree in Pharmacy, followed by 3 Master's degrees in Biotechnology, Bioinformatics, and Molecular Medicine. He performed his PhD research at Erasmus MC in the lab of Prof. Dr. Joost Gribnau, where he investigated the evolution of mammalian sex chromosomes (X&Y) during evolution and development. He then moved to the Institute Curie (Paris, France) for his post-doctoral research (with Prof. Edith Heard) where he focused on cancer genomics and epigenomics, and also adapted Naked mole rats as model animals for aging and cancer research. After completing his postdoctoral research, he started his research group at Erasmus MC in the departments of Cell Biology and Department of Genetic Identification. Eskeatnaf's current research focus includes deciphering and understanding gene regulatory networks that orchestrate normal and diseased development, understanding the role of the non-coding genome, developing novel single-cell omics techniques and analysis methods, and understanding the molecular mechanism of longevity and cancer resistance in Naked mole rats. In addition, in collaboration with Prof. Dr. Manfred Kayser (Department of Genetic Identification, Erasmus MC) he adapts single-cell omics techniques and analysis methods for forensic application. He is a coordinator of genetics and genomics course at a graduate school and a lecturer of computational biology.



**Tamas Ordog, M.D., PhD,** is Professor of Physiology and Director of the Gastroenterology Research Unit in the Division of Gastroenterology and Hepatology at Mayo Clinic in Rochester, Minnesota. He is also Genetics and Epigenetics Mechanistic Research Theme Group Leader and Epigenomics Core Director in the Center for Cell Signaling in Gastroenterology. His research focuses on the transcriptional, epigenetic, and metabolic regulation of gene expression in the lineage of interstitial cells of Cajal, gastrointestinal stromal tumors, and enteric neurons. He has also contributed to the discovery of mechanisms of epigenetic inheritance at mitosis and during stem cell differentiation and the role of epigenetic factors in diabetes, aging, immunological, fibrotic, psychiatric, and neurodegenerative diseases, as well as various cancers.

**Walther Parson, PhD,** holds an associate professorship at the Institute of Legal Medicine, Medical University of Innsbruck, Austria and an adjunct professorship at the PennState University, PA, USA. Together with his colleagues he set up the Austrian National DNA Database Laboratory in 1997 in Innsbruck, where he currently supervises the High Through-put DNA Database Laboratory and the research group Forensic Genomics. Walther Parson's research focuses on various fields of genetics and genomics, including forensic, medical and population genetics. His group was repeatedly consigned to handle international requests on Forensic DNA profiling of victims of mass fatalities (e.g., the 2004 Tsunami, the 1973 victims Chile, the 2014 Missing Mexican students), international human identification cases (e.g., the Russian Tsar family Romanov) and identification of individuals of historic interest (e.g., Friedrich von Schiller, Wolfgang Amadeus Mozart). The most recent challenge in that respect was a 20 year-long debated controversy over Kaspar Hauser, the putatively kidnapped Prince of the House of Baden (Germany). Walther discusses how the case was finally solved with the help of modern forensic genetic identification methods.

**Mrinal S. Patnaik, M.D., PhD,** is a Physician Scientist with the Division of Hematology and an Associate Professor in the Department of Medicine at the Mayo Clinic (Rochester, MN), with a specific interest in epigenetic dysregulation in myeloid neoplasms, specifically Chronic Myelomonocytic Leukemia (CMML). He has carried out important preclinical and prognostic work in CMML and have importantly demonstrated the high prevalence of epigenetic and chromatin dysregulation along with the negative prognostic impact of epigenetic mutations (more aggressive disease phenotype and shortened overall survival). This work has led to the development of the Mayo Molecular Model (Leukemia 2014); a contemporary prognostic model for patients with CMML that now is applied worldwide. In July 2015, he was awarded the NIH CTSA KL-2 scholar award to further his work in CMML and has used this time and mentoring to define the interplay in the genomic and epigenetic landscape of proliferative and dysplastic CMML subtypes.



**Dragan Primorac, M.D., PhD,** is a pediatrician, forensic expert and geneticist. He is the first recipient of the title "Global Penn State University Ambassador" and currently he serves as the Chair of the International Affairs Committee of the American Academy of Forensic Sciences. He is professor at Eberly College of Science, The Pennsylvania State University, and Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, in the United States and as professor at Medical Schools in Split, Osijek and Rijeka as well as at Department of Biotechnology, University of Rijeka, in Croatia. In October of 2016. he was appointed as a visiting professor at the College of Medicine and Forensics, Xi'an Jiaotong University, People's Republic of China. Dr. Primorac is one of the pioneers in DNA identification of skeletal human remains from mass graves. Currently, he has particular interest in metabolic bone and cartilage disorders, pain treatment, sports medicine as well as in personalized and regenerative medicine. Dr. Primorac was invited speaker at more than 150 conferences





all around the world. His work was published in most cited journals including Science and Nature and his papers have been cited more than 4300 times (Google Scholar) while h-index is 29. Currently, he is a team leader of the Croatian partner in the international consortium within EU FP7 project entitled "Multi-dimensional OMICS approach to stratification of patients with low back pain", worth 7.6 million euros. He is the cofounder and the President of The International Society of Applied Biological Sciences (www.isabs.hr). In 2017. he was elected president of The Croatian Society for Human Genetics. Dr. Primorac received 21 domestic and international awards. From 2003 to 2009 he served as Minister of Science, Education and Sports of The Republic of Croatia.

**Mechthild Prinz, PhD**, is a professor for Forensic Genetics and the director of the Masters of Science in Forensic Science Program at John Jay College of Criminal Justice in New York City. She has an MS in Biology from the University of Cologne in Germany and a PhD in Human Biology from the University of Ulm, also in Germany. Prior to joining John Jay College, Dr. Prinz worked as a forensic geneticist and laboratory director performing, and later supervising and managing, paternity and criminal casework for the Institute of Legal Medicine of the University of Cologne and the Office of Chief Medical Examiner in New York City. She is a past member of the FBI's Scientific Working Group on DNA Analysis Methods and the National Institute of Standards Organization of Scientific Area Committees, and a former president of the International Society for Forensic Genetics. She is a fellow for the American Academy of Forensic Sciences and serves as peer reviewer for prominent forensic journals and grant organizations. She has more than 30 years of experience in forensic DNA research and casework applications including Y-STR typing, low template DNA analysis, and mass disaster victim identification. Her current research focusses on the optimization of recovery, testing and interpretation for trace amounts of DNA evidence.

**Elias Puchner, PhD**, received his Diploma (M.S. equiv.) and then graduated with a PhD in Biophysics from the University of Munich in 2008 for performing research in the field of single-molecule force spectroscopy in the lab of Hermann E. Gaub. Research highlights include the development of "single-molecule cut and paste" which combines the specificity of DNA hybridization with the nm-precision of atomic force microscopy to assemble single molecules to precise arrangements. I also experimentally demonstrated that the enzyme titin kinase acts as a molecular force sensor in muscle and gets mechanically activated by force. With the support of a fellowship from the German Science Foundation, he transitioned to synthetic biology and single molecule super-resolution microscopy (PALM) during his postdoc with Wendell A. Lim and Bo Huang at the University of California. He developed quantitative PALM to be able to count single biomolecules in diffraction limited structures and gained new insights into the maturation of endocytic vesicles to endosomes. Since 2015 he leads his own single-molecule and cellular biophysics lab at the University of Minnesota where he became Associate Professor in the School of Physics and Astronomy in 2021. His lab further develops and applies optical single molecule and super-resolution microscopy techniques to gain new quantitative insights into biological processes with single molecule sensitivity and a resolution on the nanoscopic length scale.

**Keith Robertson, PhD**, earned his Bachelor's degree at Cornell University and his Ph.D. at The Johns Hopkins University in Dr. Richard Ambinder's laboratory. He did postdoctoral training with Dr. Peter Jones at the University of Southern California and with Dr. Alan Wolffe at the National Institutes of Health. His first faculty position was within the intramural program at the NIH followed by the University of Florida and currently the Mayo Clinic, where he is a Professor of Molecular Pharmacology and Experimental Therapeutics. Dr. Robertson has published over 120 manuscripts in the area of cancer epigenetics and his laboratory has been continuously funded by the NIH. He has served on numerous national and international grant review panels and is on the editorial board of the journal Epigenetics. The laboratory uses a combination of basic biochemical methods, cell biology, genome/epigenome engineering, and omics analyses to understand how epigenetic processes like DNA and histone methylation maintain normal cellular growth controls, and when deregulated or mutated in cancer, contribute to tumor initiation and progression.

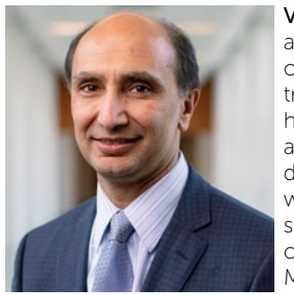
**Yi Xing, PhD** is the Francis West Lewis Endowed Chair and Founding Director of the Center for Computational and Genomic Medicine, as well as the Executive Director of the Department of Biomedical and Health Informatics at the Children's Hospital of Philadelphia (CHOP). Dr. Xing is also a Professor of Pathology and Laboratory Medicine at the University of Pennsylvania (Penn). Prior to his appointment at CHOP and Penn, Dr. Xing was a Professor of Microbiology, Immunology, and Molecular Genetics at UCLA, and served as Program Director of UCLA's Bioinformatics Interdepartmental Ph.D. Program. Dr. Xing received his BS in Molecular and Cellular Biology and BE in Computer Science and Technology from the University of Science and Technology of China (2001). He completed his PhD training in Bioinformatics with Dr. Christopher Lee at UCLA (2001-2006), and his postdoctoral training with Drs. Wing Hung Wong and Matthew Scott at the Stanford University (2006-2007). Dr. Xing has an extensive publication record in bioinformatics, genomics, and RNA biology. His work has provided fundamental insights into the function, regulation, and evolution of post-transcriptional RNA processing in mammals. His current research merges the fields of computational biology, biomedical data science, RNA genomics, human genetics, precision medicine, and immuno-oncology.

**Antti Sajantila, M.D., PhD,** works as a professor of forensic medicine in the Department of Forensic Medicine, at the University of Helsinki, and as a senior medical examiner in the Forensic Medicine Unit at the Finnish Institute of Health and Welfare. He is an Honorary Professor of Pontificia Universidad Católica del Perú in Lima. Professor Sajantila is a member of the executive board of European Council of Legal Medicine and the Steering Committee of the Independent Forensic Expert Team hosted by the International Rehabilitation Council for Torture Victims. He has served in the Advisory Board for the United Nations Human Rights Special Rapporteur for the Minnesota Protocol on the Investigation of Potentially Unlawful Death. Professor Sajantila has participated in many international medico-legal investigations,



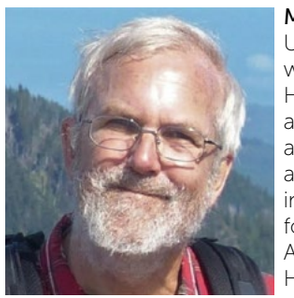


implemented on the requests of various international communities. Professor Sajantila has published over 220 articles in peer-reviewed scientific journals and co-authored books in forensic genetics, pathology and pharmacogenetics. His current research interests are ancient DNA, archeaovirology, forensic genetics and forensic pathology.



**Vijay H. Shah, M.D.,** received his undergraduate, medical, and clinical medicine training at Northwestern University. He obtained advanced clinical and research postdoctoral fellowship training in hepatology and liver disease at Yale University. He has maintained an NIH-funded program at Mayo Clinic for almost 25 years which focuses broadly on alcohol related liver disease, cirrhosis, portal hypertension and its complications with over 250 peer review publications in prestigious journals such as Journal of Clinical Investigation, Nature, Proceedings of National Academy of Science, New England Journal of Medicine and others. The basic, translational, and clinical work leans heavily on Big Data analytics. Dr. Shah is a member of the prestigious American Society of Clinical Investigation (ASCI) and Association of American Physicians (AAP). Presently, Dr. Shah serves as the Carol M Gatton and Mayo Distinguished Investigator and Chair of Department of Medicine at Mayo Clinic, where he is overseeing a Digital Transformation of the Department. In his leisure time, Dr. Shah likes to ski, play guitar, kayak, exercise, and spend time with his wife, two daughters, and his Labrador retriever.

**Anne Stone, PhD,** is Regents' Professor in the School of Human Evolution and Social Change at the Arizona State University. Currently, her research focuses on population history and understanding how humans and the great apes other primates have adapted to their environments, including their disease and dietary environments. This includes: (a) Native American population history, (b) the evolutionary history of the Great Apes, and (c) understanding the evolutionary history of mycobacteria (specifically the causative agents of tuberculosis and leprosy). Stone has been a Fulbright Fellow (1992-93), a NIH NRSA postdoctoral fellow (1997-1998), and a Kavli Scholar (2007). She is a fellow of the American Association for the Advancement of Science (2011) and a member of the National Academy of Sciences (2016). Stone currently serves as a senior editor of Molecular Biology and Evolution.



**Mark Stoneking, PhD,** received his PhD in genetics from the University of California, Berkeley in 1986. After postdoctoral work at Berkeley he held research scientist positions at the Human Genome Center at Lawrence Berkeley Laboratory and at the Cetus Corporation. He joined the faculty of the anthropology department at The Pennsylvania State University as an assistant professor in 1990, rising to associate professor in 1994 and full professor in 1998. In 1999 he left Penn State for the newly-established Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, where he supervised the Human Population History Group and was Honorary Professor of Biological Anthropology at the University of Leipzig until his retirement in June 2022. He is currently affiliated with the CNRS Laboratory of Biometry

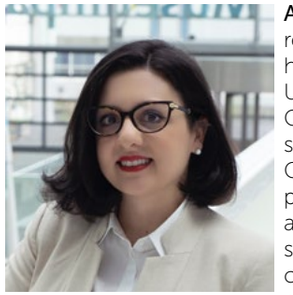
and Evolutionary Biology at the University of Lyon in Lyon, France. His research interests involve using molecular genetic methods to address questions of anthropological interest concerning the origins, migrations, and relationships of human populations, the impact of cultural practices on human genetic diversity, and the influence of selection during human evolution. He has authored more than 300 scientific papers, and a textbook, Introduction to Molecular Anthropology (Wiley, 2017).

**Andreas Tillmar, PhD,** works as a forensic geneticist at the National Board of Forensic Medicine, Sweden and as a senior lecturer and associated professor of forensic genetics at Linköping University, Sweden. He is well experienced from working over 15 years in the field. During these years he has signed more than 20,000 reports on DNA-based paternity, kinship and missing person investigations. His current tasks include technical leadership mixed with R&D. His research is focused on various topics of forensic genetics such as applying new genetic polymorphisms for complex kinship testing, applied biostatistics, population genetics and most recently investigative genetic genealogy. He is the main, senior or co-author of more than 40 peer-reviewed articles. He is the chairman of the English-Speaking Working Group (ESWG) of the International Society for Forensic Genetics (ISFG). A detailed CV can be found at https://sites.google.com/site/andreastillmar/cv.

**Natalia Tretyakova, PhD,** is a Distinguished McKnight University Professor in the Department of Medicinal Chemistry at the University of Minnesota-Twin Cities Coll. She received B.S. and MS in Chemistry at Moscow State University (Russia), then performed doctoral research in Environmental Sciences at University of North Carolina-Chapel Hill under the tutelage of Dr. James Swenberg, and received post-doctoral training in the lab of Dr. Steven Tannenbaum at the MIT. Her multidisciplinary research program employs the tools of organic synthesis, drug design, omics methodologies, mass spectrometry, molecular/cell biology, animal and population studies with the goal of understanding of the role of noncanonical DNA bases (DNA modifications) in cancer. Her studies of chemical carcinogen induced DNA modifications (DNA adducts) strive to structurally characterize and map DNA modifications in the genome, to elucidate their biological impacts, and to develop ultra-sensitive methodologies for their detection in humans. The studies of naturally occurring epigenetic marks of DNA help reveal previously unknown epigenetic mechanisms of disease and facilitate the development of novel chemical probes inhibiting DNA-modifying enzymes.

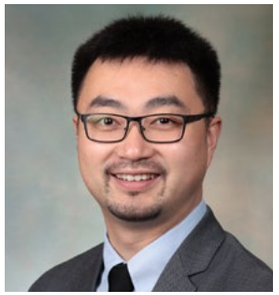




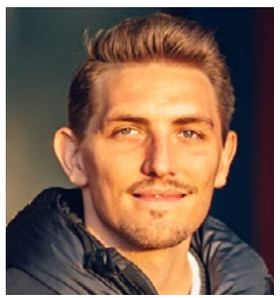


**Athina Vidaki, PhD,** is an Assistant Professor and internationally recognized expert in applied and translational epigenomics. She holds a Bachelor's in Biology from the National and Kapodistrian University of Athens, a Master's in forensic science from King's College London, and a PhD in forensic genetics by conducting studies at both the Queen Mary University of London and King's College London. Between 2016-2021, she conducted her postdoctoral studies at the Department of Genetic Identification at Erasmus Medical Center in Rotterdam, the Netherlands, where she still works. Her group consisted of both experimental and computational scientists is currently focusing on DNA methylation profiling, more specifically on how it can be used to serve justice in society as well as to improve current clinical testing. With a technology-driven focus and implementation-oriented mind, Dr Vidaki aims to (a) discover human epigenetic variation of medical and forensic relevance using existing, state-of-the-art, large-scale data, (b) create and benchmark new and innovative targeted epigenetic/epigenomic technologies, with the support of a prestigious Erasmus MC fellowship (2021-2025) and a Demonstrator grant by the Dutch Research Council (NWO, 2021-2022), as well as (c) develop and standardize robust next-generation sequencing (NGS) assays to translate her group's findings into practice, in the clinic or in court. Beyond epigenomics and via collaborations, she has also led or contributed to genomic, RNA, microbiome and immunogenomic studies.

**Liewei Wang, M.D., PhD,** trained in a national center for pharmacogenomic (PGx) research that has been engaged in the application of PGx in clinical translational studies for nearly a quarter of a century. Since assuming an independent faculty position in the Department of Molecular Pharmacology and Experimental Therapeutics at Mayo Clinic, she has developed a research program with a focus on the use of high throughput genomic technology, joined with a cell-based model system that she developed that consists of 300 lymphoblastoid cell lines (LCLs) with extensive high throughput genomic data to study mechanisms of cancer biology and therapy, including both chemotherapy and radiation therapy. Her research has also focused on understanding regulation of the PI3K-AKT pathway and its impact on response to drug therapy. As a Co-PI, she led the functional genomics activities within the Mayo-NIH Pharmacogenomics Research Network (PGRN) grant that included the projects understanding PGx of endocrine treatment in breast cancer. At the same time, her program is also studying prostate cancer therapy response including AR blocker and CYP 17 inhibitor. She is also leading a program that is developing patient derived xenografts (PDX) using breast cancer and prostate biopsy samples collected from two genome-guided prospective trials, BEAUTY for breast cancer and PROMOTE for prostate cancer. She is a member of several key programs at Mayo including the Mayo-NIH Comprehensive Cancer Center (MCCC), and the Director for the Pharmacogenomics Program of the Mayo Center for Individualized Medicine (CIM).



**Zong Wei, PhD,** is an assistant professor of physiology in the Department of Physiology and Biomedical Engineering at Mayo Clinic College of Medicine and Science. Dr. Wei joined Mayo Clinic in 2019 following postdoctoral training at the Salk Institute for Biological Studies with Ronald M. Evans, Ph.D. Research in the Epigenomics of Metabolic Diseases Laboratory led by Zong Wei, Ph.D., is focused on investigating the fundamental mechanisms of diabetes, obesity and inflammation, and identifying therapeutic targets in metabolic diseases. Using both human stem cell-differentiated organoids and mouse models, the laboratory studies the epigenomic regulation of cellular dysfunction in diabetes and metabolic diseases, identifies novel therapeutic targets in obesity and inflammation, and explores the fundamental mechanisms of transcription and chromatin biology. To address these issues, the team employs a variety of tools, which include mouse models, epigenomic and computational tools, human organoid models, functional genomics, single cell multi-omics and proteomics.



**Hugo Zeberg, PhD,** is a Swedish evolutionary geneticist at the Karolinska Institutet and at the Max Planck Institute for Evolutionary Anthropology. He did his PhD thesis in electrophysiology and computational neuroscience but has since shifted to genetics. His main research focus is the functional consequences of the admixture with Neandertals that took place ~60,000 years ago.

## SPONSOR LECTURE

**Rea Dabelic PhD,** earned her undergraduate and PhD degrees in Microbiology and Immunology from the University of Florida, where she studied interferon signaling and developed immunomodulatory therapeutics. She completed her postdoctorate in Virology at Columbia University where she studied viral immune evasion and host defense mechanisms. At 10x Genomics, Rea is the immunology market segment manager focused on accelerating the use of single-cell and spatial analyses.





## INTERDISCIPLINARY SESSION OF AMERICAN ACADEMY OF FORENSIC SCIENCES AND ISABS 2022

**Peter Ausili, M.S.,** is a retired forensic chemist who worked at the US Drug Enforcement Administration. He has 42 years of experience analyzing suspected controlled substances. He provided clandestine lab processing and safety training to chemists and law enforcement personnel and has testified in state, federal and military courts over 100 times. He is a fellow of the American Academy of Forensic Sciences (AAFS) and a member of the International Education Outreach Program (IEOP) in 20 foreign countries. He is also a former Member of the Midwestern Association of Forensic Scientists (MAFS) and the Clandestine Lab Investigating Chemists (CLIC).

**Andrej Bozhinovski, PhD,** is an Assistant at the Department of Criminal Law at the University of Zagreb Croatia and Project Assistant of the "Innocence Project of Croatia" implemented by the Faculty of Law in Zagreb and the Croatian Science Foundation. He holds Masters degree in Criminal Law from the Faculty of Law "Iustinianus Primus" of the University "St. Cyril and Methodius" in Skopje 2012. From 2016-2021, he worked as a Legal Advisor of the Association of Judges of North Macedonia, coordinating major multilateral projects aimed at promoting the reform of the criminal justice system and strengthening the independence, ethics, and transparency of the judiciary in the country. In this capacity he provided logistical and legal support in the establishment of the Advisory Council for Judicial Ethics of North Macedonia, the Judicial-Media Council, as well as assistance in drafting a new Code of Judicial Ethics for Judges and Lay Judges of North Macedonia, adopted by the Supreme Court of North Macedonia As a Criminal Justice expert, he actively cooperates with the OSCE Mission to Skopje, the OSCE-ODIHR in Warsaw, DCAF - Geneva Center for Security Sector Governance, and the CEELI Rule of Law Institute in Prague, where he provides legal expertise in projects focused on on the protection of human rights, access to justice, an independent judiciary and introducing remote trials. In 2019 was selected by the US State Department to participate in the IVLP (International Visitor Leadership Program) program for young leaders and was invited to a three-week training course on "Foreign Policy and Human Rights" in Washington DC, USA. Since 2021, he actively cooperates with the Croatian Academy of Legal Sciences and the US Embassy in Zagreb Croatia, in aiding the implementation projects aimed at protecting, assistaning vulnerable groups and victims of Human Trafficking.

**Henry Lee, PhD,** is one of the world's foremost forensic scientists. Dr. Lee has worked with law enforcement agencies from 46 countries in helping solve more than 8,000 cases. Dr. Lee is currently the director of Forensic Research and Training Center and Distinguished Chair Professor in Forensic Science of University of New Haven. He was the Chief Emeritus for Connecticut State Police from 2000 to 2010 and the Commissioner of Public Safety for the State of Connecticut from 1998 to 2000 and has served as that state's Chief Criminalist and Director of State Police Forensic Laboratory from 1978 to 2000. Dr. Lee was the driving force in establishing a modern state police communication system, community-based police services sex offender and DNA databank, Major Crime Investigation Concepts, Standardized Evidence Collection system, Laboratory Operation Standard and advanced forensic science services in Connecticut. He currently serves as advisor/consultant for more than 80 law enforcement agencies around the world. He was just appointed, in 2013, as Chief Forensic Advisor.

**Laura Fulginiti, PhD,** received her PhD from the University of Arizona in 1993. She is a forensic anthropologist at the Maricopa County Office of the Medical Examiner in Phoenix, AZ. She has adjunct faculty status with Arizona State University and a Clinical Associate Professorship in Pathology at the University of Arizona, School of Medicine. She is on the Executive Committee of the Board of Directors for the American Academy of Forensic Sciences and served as a Director on the American Board of Forensic Anthropology. She is a fellow of the American Association of Physical Anthropologists and a member of Sigma Xi. Dr. Fulginiti's research interests include enhancement techniques for the identification of unknown human remains, age-at-death and sex determination using the pubic symphysis and sternal ends of the fourth ribs, trauma analysis including direction of force in pedestrians struck by motor vehicles and understanding dismemberment patterns. She has publications addressing each of these areas of interest.

**Carl R. McClary, MS**, has been working in the U.S. Department of Justice Bureau of Alcohol, Tobacco, Firearms and Explosives Forensic Laboratory, Atlanta Forensic Science Laboratory as a Senior Questioned Document Examiner since 1996. He previously worked as a Questioned Document Examiner at the South Carolina Law Enforcement Division, Division of Forensic Sciences, Columbia, South Carolina. He finished his B.A. (Psychology) from the University of South Carolina in 1982 and became Masters of Forensic Science at the Oklahoma State University in 2020. He has had 46 appearances in different Federal Courts, Industry Operations Hearings, Magistrate and General Sessions Courts and Superior Court. He is a member of numerous professional associations and he was awarded in 2008 with a Forensic Science Award by the American Society for Testing and Materials, International for technical and administrative contributions to the Committee E30 Forensics.





**Damir Primorac, PhD,** is a full-time professor at the Faculty of Law University of Mostar, as well as an Associate Professor at the University Department of Forensic Sciences of the University of Split, Croatia, as well as member of the expert's council of the "Innocence Project of Croatia" project, implemented by the Faculty of Law in Zagreb and the Croatian Scientific Foundation. He graduated from the Faculty of Law in Split in 1993, where he obtained mr.sc. in 1997. He received his doctorate at the Faculty of Law in Zagreb in 2007. From 1996 to 2005 he was appointed as a judge at the Municipal Court in Split, and from 2005 to 2008 at the Split County Court. Meanwhile, he worked as a Deputy President of the Municipal Court in Split. He was president of the Judge's Council of the Split County Court. During 2000, he spent two months in vocational training in the United States (Connecticut and Illinois). He is a Honorary citizen of Crystal Lake, Illinois, USA. In 2003 he passed a notary exam. From 1999 to 2005, he was the President of the Civil Service Tribunal for County Offices in the Zadar County, Šibenik-Knin County, Splitsko-Dalmatinska County, and Dubrovačko-Neretvanska County. At the end of 2008 he founded the Law Firm "Primorac i partneri" d.o.o. headquartered in Split and the Zagreb office, and since then has been working as a attorney at law. By resolution of the Croatian Bar Association, he was recognized by his specialty in the field of criminal law. He is a regular member and a member of the Presidency of the Croatian Academy of Sciences. Furthermore, he is a principal lecturer for the course "Criminal Procedure Law II" at the University Department of Forensic Sciences of the University of Split and for the course "Sport Misdemeanor Law at the Faculty of Law", University of Split. Lecturer at the Postgraduate Doctoral Study in Legal Sciences – criminal law sciences at the Faculty of Law, University of Mostar, the holder of two courses - Criminal Procedure Law and Criminalistics. He is a full member of the American Academy of Forensic Sciences and from 2000 to 2016 he was a member of the American Judges Association. He is a member of the Election Complaints Commission of the Croatian Football Association. From 2007 to 2018 he was the president of the Arbitration of the Croatian Football Association, from 2013 to 2018. President of the Appeals Commission of the Football Association of the County of Splitsko-dalmatinska. From 2015 to 2016 he was a member of the Pardon Commission of the Republic of Croatia. Professor Primorac is also a veteran of the Homeland War.

**Lucija Sokanovic, PhD,** is an Assist. Prof. at the Chair for Criminal Law, University of Split. After graduating from the Faculty of Law in Split, she worked at the Municipal Court in Kastel Luksic and at the Commercial Court in Split. She passed her Bar Exam in 2002. In 2014 she attained her PhD degree at the Faculty of Law, University of Zagreb (Doctoral Studies in Criminal Sciences) with the dissertation "Fraud in Criminal Law". She teaches courses in Criminal Law, Commercial Criminal Law, Criminal protection of the environment and Sports Criminal Law. She authored and co-authored published articles in said fields. She was a scholar of the Freie Universität, Berlin and Max-Planck-Institut für ausländisches und internationales Strafrecht, Freiburg im Br. She received the University Department of Forensic Science's award for the best quality teaching 2015/16, and the Award of the Student Choir of the Faculty of Law of the University of Split 2017. Assistant Professor Sokanovic is a member of the expert's council of the „Innocence Project of Croatia"implemented by the Faculty of Law University of Zagreb and the Croatian Science Foundation.

**Andrea Zaferes, PhD,** a medicolegal death investigator, is familiar with the handling of aquatic cases from the scene to the courtroom. She trains law enforcement, medical examiners/coroners, forensic pathologists, dive teams, domestic violence and trafficking crime workers, medical personnel, and jurisprudence members to recognize, document, and investigate homicide, death, assault, and abuse cases that involve drowning, aquatics, and other forms of asphyxiation. She assists in analyzing and building such cases in the U.S. and abroad, and has developed best practices for their investigation. She has presented in more than 130 forensic and 100 rescue/diving conferences, has been teaching dive teams around the world for more than 30 years to recover submerged evidence and bodies, and is an author. She is a pro bono consultant for such organizations as the National Center for Missing and Exploited Children, and the New York State Department of Criminal Justice Missing Person Cold Case Review Panel, the National Center Fatality Review Program Drowning Scene Investigation Report Committee, and works with Lifeguard Systems, Dutchess County Medical Examiner's Office, and Respond Against Violence. She is a fellow of the American Academy of Forensic Sciences and a member of the International Association of County Coroners and Medical examiners, and an associate member of the National Association of Medical Examiners. She helps sets standards and best practices for aquatic scene, abuse, injury, and death investigation.





# ANTHROPOLOGICAL SESSION "BIOANTHROPOLOGY AND GLOBAL HEALTH IN THE TIMES OF CRISIS"

**Luka Bočkor, PhD,** is a research associate at the Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia. He obtained his PhD in Life and Biomolecular Sciences at the International Centre for Genetic Engineering and Biotechnology (Trieste, Italy) in 2015 in the field of molecular genetics and gene therapy. His areas of scientific interest are application of molecular genetics and biotechnology in prevention and treatment of non-communicable human diseases and study of normal biological processes. He has published 17 scientific papers in different fields of molecular biology and biomedicine (molecular genetics, gene therapy, genome editing, bacteriology).

**Noël Cameron**, **PhD,** DSc, is Professor Emeritus of Human Biology at Loughborough University, UK. His post-graduate education was at Loughborough and London Universities where he initially studied for an MSc in Human Biology and then pursued a PhD in Medicine at London University under the supervision of Professor James Tanner at the Institute of Child Health. He was subsequently appointed to a lectureship and undertook research in normal and abnormal child growth. He spent from 1984 to 1997 as Associate (1987) and then Full Professor of Anatomy and Human Biology (1994) in the Department of Anatomy of the University of the Witwatersrand, South Africa. He initiated the Birth to Twenty (Bt20) birth-cohort study in Soweto and Johannesburg in 1991 which has become the longest running and most detailed longitudinal study of child health and growth in any developing country. He returned to the UK in 1997 as Professor of Human Biology at Loughborough University and was made a Fellow of the Royal Society of Biology in 1998 for his significant contribution to research in human biology. He continues to be involved in a variety of birth cohort studies including the Croatian Islands Birth Study initiated in 2015. Longitudinal data from these birth cohort studies facilitate his research into the early determinants of risk for non-communicable disease of lifestyle such as obesity, Type 2 diabetes, and cardio-vascular disease.

**Miran Čoklo, M.D., PhD,** is the Head of the Laboratory for chemical analytics and Deputy Head of the Centre for Applied Bioanthropology, at the Institute for Anthropological Research (INANTRO), Zagreb, Croatia. He received his PhD in 2009 at the Rijeka University School of Medicine in the field of forensic toxicology. Since 2014 he is employed at the Institute for Anthropological Research with analytical chemistry in anthropology, including the interplay between toxicological burden, human microbiota and occurrence of disease, being in his research focus. He was involved in the NIH project „Integrated GWAS and EWAS of Cradiometabolic Traits in an

Island Population"dealing with the influence of toxicological burden (in particular PFAS) on the occurrence of the metabolic syndrome on the Eastern Adriatic Island of Hvar. Currently, he is the leader of the INANTRO research group within the HORIZON2020 project "Unravelling Data for Rapid Evidence-Based Response to COVID-19 (unCoVer)". He is also the PI of the international collaborative project "The impact of COVID-19 pandemic on the determinants of food choice in the adult population of Croatia and Belgium (CFC CRO-BE)". Regarding the COVID-19 pandemic, he is focused on the influence of toxicological burden and gut microbiota on the severity of the clinical course and outcome of COVID-19, as well as the occurrence of the post-COVID syndrome.

**Vlatka Cubric Curik, PhD**, is a Professor of Animal Genetics (BSc) and Molecular Genetics of Animals (MSc) at the University of Zagreb Faculty of Agriculture (Croatia). Her scientific interests and publications concern animal breeding and genetics, laboratory methods for molecular biodiversity, analysis of molecular genetic data, study of the population of domestic animals and their wild relatives, archaeogenetics. She is the editorial member and reviewer of Frontiers in Genetics, Livestock Science, Genes, Small Ruminant Research, Diversity. She is the head of the laboratory of archaeogenetics at the University of Zagreb, Faculty of Agriculture.

**Ino Čurik, PhD,** is a professor of animal breeding, conservation genetics and population genetics at the University of Zagreb, Faculty of Agriculture (Croatia). His scientific interests and publications concern conservation genetics (estimation of inbreeding, effective population size, diversity and inbreeding depression), quantitative genetics (quantitative aspects of coat colour inheritance, effects of mitogenome on quantitative variation) and archaeogenetics (genomic changes during domestication). Editorial board member of Frontiers in Genetics (associate editor), Livestock Science, Genes, and Journal of Bioanthropology. Recipient of the National Science Award of the Republic of Croatia for exceptional scientific achievements in the field of biotechnology.

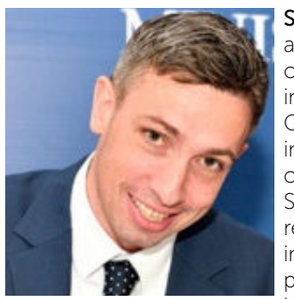
**Florin Grigorescu**, **M.D., PhD**, s the leader of molecular genetics team at University of Montpellier, France known as endocrinologist and nutritionist for his contribution in purification of insulin receptor kinase from human erythrocyte and identification of defects in genetic syndrome of severe insulin resistance with Acanthosis Nigricans. Retiring as researcher from INSERM in 2019, he is currently fellow of the Institute Convergences Migrations (ICM) at Collège de France and from 2003, honorary professor for genetics at Carol Davila university, Bucharest Romania. Awarded with JB Morganni medal in Italy (Padova) in 1986 and by the National Academy of Medicine in Paris in 2001, he oriented research towards genetics of complex disorders like metabolic syndrome and polycystic ovary syndrome (PCOS) in women, searching for culprit genes by fine-scale haplotype mapping and whole genome screening. In the last several years he coordinated the MEDIGENE European program (awarded by Etoiles de l'Europe prize in 2017) over Mediterranean countries on insulin





resistance using combined approaches in population genetics and genetic anthropology. At INSERM he studied molecular mechanisms of insulin receptor activation using synthetic phosphorylated peptides contributing to understanding the cognitive surface of insulin receptor and IRS-1 in cellular signaling. He also described the first mutation in IGF-1 receptor gene and contributed to the description of FTO gene in PCOS. Florin Grigorescu settled the Laboratory of Molecular Endocrinology in Montpellier, France contributing to the discovery of genes involved in Berardinelly-Seip Congenital Lipodysptophy and Alström syndrome. His current interest is focused on genetic variation in native and immigrant populations in Europe explaining metabolic alterations, effects on life span and interaction with nutritional factors explored by a novel program (MEDIPAD) for nutritional investigation in Mediterranean populations.

**Damir Marjanović, PhD,** is currently professor at several universities in Bosnia and Herzegovina and visiting professor at several academic abroad Institutions. He was long term head of the Laboratory for forensic genetics at the Institute of Genetic Engineering and Biotechnology Sarajevo and he is currently senior scientific assistant at the Institute for Anthropological researches, Zagreb, Croatia. He was also employed as the expert in the field of forensic genetics by EU and OSCE (Kosovo), European Agency for Reconstruction (Serbia) and International Commission on Missing Persons (Bosnia and Herzegovina), but also, he was working for the Institute Rudjer Bošković and Genos scientific company (Croatia). In the 2011 he was awarded as the Scientist of the Year in Bosnia and Herzegovina. His main scientific field of interest is forensic, population and molecular genetics and molecular anthropology. He is author and coauthor of 14 book chapters, more than 90 scientific and review articles and abstracts in CC, WOS, SCOPUS journals and more than 250 scientific papers and abstracts in other journals and scientific publications. He was cited around more than 2800 times Also, he was invited speaker on more than 60 different international and national scientific events all over the world.and he participated in more than 20 science national and international projects (he was project coordinator for 11 of them) supported by the respective ministries and foreign institutions. Between 2012-2013 he was Minister of education and science, Canton Sarajevo, Bosnia and Herzegovina and he is acting member of the House of Peoples of the Parliament of the Federation Bosnia and Herzegovina, Parliament of Canton Sarajevo and president of the Commission for Education and Science within Cantonal Parliament.



**Saša Missoni, PhD,** is a scientist engaged in interdisciplinary anthropological research and the director of the Institute of Anthropology in Zagreb. His main research focus is the investigation of metabolic syndrome and obesity in the Croatian island populations and their prevention, especially in the period of growth and development of children. Some of his projects include: the Croatian Islands' Birth Cohort Study, project „Genetic and environmental factors of insulin resistance syndrome and its long-term effects complications in immigrant Mediterranean populations (MEDIGENE)", NIH project „Integrated GWAS and EWAS cardiometabolic properties in the island population", as well as the project „Individualized approach in predicting the onset and development of type 2 diabetes (IRI)". One of the most important projects that can be highlighted is the infrastructure project of construction and equipping of the Center for Applied Bioanthropology, funded by the European Regional

Development Fund. Saša Missoni is the President of the International Society for the Study of Human Growth and Clinical Auxology - ISGA (since 2017) and Vice President of the International Society of Anthropological and Ethnological Sciences – IUAES (since 2018), and he is also the president of the Croatian-Israeli Business Club. Dr, Missoni is a member of the Board of the Croatian Society for Human Genetics and of numerous other international societies. Due to the organization of numerous international congresses in Croatia, he was appointed Ambassador of Croatian Tourism by the Croatian Tourist Board. In 2019 he was appointed by the Ministry of Science and Education as the representative of Croatia in The International Consortium for Personalized Medicine (ICPerMed) and in 2021 as a Deputy National Representative to the European Commission in Working Group 1 + MG WG12 The Genome of Europe (GoE).

**Jelena Šarac, PhD,** is an anthropologist and a research associate at the Center for Applied Bioanthropology, Institute for Anthropology, Zagreb, Croatia. Her research interest involves anthropological investigation of maternal and child health, with special emphasis on the development of complex diseases, as well as evolutionary biology and population genetics. Her early work was focused on the anthropological analysis of genetic diversity and population structure in the eastern Adriatic and the wider area of Croatia and Europe based on molecular genetic markers of DNA (mitochondrial DNA and Y chromosomes) and the study of microevolutionary, historical and cultural processes that shaped the genetic landscape of these populations. In the last years her focus shifted towards the identification of risk factors for the development of complex non-communicable diseases (especially metabolic syndrome, diabetes mellitus and obesity) in early childhood, adolescence and adulthood and means of their prevention, through studies performed on Croatian islands. The investigation of maternal and child health in Dalmatia, Croatia and the identification of early risk factors affecting future well-being of the child have been in the centre of her latest work, based on her active involvement in the "Croatian Islands' Birth Cohort Study (CRIBS)". From 2010 onwards she taught several Anthropology undergraduate courses at the Faculty of Humanities and Social Sciences and the Faculty of Science and she is an author or co-author of more than 40 scientific publications.

**Serena Tucci, PhD,** is Assistant Professor of Anthropology and Principal Investigator of the Human Evolutionary Genomics Laboratory at Yale University. Dr. Tucci's research addresses fundamental questions in human evolution and population history using DNA from present-day and ancient humans. Her interdisciplinary approach combines expertise from anthropology, population genetics, and computational biology, to reconstruct past demographic events and disentangle the genetic basis of human adaptation. By integrating field work, laboratory work and cutting-edge computational methods, her work sheds light on mechanisms of evolutionary change, and on the genetic legacy that extinct humans - such as Neandertals and the enigmatic Denisovans - left in the genomes of human populations in Island Southeast Asia and Oceania. Prior to joining Yale, she conducted postdoctoral research at the Department of Genome Sciences at the University of Washington and the Lewis-Sigler Institute for Integrative Genomics at Princeton University. She was supported by the Lewis and Clark Fund for Exploration and Field Research from the American Philosophical Society. Tucci received her Ph.D. in Evolutionary and Environmental Biology from the University of





Ferrara in Italy, where she was awarded the Young Investigator Fellowship in 2013 and 2015.

**John Vena, PhD,** was appointed as Professor and Founding Chair of the Department of Public Health Sciences at the Medical University of South Carolina in 2014 and became Professor Emeritus in July 2020. A fellow of the American College of Epidemiology and the American Epidemiological Society, Dr. Vena's areas of research expertise include cancer epidemiology, community-based research, environmental health, epidemiology, occupational health, and reproductive and developmental health. He serves as a member of the American Public Health Association, the Society for Epidemiologic Research and the International Society for Environmental Epidemiology. Dr. Vena has 42 years of experience in epidemiology and community–based participatory research (CBPR). He coordinated all the new curriculum development in the Department of Public Health Sciences including three new MPH degree programs that started in August 2015. He has experience in environmental epidemiology and graduate training, and from 1999-2003 he was part of a team as co-investigator on NIH grants to pioneer integration of biomarkers in epidemiology analytic studies to look at gene-environment interactions, exposure assessments and the use of Geographic Information Systems in epidemiologic research. Current grant activities are on the topics of environmental influences on children's heath (ECHO: https://www.nih.gov/echo) environmental determinants of cancer, chronic kidney disease and systemic lupus erythematosus (SLE); physical activity, stroke and cognitive function; stress and cardio-metabolic disease in police; and health effects of persistent organic pollutants. He was the PI of the ECHO NFG cohort study of Mothers and Children from 2016-2020 which is still underway.

**Maria Zafiropoulou, PhD,** holds a PhD in Economics and Management of Health and Social Organizations from the Institute of Administration of Enterprises (France) and Msc degrees in political sciences, health law and Regional Development of Social Entreprises in University of Lille. She has been a fellow in France, in the National School of Public Health (EHESP). She is a jurist specialized in health and social policies and gerontologist, and actually works as associate member of Hellenic Mediterranean University and is the cofunder of co2gether.gr. She has a long-lasting experience as a contractual professor in different European and African universities. Since 2020 she works as an advisor of the Region of Western Greece for FEAD programs while Maria she was the vice director of the General Hospital of "Agios Andreas" in Patras from 2018 to 2021. She has coordinated more than 40 interdisciplinary research projects in health, cultural and social sectors in Greece, France, Belgium and Switzerland and has attracted significant competitive research funding. Since 2010 she acts as an advisor to the European Commission and as an expert in different sectoral and transversal groups of interest such as CHAFEA, European Skills, Competencies and Occupations (ESCO), European Social Fund (ESF), OECD and WHO, numerous foundations and NGOs in Greece and in Brussels. She has been a consultant to Romanian, French and Greek ministries. Her research is oriented on building resilience mechanisms during crisis, on upskilling of vulnerable groups and on gerontology integration and empowerment.

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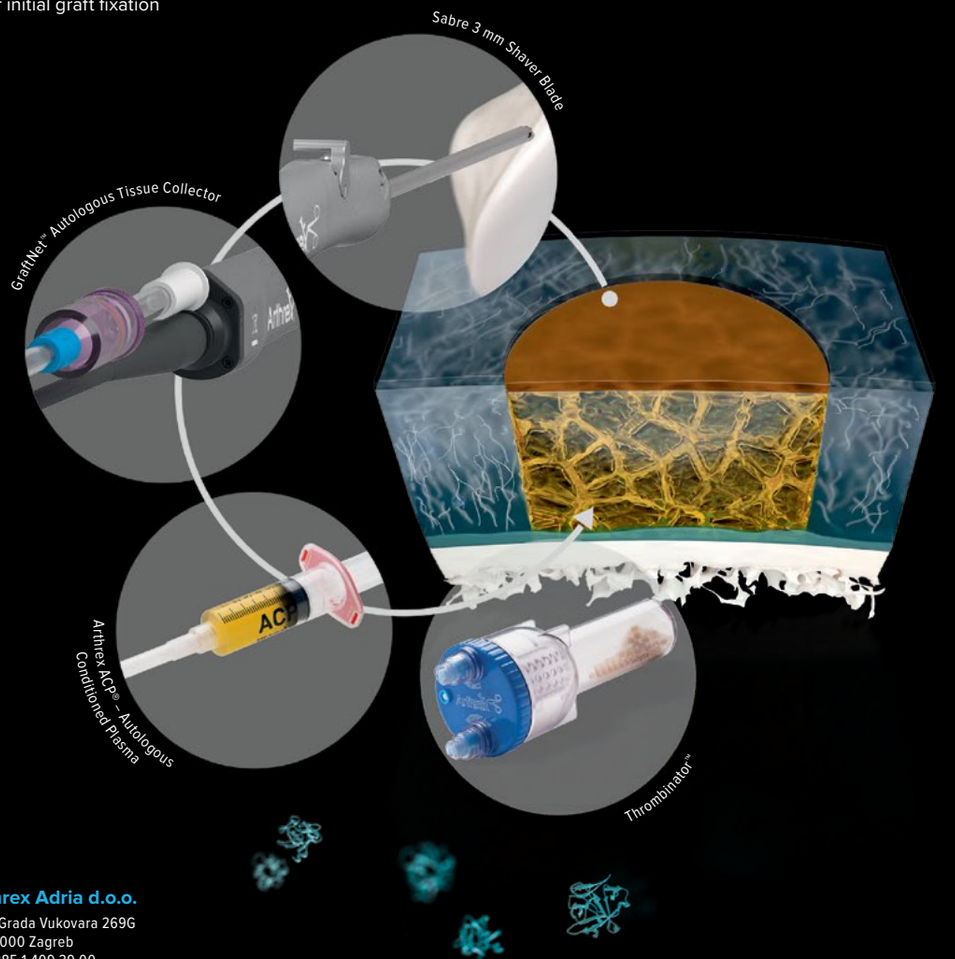


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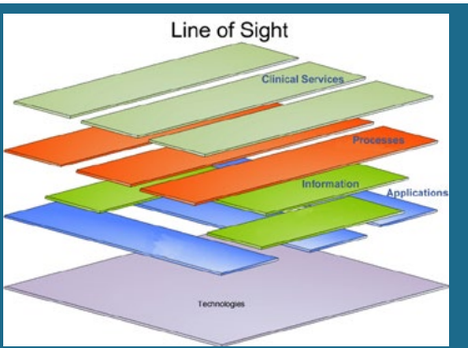


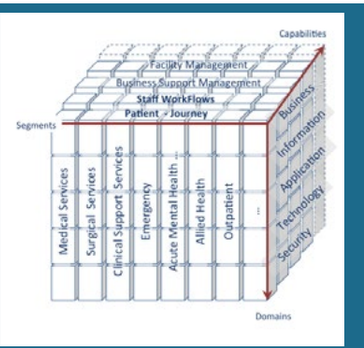


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