PROGRAM AND ABSTRACTS

The Tenth ISABS Conference
on Forensic and Anthropologic Genetics
and Mayo Clinic Lectures in Individualized Medicine

June 19-24, 2017, Dubrovnik, Croatia

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(dodati!)

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PROGRAM AND ABSTRACTS

THE TENTH ISABS CONFERENCE ON FORENSIC AND ANTHROPOLOGIC GENETICS AND MAYO CLINIC LECTURES IN INDIVIDUALIZED MEDICINE

JUNE 19-24, 2017
Hotel Dubrovnik Palace
Dubrovnik
CROATIA

www.isabs.hr info@isabs.hr

DUBROVNIK PALACE HOTEL – LAY OUT

10TH FLOOR

- MARE CONFERENCE HALL
- EXHIBITION AREA
- REGISTRATION DESK
- PREVIEW AND SLIDE AREA
- BAGS' PICK UP AREA
- INTERVIEW CORNERS

9TH FLOOR - LOBBY

- POSTER PRESENTATION AREA
- PRESS CENTER (DUBRAVA CONFERENCE HALL)
- INTERVIEW C ORNERS

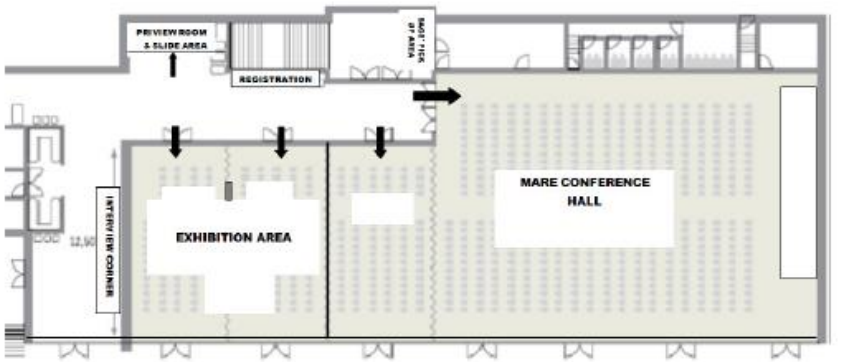
7TH FLOOR

- LUNA CONFERENCE HALL

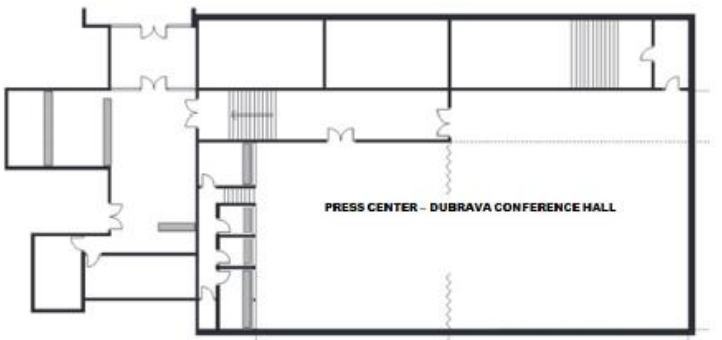
3RD FLOOR

- LEVANAT CONFERENCE HALL

10TH FLOOR



9TH FLOOR – LOBBY



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ISABS - THE FIRST TWENTY YEARS

Welcome to the Tenth International Society for Applied Biological Sciences (ISABS) Conference on Forensic and Anthropologic Genetics and Mayo Clinic Lectures in Individualized Medicine, Dubrovnik, Croatia, June 19-24, 2017.

This jubilee event commemorates the twentieth anniversary of our conference series. The idea for the conference was conceived out of necessity to bring to Croatia and broader region insights into the rapidly developing genetic techniques to forensic and clinical applications.

The impact of the conferences and the resulting professional ties on applied genetics in the region has been substantive. It affected numerous research and educational efforts being crowned in 2009 with the opening of the Center for Forensic Sciences at the University of Split, currently the only institution that offers the specialized graduate program in the region. In addition, ISABS, our parent society, and the American Academy of Forensic Sciences agreed recently to forge scientific and academic ties to provide even more opportunities and access for the budding scientists.

As genetic technology in forensics overlaps with genetic anthropology, for the past decade we have included anthropologic sessions into the program. At the same time, Mayo Clinic joined the effort. It provided the critical link into 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 anthropologic genetics, of our programs. We feel this integration of the three areas, united by technology and applicative intent, provides an unprecedented opportunity for crosspollination. The more recent conferences in the series have amply vindicated this approach.

Not everything has been successful from the beginning. While the initial intent to encompass clinical applications was justified, the field simply had not been defined well enough to facilitate coherent programs. As personalized/individualized/precision medicine became a field in itself, recent conferences emphasize this area.

We have been fortunate with the fantastic support by the conference speakers, all undisputed leaders of their respective fields. Our mission would not have been possible without the loyal support by our sponsors. Our sincere heartfelt gratitude to them all!

For the last conference we passed the mantle of program directorship to Manfred Kayser and Tamás Ördög. They did an excellent job then and--we invite you to judge for yourself--for this conference too.

Building this conference series has not been always easy, but we believe the effort has been worthwhile. As we will enter the third decade of the series, we look forward to the new faces, new topics, continued enthusiasm and even deeper impact than that of the first two decades.

Welcome!

Dragan Primorac, Moses Schanfield and Stanimir Vuk-Pavlović
Conference Founders

WELCOME TO THE TENTH ISABS CONFERENCE ON FORENSIC AND ANTHROPOLOGIC GENETICS AND MAYO CLINIC LECTURES IN INDIVIDUALIZED MEDICINE!

The conference is next in the series of biennial events organized by the International Society for Applied Biological Sciences, a society dedicated to the promotion of applied molecular biology (www.isabs.hr). This conference is organized with participation of Mayo Clinic, George Washington University, Penn State University, The Henry C. Lee Institute of Forensic Science, University of New Haven, Universities of Zagreb, Split, Osijek and Rijeka, Ruđer Bošković Institute, Croatian Academy of Engineering, Croatian Forensic Association, International Burch University, University of Botswana, Croatian Society of Human Genetics, Chindren's Hospital Srebnjak, Genos Ltd, Institute for Anthropological Research, St. Catherine Hospital, Unfallkrankenhaus Berlin, Faculty of Law Zagreb, Ivan Vučetić Forensic Science Center, Croatian Academy of Technical Sciences, Croatian Academy of Legal Sciences, City of Split and Split-Dalmatia County. As in the past, this conference is organized under the auspices of the Croatian Academy of Sciences and Arts. Croatian Medical Journal, the official journal of ISABS, will publish the conference papers in its tenth special ISABS edition.

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 cutting-edge analytical methodology in forensic science. 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 of the human genome. In 2011, the conference discussed the new molecular methods in early cancer diagnosis, new approaches to AIDS treatment, non-invasive prenatal diagnostics, gene and cell therapy, phenotype prediction from genetic information, identification of victims of mass disasters, plant and animal DNA analysis, cold-case forensics, and selected topics in molecular anthropology. In 2013 we included genetics applied to crossing of forensic science, anthropology and translational medicine, and also lectures from Nobel Laureates Aaron Ciechanover, Robert Huber and Ada Yonath. 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 ephasys on new knowledge in Next Generation Sequencing (NGS) in forensics, DNA investigative intelligence, and advancements in forensic DNA routine. Also, the Nobel Laureate Robert Huber held a lecture.

This year we are pleased to

As before, the conference is structured to allow close interaction of the international faculty and attendees. Together with formal presentations, there will be other social occasions that are ment to enhance opportunities for scientific intercourse, but also to introduce the participants to the city of Dubrovnik, one of the best known tourist destinations in Croatia due to its cultural and historical attractions and well developed tourist and sport offers.

Enjoy!

Manfred Kayser and Tamás Ördög
Program/Conference Directors

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 appellation 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 century, 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 variety of archaeological, historical and cultural monuments are 1,940 m long defensive walls (from 1979 inscribed into the UNESCO World Heritage List) which 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".

10TH ISABS CONFERENCE, DUBROVNIK, CROATIA, JUNE 19-24, 2017

• *Organizer*

International Society for Applied Biological Sciences
URL: http://www.isabs.hr

The conference is organized under the auspices of the Croatian Academy of Science and Arts.

• *ISABS Founders*

Dragan Primorac (The Pennsylvania State University and University of New Haven, USA; St. Catherine Hospital, Croatia; Universities of Split, Rijeka and Osijek, Croatia)
Moses Schanfield (George Washington University, Washington, DC, USA)
Stanimir Vuk-Pavlović (Mayo Clinic College of Medicine, Rochester, MN, USA)

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Dubrovnik
Roberta Šimunović

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INTERNATIONAL SOCIETY FOR APPLIED BIOLOGICAL SCIENCES

Registration number: 21003655
Date of registration: August 27, 2004

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Ada Yonath (Nobel Prize in Chemistry 2009; Weizmann Institute of Science, Rehovot, Israel)
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Lara Primorac (XV Gymnasium, Zagreb, Croatia)
Matea Primorac (XV Gymnasium, Zagreb, Croatia)

The Young Investigator Awards

Recipients of the 2017 Young Investigator Awards

- Sabriya Syed, USA (Personalised Medicine)
- Goran Josipović and Vladimir Zanki, Croatia (Personalised Medicine)
- Atina Vidaki, The Netherlands (Forensic Genetics)
- Mateja Hajdinjak, Germany (Anthropological Genetics)

Recipients of the 2015 Young Investigator Awards

- Dora Polšek, Croatia (Molecular therapy)
- Barbara Zajac, Germany (Genetic analysis of forensic non-human material)
- Niraj Rai, India (Molecular Anthropology)

Recipients of the 2013 Young Investigator Awards

- 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 Awards

- Rebecca S Just, USA (Genome-based applications in forensic science)
- Mark Barash, Australia (Forensic DNA phenotyping)
- Renato Polimanti, Italy (Molecular anthropology)
- Martina Smolić, Croatia (Molecular therapy)

Recipients of the 2009 Young Investigator Awards

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

Recipients of the 2007 Young Investigator Awards

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

Recipients of the 2005 Young Investigator Awards

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

- Mirela Baus Lončar, Germany (Molecular and Cellular Medicine)

Recipients of the 2003 Young Investigator Awards

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

Recipients of the 2001 Young Investigator Awards

Forensic IdentityTesting: Frontiers in Molecular and Cellular Medicine:

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

The High School "Future Scientist Award"

Recipients of the 2017 Future Scientist Award

- Filip Bognar, XV. Gymnasium, Zagreb, Croatia
- Lovro Jančić, Karlovac Gymnasium, Karlovac, Croatia
- Rej Kovačević, VII. Gymnasium, Zagreb, Croatia
- Lara Primorac, XV. Gymnasium, Zagreb, Croatia
- Magda Topić, XV. Gymnasium, Zagreb, Croatia
- Borna Branimir Vuković, V. Gymnasium, Zagreb, Croatia

Scientific Program Information

Certificate of Attendance
Confirmations of attendance will be issued at the registration desk.

Young Investigator Awards
The ISABS 2017 Young Investigator Award Committee reviewed all abstract submitted for YIA and selected three recipients who will receive Young Investigator Award Certificate, the prize of 500 € and the podium presentation of their work at the special session on Wednesday, June 21st in the evening.

Future Scientist Award
Future Scientist Award is a joint project of the International Society of Applied Biological Sciences (ISABS), Ministry of Science and Education and the Croatian Education and Teacher Training Agency, which awards the best Croatian high school students' papers (essays) in the field of human biology, genetics and chemistry. The awarded high school students will receive The ISABS Future Scientist Award, the appropriate certificate, cash prize of 1,000 kuna and free registration for participation in all sessions of the conference including lectures of the Nobel laureates, the opening ceremony of the event and gala dinner. Furthemore, their mentors will be awarded free participation in the conference and an appropriate certificate. Winners are required to display their work at the conference in the form of posters.

Credits
Croatian Medical Chamber has approved to award 20 points to participants or 30 to lecturers of the 10th ISABS Conference on Forensic and Anthropologic Genetics. The CMC Credits are intended for medical doctors, members of Croatian medical Chamber towards the maintenance of the physician's license. CMC credits are valid for all other medical doctors in compliance with their national policy.

Sponsor Exhibition
Setup: June 19, 2017
Dismantling: June 24, 2017 by noon

Poster Setup
Monday, June 19, 2017
Tuesday, June 20, 2017 untill noon
Poster board numbers can be found in the author's index at the back of this brochure. Staff at the registration & info desk will help you locate the board.

Poster Sessions
Tuesday, June 20, 2017, 11:30 a.m. to 1:30 p.m.
Thursday, June 22, 2017, 12:00 p.m. to 1:30 p.m.
Friday, June 23, 2017, 12:00 p.m. to 1:30 p.m.

If unable to be present at your poster at this time, please leave a note on your poster stating date and time of your presence.

Poster Removal
Friday, June 24, 2017, until noon
Posters left on boards after noon on Friday will be discarded.

Program Changes
Oganizers assume no liability for any changes in the program due to external or unforeseen circumstances.

Registration Desk Hours

Sunday, June 18, 2017	8:00 a.m. – 5:00 p.m.
Monday, June 19, 2017	8:00 a.m. – 5:00 p.m.
Tuesday, June 20, 2017	8:00 a.m. – 5:00 p.m.
Wednesday, June 21, 2017	8:00 a.m. – 5:00 p.m.
Thursday, June 22, 2017	8:00 a.m. – 5:00 p.m.
Friday, June 23, 2017	8:00 a.m. – 12:00 p.m.

Exhibit Hall Hours

Monday, June 19, 2017	1:30 p.m. – 5:00 p.m.
Tuesday, June 20, 2017	9:00 a.m. – 5:00 p.m.
Wednesday, June 21, 2017	9:00 a.m. – 5:00 p.m.
Thursday, June 22, 2017	9:00 a.m. – 5:00 p.m.

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

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

Message Center will be available at registration desk.

Service Center provides photocopying, typing, and computer printouts at cost.

Smoking Policy: The 10th ISABS Conference on Forensic and Anthropologic and Genetics and Mayo Clinic Lectures in Individualized Medicine is declared as a non-smoking-event.

Special requirements: Registrants with special physical, communication and dietary needs should contact official service agency of the conference in advance.

Conference staff will be pleased to help you with questions you may have. Recognize them by special name badge they will be wearing.

Podcast: Lectures will be available at www.podcast.isabs.hr

General Information

Badges will be provided to participants, accompanying persons, exhibitors and press. Badges are required for admission to all conference facilities, scientific and social events during the duration of the conference. Security guards are in charge of checking badges at the conference venue. Individual without an official meeting badge will be directed to the registration desk to register or, if already registered, to purchase a replacement badge at the cost of 10 €.

Bank Services: Official currency in Croatia is the Croatian kuna (HRK). Aproximate exchange rates are 1 EUR = 7.43 HRK and 1 USD = 7.01 HRK. As exchange rates change daily, for the actual exchange rate during the Conference, please inquire at the Registration & Info Desk.

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

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

Electricity Supply: 220-240 V, 50 Hz.

Travel and Helath Insurance: Participants are advised to make their own arrangements pertinent to health and travel. By registering for the 10th ISABS Conference on Forensic and Anthropologic 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 Palace Dubrovnik assume any liability whatsoever.

Restaurants: Most restaurants in Dubrovnik are opened 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.

Shops in Dubrovnik are usually opened from 8:00 – 21:00, Monday to Friday, and from 8:00 – 15:00 on Saturdays. Some are opened on Saturday afternoon. Most shops accept major credit cards.

Taxi: Numerous Taxi stands are located throughout Dubrovnik city centre and in front of the hotels. All 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. Just a few minutes' drive northwest of medieval Dubrovnik Old Town, the cinematic location offers a phenomenal vista of the Elafiti Islands from every space. Breathtaking Adriatic Sea views from every room, a fresh new contemporary interior design scheme and intuitive service are key ingredients for

a perfect five-star seaside escape at the multi-award-winning Hotel Dubrovnik Palace. Hotel address: Masarykov put 20, 20000 Dubrovnik.

INVITED SPEAKERS

- ***Nobel Lectures***

Robert Huber (Nobel Prize in Chemistry 1988; Max-Planck-Institute, Martinsried, Germany)
Harald zur Hausen (Nobel Prize in Medicine 2008; German Cancer Research Center, University of Heidelberg, Heidelberg, Germany)
Ada Yonath (Nobel Prize in Chemistry 2009; Weizmann Institute of Science, Rehovot, Israel)

- ***Inaugural Plenary Session***

Gianrico Farrugia (Mayo Clinic, Jacksonville, FL, USA)
Eske Willerslev (University of Cambridge, United Kingdom and University of Copenhagen, Denmark)
Manolis Kellis (MIT Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA, USA and The Broad Institute of MIT and Harvard, Cambridge, MA, USA)
Walther Parson (Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria)

- ***Special Plenary Lectures***

Anthony Atala (The Wake Forest Institute for Regenerative Medicine, Wake Forest University, Winston-Salem, NC, USA)
Turi King (University of Leicester, Leicester, United Kingdom)
Jan van Deursen (Mayo Clinic, Rochester, MN, USA)

- ***Individualized Medicine Program***

Amelie Bonnefond (Institut Pasteur de Lille, Lile, France)
Jiří Damborský (Masaryk University, Brno, Czech Republic)
Nilufer Ertekin Taner (Mayo Clinic, Rochester, MN, USA)
Magnus Essand (Uppsala University, Uppsala, Sweden)
William Faubion (Mayo Clinic, Rochester, MN, USA)
Arezou Ghazani (Harvard Medical School and Dana-Faber Cancer Institute, Boston, MA, USA)
Zdenko Herceg (Int. Agency for research on Cancer, Lyon, France)
Heidi Nelson (Mayo Clinic, Rochester, MN, USA)
Eric Klee (Mayo Clinic, Rochester, MN, USA)
Gordan Lauc (University of Zagreb & Genos Glycoscience Research Laboratory, Zagreb, Croatia)
Grzegorz Nowakowski (Mayo Clinic, Rochester, MN, USA)
Tamas Ordog (Mayo Clinic, Rochester, MN, USA)
Leonard Petrucelli (Mayo Clinic, Jacksonville, FL, USA)
Dieter Saur (Technische Universität München, München, Germany)
Tim Spector (King's College London, London, England, United Kingdom)
Stephen Thibodeau (Mayo Clinic, Jacksonville, FL, USA)
Raul Urrutia (Mayo Clinic, Rochester, MN, USA)
George Vasmatzis (Mayo Clinic, Rochester, MN, USA)
Zhiguo Zhang (Institute for Cancer Genetics, Columbia University, NY, USA)
Vlatka Zoldoš (University of Zagreb Faculty of Science, Zagreb, Croatia)
Eric Wieben (Mayo Clinic, Rochester, MN, USA)

- ***Forensic Genetics and Anthropological Genetics Program***

Frederick Bieber (Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA)
Wojciech Branicki (Jagiellonian University, Kraków, Poland)
Bruce Budowle (University of North Texas, Health Science Center, Fort Worth, TX, USA)
Sandy Calloway (UC Davis, Davis, CA and Children's Hospital Oakland Research Institute, Oakland, CA, USA)
Henry Erlich (Children's Hospital Oakland Research Institute, Oakland, CA, USA)
Wolfgang Haak (Max Planck Institute for the Science of Human History, Jena, Germany)

Diana Hall (University Centre of Legal Medicine, University of Geneva and University of Lausanne, Switzerland)
Mitch Holland (The Pennsylvania State University, State College, PA, USA)
Mattias Jakobsson (Uppsala University, Uppsala, Sweden)
Manfred Kayser (Erasmus University Medical Center, Rotterdam, The Netherlands)
Peter de Knijff (Leiden University Medical Centre, Leiden, The Netherlands)
Michael Kobor (University of British Columbia, Vancouver, Canada)
Henry Lee (University of New Haven, New Haven, CT, USA)
Jessica Metcalf (Colorado State University, Fort Collins, CO, USA)
Matthias Meyer (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany)
Rasmus Nielsen (University of California Berkeley, Berkley, CA, USA)
Timothy Palmbach (University of New Haven, New Haven, CT, USA)
Antti Sajantila (Department of Forensic Medicine, University of Helsinki, Helsinki, Finland)
Pontus Skoglund (Harvard University, Boston, MA, USA)
Elmar Tobi (Wageningen University and Research, Wageningen, The Netherlands)
Chris Tyler-Smith (Wellcome Trust Sanger Institute, Hinxton, United Kingdom)

10TH ISABS CONFERENCE ON FORENSIC AND ANTHROPOLOGIC GENETICS AND MAYO CLINIC LECTURES IN INDIVIDUALIZED MEDICINE

Hotel Palace Dubrovnik
Dubrovnik, Croatia,
June 19-24, 2017

SCIENTIFIC PROGRAM

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

Monday, June 19

09:30 Opening ceremony

Opening session (D. Primorac, chair)

10:30 **Gianrico Farrugia** (Jacksonville, FL, USA): Individualized Medicine: From Promise to Practice

11:15 **Eske Willerslev** (Cambridge, UK and Copenhagen, Denmark): What we can learn from ancient genomics

12:00 **Manolis Kellis** (Cambridge, MA, USA): Interpreting the "Dark Matter" of the Human Genome in Complex Diseases

12:45 **Walther Parson** (Innsbruck, Austria): From Forensic Genetics to Forensic Genomics

1:30 **Adjourn**

1:30 ***The Third American Academy of Forensic Sciences (AAFS) and International Society for Applied Biological Sciences (ISABS) Joint Session (S. Ballou, chair)***

Personalized Medicine: Approaches and Applications (S. Vuk-Pavlović, chair)

2:30 **Stephen Thibodeau** (Rochester, MN, USA): The All of Us Research Program: Biobanking for 1 Million Participants

3:00 **Magnus Essand** (Uppsala, Sweden): Cancer Gene and Immunotherapy

3:30 **Gordan Lauc** (Zagreb, Croatia): High-throughput Glycomics for Patient Stratification: What Did We Learn from the First 50,000 Analyses?

4:00 **Vlatka Zoldoš** (Zagreb, Croatia): Epigenetic Editing Using the CRISPR/Cas9 System and Its Applications in Precision Medicine

4:30 **Raul Urrutia** (Rochester, MN, USA): Epigenetics of Pancreatic Cancer

Special Invited Lecture: Individualized Medicine (T. Ordog, chair)

5:30 **Adjourn**

Tuesday, June 20

8:30 ***The Fifth Days of Human Genetics – Prof. Ljiljana Zergollern-Čupak (D. Primorac and I. Barišić, chairs)***

Anthropological Genetics (M. Schanfield, chair)

8:30 **Mattias Jakobsson** (Uppsala, Sweden): Into Europe from the South - Genetic Impact of Hunter-Gatherers and Farmers

9:00 **Wolfgang Haak** (Jena, Germany): Into Europe from the East - Genetic Impact of Steppe Nomads

9:30 **Rasmus Nielsen** (Berkley, CA, USA): The Genetic Basis of Human Physiological Adaptation

10:00 **Chris Tyler-Smith** (Hinxton, UK): Genetic Evidence of Classic Selective Sweeps

Selected oral presentations

10:30 **Mateja Hajdinjak** (Leipzig, Germany): Reconstructing the population history of late Neandertals

10:45 **Leonardo Arias Alvis** (Leipzig, Germany): Genetic perspectives on population dynamics and cultural interactions in Northwestern Amazonia

Special Invited Lecture: Identification of Historic Persons through Genomics (M. Kayser, chair)

11:00 **Turi King** (Leicester, England, UK): The Incredible but True Identification Story of King Richard III

11:30 **Adjourn**

11:30 ***Poster Session 1***

12:00 ***Joint Session: ISABS, Croatia/USA and Regiomed Kliniken, Germany/Croatia: Why Personalised Medicine is the Way Forward (J. Brachmann and D. Primorac, chairs; Š. Anđelinović and Z. Đogaš, co-chairs)***

Complex and Undiagnosed Diseases: Genomics and Epigenomics (M. Kellis, chair)

1:30 **Leonard Petrucelli** (Jacksonville, FL, USA): Genomics and Epigenomics of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

2:00 **Amelie Bonnefond** (Lile, France): Genetics of Type 2 Diabetes

2:30 **Tamas Ordog** (Rochester, MN, USA): Metabolic Control of Transcriptional Memory

3:00 **Jiří Damborský** (Brno, Czech Republic): PredictSNP: Automated and Accurate Platform for Prediction of Disease-related Mutations for Personalized Medicine

3:30 **Eric Klee** (Rochester, MN, USA): Solving Diagnostic Odyssey Cases through Genomics

Selected oral presentations

4:00 **Hongyan Wang** (Shanghai, China): Exploring genetic landscape of neural tube defects using targeted next generation sequencing

4:15 **Ivona Sansović** (Zagreb, Croatia): Chromosomal microarray in clinical diagnostics - a study of 337 patients with congenital anomalies and developmental delays/intellectual disability

Phillips workshop (M. Schanfield, chair)

4:30 **Nevenka Dimitrova** (Philips): Assessment of information flow and biological pathway dysregulation in cancer using multi-omics data

5:30 **Adjourn**

Special Plenary Lecture: Tissue Engineering (D. Primorac, chair)

8:15 **Anthony Atala** (Winston-Salem, NC, USA): The Future of Regenerative Medicine

9:00 **Adjourn**

Wednesday, June 21

Philips lecture (D. Primorac, chair)

7:45 **Jeroen Tas** (Philips): Digital Transformation in Health

Forensic Genetics (W. Parson, chair)

8:30 **Henry Lee** (New Haven, CT, USA): DNA and Other Advanced Technologies for Crime Scene Investigation

9:00 **Frederick Bieber** (Boston, MA, USA): DNA on Trial: Interpretation of Complex Mixtures and Courtroom Admissibility

9:30 **Timothy Palmbach** (New Haven, CT, USA): Use of DNA for Border Security, Counterterrorism, and Human Rights

10:00 **Diana Hall** (Geneva/Lausanne, Switzerland): DIP-STRs for Forensic Mixture Deconvolution

10:30 **Manfred Kayser** (Rotterdam, The Netherlands): Recent Advances in Appearance Genetics and Impact on Forensic DNA Phenotyping

Selected oral presentations

11:00 **Hwan Young Lee** (Seoul, Korea): Investigation of smoking-related DNA methylation changes in Korean adults

11:15 **Kenneth Kidd (**New Haven, USA): Microhaplotypes are ready for implementation in casework

11:30 **Adjourn**

Nobel session (D. Primorac, chair)

12:15 **Robert Huber** (Martinsried, Germany): Nobel Prize in Chemistry 1988

1:00 **Paul Modrich** (Durham, NC, USA): Nobel Prize in Chemistry 2015

1:45 **Harald zur Hausen** (Heidelberg, Germany): Nobel Prize in Medicine 2008

2:45 **Ada Yonath** (Rehovot, Israel): Nobel Prize in Chemistry 2009

3:30 **Adjourn**

6:00 ***Nobel Spirit***

8:30 ***Conference Reception and Dinner Presentation of Young Investigator Awards***

Thursday, June 22

Joint Session: NGS technology in Forensics, Anthropology, and Medicine (M. Kayser, chair)

8:30 **Eric Wieben** (Rochester, MN, USA): Next-generation Sequencing Technologies and Challenges

9:00 **Matthias Meyer** (Leipzig, Germany): Tailor-made NGS for Ancient DNA Analysis

9:30 **Henry Erlich** (Oakland, CA, USA): Next Generation Sequencing of Clinically Informative DNA Mixtures

10:00 **Mitch Holland** (Pennsylvania, USA): Next Generation Sequencing of mtDNA Heteroplasmy in Forensics and Medicine

10:30 **Sandy Calloway** (Oakland, CA, USA): Next Generation Sequencing of Mixtures in Forensic and Prenatal DNA Testing

11:00 **Peter de Knijff** (Leiden, The Netherlands): Next Generation Sequencing of STRs for Individual Identification

Selected oral presentations

11:30 **Lingqian Wu** (Changsha, China): Development of a novel whole genome sequencing method for non-invasive prenatal detection of fetal submicroscopic chromosome anomalies

11:45 **Viviane Slon** (Leipzig, Germany): Retrieving Neandertal and Denisovan DNA from Late and Middle Pleistocene sediments

12:00 **Adjourn**

12:00 ***Poster session 2***

Cancer Genomics and Epigenomics (Z. Herceg, chair)

1:30 **George Vasmatzis** (Rochester, MN, USA): Genomic Biomarkers of Cancer

2:00 **Arezou A. Ghazani** (Boston, MA, USA): It Takes Two: The Complementary Roles of Constitutional and Tumor Genomics in Cancer Therapy

2:30 **Zhigou Zhang** (New York, USA): Onco-histone Mutations Reprogram Cancer Epigenomes

3:00 **Grzegorz Nowakowski** (Rochester, MN, USA): Individualized Therapy of Aggressive B-cell Lymphoma

3:30 **Dieter Saur** (Munich, Germany): Genetic Animal Models of Carcinogenesis

Selected oral presentations

4:00 **Hansjörg Rothe** (Klinikum Coburg GmbH, Germany): Ecto-5' -Nucleotidase CD73 (NT5E), vitamin D receptor and FGF23 gene polymorphisms may play a role in the development of calcific uremic arteriolopathy in dialysis patients - Data from the German Calciphylaxis Registry

4:15 **Dean Kaličanin** (Split, Croatia): Genome-wide association analysis of food intolerance in patients with Hashimoto's thyroiditis

4:30 **Adjourn**

Special Plenary Lecture: Fundamental mechanisms of aging and aging-related diseases (T. Ordog, chair)

8:15 **Jan van Deursen** (Rochester, MN, USA): Fundamental Mechanisms of Aging and Aging-related Diseases

9:00 **Adjourn**

Friday, June 23

Joint Session: Epigenetics in Forensics, Anthropology, and Medicine (T. Spector, chair)

8:30 **Michael Kobor** (Vancouver, Canada): Geographic Structure of Epigenetic Variation
9:00 **Wojciech Branicki** (Krakow, Poland): Epigenetic Estimation of Age in Forensics
9:30 **Zdenko Herceg** (Lyon, France): Epigenetics and Cancer: Searching for Angels and Devils around Our Genes
10:00 **Elmar Tobi** (Wageningen, The Netherlands)
10:30 **Nilufer Ertekin Taner** (Jacksonville, FL, USA): Genetics and Epigenomics of Alzheimer's Disease and Progressive Supranuclear Palsy
11:00 **William Faubion** (Rochester, MN, USA): Epigenetics of Inflammatory Bowel Disease

Selected oral presentations

11:30 **Sabriya Syed** (Rochester, USA): EZH2-dependent epigenetic reprogramming controls a developmental switch between modes of gastric neuromuscular regulation
11:45 **Athina Vidaki** (Rotterdam, The Netherlands): Epigenetic Discrimination Of Monozygotic Twins From Blood And Saliva In The Forensic Scenario
12:00 **Adjourn**

12:00 ***Poster session 3***

Joint Session: Microbiome Analysis in Forensics, Anthropology, and Medicine (W. Faubion, chair)

1:30 **Tim Spector** (London, UK): Gut Microbiome in Twins
2:00 **Heidi Nelson** (Rochester, MN, USA): Microbiome Analysis in Clinical Medicine
2:30 **Antti Sajantila** (Helsinki, Finland): Bones as the Key to DNA Virus History and Epidemiology
3:00 **Jessica Metcalf** (Fort Collins, CO, USA): Estimating Postmortem Interval with Microbiomes

Selected oral presentations

3:15 **Pero Dimsoski** (Miami, USA): Genotyping horse epithelial cells from fecal matter by isolation of PCR products (IPCRP) method

Special Invited Lecture: Microbial Forensics (D. Primorac, chair)

4:00 **Bruce Budowle** (Fort Worth, TX, USA): The Expanding Field of Microbial Forensics
4:30 ***Closing remarks***
5:00 **Adjourn**

ABSTRACTS OF INVITED LECTURES

POST-GWAS FUNCTIONAL ANALYSES OF VARIANTS (AND PROXY GENES) ASSOCIATED WITH TYPE 2 DIABETES

Bonnefond A

CNRS UMR 8199, European Genomic Institute for Diabetes (EGID), Institut Pasteur de Lille, University of Lille, Lille, France

Type 2 diabetes (T2D) is a complex genetic metabolic disorder which has developed into major health problem responsible for early morbidities (e.g. severe vascular complications and cancers) and mortality, with a burden increasing globally. T2D results from the progressive alteration of insulin secretion from pancreatic beta cells on a background of impaired insulin action in sensitive organs and tissues. Whilst the environment is the key risk factor for T2D at the population level, one remarkable feature is the persistence of considerable individual disease risk amongst people sharing same environment. Estimates of T2D heritability range from 40 to 70%. Genome-wide association studies (GWAS) have identified >100 loci independently contributing to T2D risk. Despite this dramatic success, there has been a considerable gap between the knowledge of the genetic contribution of these loci and the understanding of how these loci physiologically impact the disease: indeed, association does not mean causality. Therefore, translational implications for precision medicine and for the development of novel treatments have been disappointing, due to the poor knowledge of how these loci impact T2D pathophysiology.

EPIGENETIC ESTIMATION OF AGE IN FORENSICS

Branicki W

Jagiellonian University, Malopolska Centre of Biotechnology, Krakow, Poland

The investigators to track an unknown perpetrator have for many years used information about ancestry, appearance and age provided by a witness of a crime. The recent advances in genomics opened up possibility to retrieve these data solely from DNA. Age prediction has a special meaning as a forensic intelligence tool. Informative itself human age can also be used to draw more accurate forensic sketch of an unknown individual by indicating natural aging processes like hair loss, hair greying or wrinkle formation. Forensic experts have for many years sought a reliable molecular method for age estimation but the real breakthrough has come lately with the advance of epigenetics. The recent progress in analysis of the human methylome has enabled selection of multiple CpG sites showing correlation with chronological age. Practical application of DNA methylation analysis in forensics depends on successful validation of the most relevant age predictors and the most accurate prediction models. They should be applicable to casework studies involving typical forensic specimens like bloodstains, saliva, semen or bones. Quantitative methodology is necessary for determining DNA methylation status and its precision is crucial for prediction accuracy. Several age prediction methods based on DNA methylation analysis are available in forensics. They have been developed using various markers and analytical methods including pyrosequencing, SNaPshot, EpiTYPER and massively parallel sequencing. Their proper implementation in forensic DNA laboratories and correct interpretation of the obtained results is critical for reliability of age estimations.

MICROBIAL FORENSICS FOR MICROBIAL AND HUMAN IDENTIFICATION IN CRIMINAL AND CIVIL INVESTIGATIONS

Budowle B

University of North Texas Health Science Center

Microbial forensics originally was defined as the discipline of applying scientific methods to the analysis of evidence related to bioterrorism, biocrimes, hoaxes, or the accidental release of a biological agent or toxin for attribution purposes. Since its inception in the aftermath of the anthrax letter attacks, the field has focued on microorganism and toxin detection and those incidents considered bioterrorism or biocrime. In recent years, technology, particularly massively parallel sequencing, and bioinformatics have advanced such that is now relatively straightforward to characterize the whole genome of microorganisms from single homogeneous to complex metagenomic samples. These same technical capabilities allow for expanded applications beyond bioterrorism and biocrime. Human identity applications also can be considerd, such as human identification, body fluid characterization, postmortem interval estimation, geolocation and biocrimes involving tracking of infectious agents. To be more consistent with these expanded capabilities, microbial forensics should be defined as the discipline of applying scientific methods to the analysis of microbial evidence in criminal and civil cases for investigative purposes. This presentation will describe the past and state-of-the-art capabilities of microbial forensics to assist law enforcement in developing investigative leads.

PROBE CAPTURE NEXT-GENERATION SEQUENCING OF DEGRADED AND MIXED DNA SAMPLES IN FORENSIC TESTING

Calloway C[1,2], Bose N[1,2], Gordon R[1], Taylor C[1,2], Shih S[1,2], Vohr S[3], Almada G[1,2], Gonscalves AB[1], Sensabaugh G[4], Green R[3], Henry Erlich H[1]

[1]Children's Hospital Oakland Research Institute, University of California San Francisco Benioff Children's Hospital, Oakland, California, USA
[2] Forensic Graduate Group, University of California, Davis, California, USA
[3]University of California, Santa Cruz, California, USA
[4]University of California, Berkeley, California, USA

Forensic biological samples can often be highly compromised (degraded, limited, or mixed). Alternative markers such as nuclear Single Nucleotide Polymorphisms (SNPs) or mitochondrial DNA (mtDNA) can be analyzed for increased genotyping success in cases where STR analysis using conventional electrophoretic methods fail. Next-generation sequencing (NGS) methods have been developed which have the potential to overcome many of the limitations of conventional methods used for analyzing mtDNA and SNP markers. Recently, we have developed probe capture NGS systems for massively parallel sequencing the entire mitochondrial genome and over 450 nuclear polymorphisms for the analysis of highly degraded, limited and mixed DNA samples. This approach uses DNA probes to enrich targeted regions from randomly fragmented DNA libraries for clonal, massively parallel sequencing, thereby maximizing recovery of short DNA fragments characteristic of forensic samples. The clonal sequencing aspect of NGS allows for analysis of the components of a mixture separately and by counting the number of sequence reads assigned to each individual contributor of the mixture. We have successfully applied this system to sequence the entire mitochondrial genome of limited and highly degraded DNA from hair and bones as well as mixtures. Mitochondrial DNA mixtures were analyzed using both frequency and phylogenetic based approaches. A software program (mixemt) was developed which uses an expectation-maximization algorithm to resolve major and minor haplotypes in a mtDNA mixture using phylogenetic information and applied to mtDNA mixture analysis. We have also demonstrated proof of concept of the nuclear SNP capture NGS system for analyzing degraded DNA from telogen hairs and bones as well a mixed DNA samples.

PREDICTSNP: AUTOMATED AND ACCURATE PLATFORM FOR PREDICTION OF DISEASE-RELATED MUTATIONS FOR PERSONALIZED MEDICINE

Bendl J[1], Stourac J[1], Musil M[1], Wieben E[2], Zendulka J[3], Brezovsky J[4], **Damborsky J[4]**

[1]Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, [2]Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, Minnesota, [3]Department of Information Systems, Faculty of Information Technology, Brno University of Technology, [4]Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne's University Hospital Brno

Understanding the functional impact of genetic variations that play a key role in the disease development is one of the objectives of the human genetics and personalized medicine [1]. The difficulty of interpretation of noncoding variants caused that the main attention has been paid to protein coding regions constituting only 1-2% of the genome. Although several tools aiming at predictions on whole genome have been published recently, they report performance metrics measured on inhomogeneous datasets compiled from highly unequal number of mutations from each genome region. As a result, the performance is skewed by the regions with the highest number of representatives in variation databases. We have addressed this problem by construction of several well balanced data sets combining neutral and deleterious mutations. These datasets enabled extensive validation of existing tools and construction of automated classifiers PredictSNP 1.0 [2] and PredictSNP 2.0 [3], combining prediction from several individual tools into a single consensual prediction. The PredictSNP 1.0 predicts effect of mutations at the amino acid level and combines the results from six established prediction tools: MAPP, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT and SNAP. The PredictSNP 2.0 predicts effect of mutations at the DNA level tailored to the specific characteristics of regions in the genome and integrates predictions from five prediction tools CADD, DANN, FATHMM, FunSeq2 and GWAVA. Moreover, we provide access to experimentally validated information from eight genetic databases. Web interface of PredictSNP 1.0 and 2.0 enables an easy access to the consensus and all integrated tools providing their unified confidences derived from their performance on the datasets. The server is available at loschmidt.chemi.muni.cz/predictsnp.[1] Schork, N.J., 2015: Personalized Medicine: Time for One-Person Trials. Nature 520: 609–611.[2] Bendl, J., et al., 2014: PredictSNP: Robust and Accurate Consensus Classifier for Prediction of Disease-Related Mutations. PLOS Computational Biology 10: e1003440.[3] Bendl, J., et al., 2116: PredictSNP 2: A Unified Platform for Accurate Evaluation of SNP Effect by Exploiting Different Characteristics of Variants in Distinct Genomic Regions. PLOS Computational Biology 12: e1004962.

MASSIVELY PARALLEL SEQUENCING (MPS) OF STRS FOR INDIVIDUAL IDENTIFICATION

van der Gaag KJ[1], de Leeuw RH[2], Sijen T[1], Storts DR[3], **de Knijff P[2]**

[1]Netherlands Forensic Institute, Division Biological Traces, 2490 AA The Hague, The Netherlands, [2]Leiden University Medical Centre, Department of Human Genetics, 2300 RC Leiden, The Netherlands, [3]Promega Corporation, WI 53711 Madison, USA

Forensic DNA analysis predominantly involves identification of human donors by analysis of short tandem repeats (STRs)using Capillary Electrophoresis (CE). We explored the use of Massively Parallel Sequencing (MPS) as an alternative technology to analyse STRs. We sequenced forensic autosomal STRs and Y-STRs in a large number of globally dispersed human individuals using prototype versions of Promega PowerSeq™ systems and directly compared the newly obtained MPS-based genotypes with CE-obtained genotypes in the same individuals. Regarding fragment length, complete concordance between the MPS and CE-based data was found. As expected, MPS presented much more genetic variation due to repeat motif sequence heterogeneity combined with additional SNPs in flanking regions of the PCR products. We will present an in-depth analysis of the MPS-based results and, where relevant, highlight the additional (discrimination) power of MPS compared to CE. We will also show the relevance of this additional genetic variation combined with increased detection sensitivity in deconvoluting complex mixed forensic crime scene samples.

THE ANALYSIS OF MIXTURES USING NGS: APPLICATIONS TO NON-INVASIVE PRENATAL TESTING FOR BETA-THALASSEMIA AND SICKLE CELL ANEMIA

Erlich H[1], Carlberg K[2], Bose N[1], La A[2], Calloway C[1]

[1]Children's Hospital Oakland Reserch Institute, 5700 Martin Luther King Jr. Way, Oakland, California, US, [2]UCSF Benioff Children's Hospital Oakland, 747 52nd Street, Oakland, California, 94609, US

The development of massively parallel and clonal next generation sequencing (NGS) has enabled the digital analysis of the composition of mixtures by counting sequence reads representing the individual components of the mixture. Our initial application of NGS used amplicon based sequencing of the highly polymorphic HLA genes with the 454 technology to quantitatively analyze maternal cells in the child's circulation, kidney donor DNA in the plasma of a transplant recipient, and fetal DNA in the plasma of a pregnant woman. Subsequently, we applied a NimbleGen hybrid capture probe panel followed by NGS on the Illumina MiSeq platform to analyze mitochondrial DNA in forensic mixtures as well as fetal DNA in maternal plasma. The hybrid capture method is well suited to the analysis of the small DNA fragments characteristic of cell free DNA as well as of many forensic specimens.The analysis by NGS of fetal DNA in maternal plasma has been applied to the diagnosis of chromosomal aneuploidies by a number of companies for several years. We have applied it to the diagnosis of the autosomal recessive diseases, sickle cell anemia and beta thalassemia, by using a capture probe panel that covers the beta-globin gene and linked SNPs as well as >450 genomic polymorphisms used to estimate the fetal fraction. The fetal fraction is estimated by counting paternally transmitted sequence reads for alleles present in the fetus but absent in the mother. In our studies of contrived mixtures and plasma samples, around 25% of the polymorphisms reveal this informative pattern and this subset is used to estimate fetal fraction. The fetal beta-globin genotype is inferred by counting sequence reads corresponding to the beta-globin 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. This system promises to provide a robust non-invasive test for sickle cell anemia and beta-thalassemia and represent a model approach for other autosomal recessive diseases.

SYSTEMS BIOLOGY APPROACHES FOR THERAPEUTIC AND BIOMARKER DISCOVERIES IN NEURODEGENERATIVE DISEASES

Ertekin-Taner N

Mayo Clinic, Jacksonville, Florida, USA

Many neurodegenerative diseases, including Alzheimer's disease (AD) and progressive supranuclear palsy (PSP), are characterized by abnormal accumulation of endogenous proteins in the brain, which are thought to be central to the triggering of a cascade of pathophysiologic events that eventually culminate in cell death and clinical syndromes of dementia and/or movement disorders. These neurodegenerative diseases are likely influenced by a multitude of genetic and environmental risk factors and their complex interplay. The array of risk factors that lead to these diseases are also likely to be heterogeneous amongst the patients, which further complicates the search for drug targets, biomarkers and their potential downstream beneficial use in any given patient. For this reason, drug target and biomarker discovery efforts in neurodegenerative diseases have to focus on identification of both molecular mechanisms that are *commonly perturbed* between patients, as well as those mechanisms that may underlie the *heterogeneity* in diseases. To overcome this massive challenge, we and others are utilizing large-scale generation and analyses of multi-omics data from well-phenotyped human post-mortem and ante-mortem cohorts, as well as model systems. We aim to integrate multi-omics and clinical endophenotype data to build a model(s) of disease that captures these common and heterogeneous pathomechanisms; ultimately yielding novel drug targets and biomarkers. Using genome-wide gene expression data from brain tissue of patients with AD and PSP, we identified differentially expressed genes and co-expression networks, which associate with these diseases and their specific neuropathologies. These networks are enriched for transcripts that are primarily expressed in specific central nervous system (CNS) cell types, which may be informative about vulnerable cell populations in these conditions. Further, we observe enrichment of these networks for certain biological processes which implicate a number of molecular pathways that are perturbed in AD and PSP. We evaluate these networks for presence of disease risk genes and regulatory variants which provides additional evidence for their role in conferring disease. Our findings suggest that these co-expression networks and key molecules within these networks can serve as novel therapeutic and biomarker targets in neurodegenerative diseases.

PERSONALIZED CANCER IMMUNOTHERAPY

Essand M

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Immunotherapy of cancer has emerged as one of the most promising new developments in medicine. This is mainly due to the introduction of immune checkpoint inhibitors (anti-CTLA4, anti-PD1 and anti-PDL1 antibodies), which kill cancer cells through activation of anti-tumor cytotoxic T lymphocytes (CTLs). Their action has demonstrated that the balance between immune activity and immune suppression in cancer tissues is a key determinant of success. Pre-existence of CTLs recognizing tumor-associated antigens is required to achieve a good response. Although response rates are high, most cancer patients do not yet benefit from immune checkpoint inhibitors. If anti-tumor CTLs are not pre-existing, but an antigen that could be recognized by anti-tumor CTLs has been identified, T-cells isolated from peripheral blood of cancer patients can be genetically engineered with a new receptor targeting the antigen. Upon adoptive transferred back to the patient, the engineered T-cells specifically recognize and kill cancer cells presenting the target antigen. If the target antigen is a cell surface antigen so called chimeric antigen receptor (CAR)-engineered T-cells are developed. They recognize target cells in an HLA-independent manner, similar to antibody recognition, but the action of killing is T-cell mediated. If the antigen is intracellular, T cell receptor (TCR)-engineered T-cells can be developed to recognize peptides derived from the antigen in the context of HLA presentation. The advancements in deep and ultra-deep sequencing means that tumor material or liquid biopsies from cancer patients can be sequenced for identification of patient-specific neoantigens generated by somatic missense mutation in cancer tissues. Neoantigens are truly cancer cell-specific and represent the ultimate targets for personalized cancer immunotherapy. Upon verification, neoantigens can be developed as patient-specific cancer vaccines. Alternatively, neoantigen-specific T cells can be cloned and their TCRs sequenced. Personalized TCR-engineered T-cells recognizing the neoantigenic epitope can then be developed for adoptive transfer immunotherapy.

INDIVIDUALIZED MEDICINE: FROM PROMISE TO PRACTICE

Farrugia G

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We are now 13 years out from the publication of the first human genome sequence. After an initial relatively slow start, genomics and related omics, is now a powerful research tool and is now also part of routine clinical practice. The precision medicine initiative in the United States announced by the then President Obama in conjunction with the NIH and the Million European Genomes Alliance in Europe, highlight the opportunities to use 'omics' to markedly accelerate our discovery, translation and clinical endeavors. The now increasingly rapid transition from research into clinical practice is spearheaded by pharmacogenomics. Pharmacogenomics is a significant evolution from pharmacogenetics. While in pharmacogenetics we were limited to looking at the relationship between single or a few genes and a particular drug, pharmacogenomics coupled with next generation sequencing allows a much broader investigation of the relationship between multiple genes and multiple drugs. The interaction between drugs commonly used such as pain medications and genes such as CYP2D6 may help determine the use of the right drug at the right dose at the right time improving patient outcomes and reducing side effects. The use of next generation sequencing has also changed the diagnostic odyssey workup, leading to new diagnoses and already transitioning to metabolomics as a stepping stone to new therapeutics. Epigenomics is also making the transition from being solely focused on cancer to expanding to other fields including new laboratory tests and screening tools. Pharmacoepigenomics is an even newer field, studying how epigenetically determined variability provides a mechanism by which adaptive responses are "remembered" and predict future responses in a variety of areas including drug response. Individualized medicine and next generation sequencing has also accelerated the movement of microbiome research to the practice including new test development and treatment of common diseases. For next generation sequencing and 'omic' approaches to have the maximal impact on understanding and treating a variety of diseases we will continue to need clean input (standardized samples), and large investments in bioethics, bioinformatics and IT and well-coordinated biobanking and biorepositories.

EPIGENETICS OF INFLAMMATORY BOWEL DISEASE

Faubion WA

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Regulatory T (Treg) cells expressing the transcription factor FOXP3 play a pivotal role in maintaining immunologic self-tolerance. We and others have previously shown that EZH2 is recruited to the FOXP3 promoter and its targets in Treg cells. To further address the role for EZH2 in Treg cellular function, we have now generated mice that lack EZH2 specifically in Treg cells ($EZH2^{\Delta/\Delta}FOXP3^{+}$). We find that EZH2 deficiency in $FOXP3^{+}$ T cells results in lethal multi-organ autoimmunity. We further demonstrate that $EZH2^{\Delta/\Delta}FOXP3^{+}$ T cells lack a regulatory phenotype *in vitro* and secrete pro-inflammatory cytokines. Of special interest, $EZH2^{\Delta/\Delta}FOXP3^{+}$ mice develop spontaneous inflammatory bowel disease. Guided by these results, we assessed the FOXP3 and EZH2 gene networks by RNA-Seq in isolated intestinal $CD4^{+}$ T cells from patients with Crohn's disease. Gene network analysis demonstrates that these $CD4^{+}$ T cells display a Th1/Th17-like phenotype with an enrichment of gene targets shared by FOXP3 and EZH2. Combined, these results suggest that the inflammatory milieu found in Crohn's disease could lead to or result from deregulation of FOXP3/EZH2-enforced T cell gene networks contributing to the underlying intestinal inflammation.

INTO EUROPE FROM THE EAST – GENETIC IMPACT OF STEPPE NOMADS

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Recent studies in archaeogenetics have stirred the teacup of European prehistory by providing direct insights into the biological relatedness and ancestry of prehistoric individuals associated with particular archaeological cultural phenomena. Accompanying progress in genomic data analysis at population level has transformed the way in which we approach the wealth of new and contextual information from the diverse neighboring disciplines such as anthropology, archaeology and linguistics. I will illustrate the power and limitations of genome-wide ancient DNA focusing on a major migration starting ~5000 years ago. While the preceding Middle Neolithic period sees a resurgence of western hunter-gatherer ancestry throughout much of Europe, the contemporaneous Yamnaya pastoralists of the Russian steppes share ancestry with both the preceding eastern European foragers and a fourth component from south of the Caucasus, which is very different from early farmers in the Levant. With the emerging Bronze Age this blend of 'steppe ancestry' expands westward reaching Central Europe ~4,500 years ago. Individuals associated with the Corded Ware complex traced ~75% of their ancestry to the Yamnaya, which persisted in subsequent Bronze Age Europeans and is ubiquitous in present-day Europeans. The arrival of 'steppe ancestry' documents a (second) major expansion into Europe in the 3rd millennium BCE and requires reconciliation with archaeological record as the nature and mode of this expansion is less clear than the preceding 'Neolithic transition'. Consequently, the magnitude of the second expansion also has bearings on the spread of Indo-European language groups, supporting an older (but vital) alternative to the language-farming dispersal hypothesis. Intriguingly, the recent findings of plague-related agents in Final Neolithic and Bronze Age individuals from central and eastern Europe extend the scope of explanations to also include paleo-epidemiological scenarios.

DIP-STRS FOR FORENSIC MIXTURE DECONVOLUTION

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DIP-STRs are compound markers formed by a Deletion/Insertion Polymorphism linked to a microsatellite. These markers enable the deconvolution of unbalanced DNA mixtures from two individuals of any sex, up to 1,000-fold excess of the major contributor. Here, we discuss three potential applications of DIP-STRs in forensics: crime caseworks, early non-invasive prenatal paternity tests, and individual ancestry inference.DIP-STRs applied to eight cases provided additional investigative leads enabling the analysis of unbalanced DNA mixtures from the same sex or female minor/male major DNA mixtures and the discrimination of two brothers from a trace containing high levels of female DNA. Positive results were obtained for traces with 16,000-fold excess of major DNA collected up to several months after an aggression.In addition, the analysis of circulating DNA from 50 pregnant women showed that DIP-STRs can detect the fetal DNA transmitted from the father as early as 10 weeks of pregnancy. Finally, an initial set of 24 markers analysed with the STRUCTURE Bayesian algorithm is sufficient to discriminate between the five major HGDP-CEPH reference populations of Africa, Europe, East Asia, Oceania and Native America.In conclusion, DIP-STRs significantly contribute to the analysis of mixed traces that are difficult to exploit with standard methods. Moreover, DIP-STRs can potentially be used for early non-invasive prenatal paternity test to help all those women who get pregnant during the time of a sexual abuse. Finally, these markers may help direct the course of investigations in the absence of suspect match by providing the ancestry estimate of an unknown DNA.

EPIGENETICS AND CANCER: SEARCHING FOR ANGELS AND DEVILS AROUND OUR GENES

Herceg Z

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Recent advances in epigenetics and epigenomics have a tremendous impact on our understanding of biological phenomena and have galvanized the research of complex diseases, notably cancer. The epigenome can be viewed as an interface between the genome and environment, therefore aberrant epigenetic events associated with environmental stressors are likely to play an important role in the onset and progression of different human malignancies. Aberrant epigenetic events influence critical cellular events (such as gene expression, DNA repair, genome stability, carcinogen detoxification, and cell death), deregulation of which is known to promote cancer development. There is also growing evidence that epigenetic changes may be risk factor-specific ("fingerprints") that should prove instrumental in the discovery of new biomarkers in cancer. I will review the state of the science of epigenetics associated with environmental stressors and cancer risk, highlighting key developments in the field. Recent conceptual and technological advances in epigenetics and ongoing efforts aiming to identify epigenetic targets that could be exploited in cancer prevention and therapy as well as molecular epidemiology will also be discussed.

RATES, DAMAGE, AND DRIFT: CONSIDERATIONS WHEN ASSESSING MTDNA HETEROPLASMY

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Resolution of mitochondrial (mt) DNA heteroplasmy is possible when applying a massively parallel sequencing (MPS) approach. Prior to implementation in forensic investigations, weight estimates, reporting thresholds, and interpretation criteria for matching heteroplasmic sequences will need to be established that address a number of important topics, including rates of heteroplasmy, DNA damage, and drift of sequence variants in sample types such as human hair shafts. This paper will present findings in each of these areas. Error rates and system noise were used to establish analytical and reporting thresholds for heteroplasmy when using the MiSeq from Illumina. Rates of heteroplasmy in a large dataset of Europeans revealed that 41% of the population exhibits heteroplasmy, with little correlation to gender or age. Heteroplasmy is observed in a relatively small proportion of the mtDNA control region. Weight estimates are dependent on the absence of linkage between heteroplasmy and sample haplotype, and on individual rates at each nucleotide position. DNA damage, occurring through exposure to environmental insults and even during storage of DNA samples, impacts the interpretation of MPS mtDNA data when assessing heteroplasmy and must be mitigated through replicate testing and by other means. Drift of heteroplasmic sequence variants in human hair shafts is considerable and includes unexpected sites of heteroplasmy or DNA damage. As a whole, these considerations have been carefully evaluated so that reliable analysis of mtDNA heteroplasmy can be reported in forensic investigations.

NEW WAYS OF VISION: PROTEIN STRUCTURES IN TRANSLATIONAL MEDICINE AND BUSINESS DEVELOPMENT, MY EXPERIENCE

Robert Huber

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My lecture will start out with very brief remarks on the history of protein crystallography and continue with our studies since 1970 on proteolytic enzymes and their control. Proteolytic enzymes catalyse a very simple chemical reaction, the hydrolytic cleavage of a peptide bond. Nevertheless they constitute a most diverse and numerous lineage of proteins. The reason lies in their role as components of many regulatory physiological cascades in all organisms. To serve this purpose and to avoid unwanted destructive action, proteolytic activity must be strictly controlled. The regulatory principles unveiled by structural studies offer new opportunities for therapeutic purposes as illustrated with components of the blood coagulation cascade, with dipeptidylpeptidase IV in diabetes, with the proprotein convertase furin for novel antibiotics, and the essential intracellular protease, the proteasome in cancer and autoimmune disorders. I then will let you share my experience with the foundation and development of two biotech companies with different business models, but both based on basic academic research in structural biology: Proteros (www.Proteros.com) offers enabling technology services for Pharma- and Crop science companies imbedding all steps of the workflow molecular and structural biology can provide and commands and uses its platform for the generation of leads from identified targets to in vivo Proof of Concept (PoC). Suppremol (www.Suppremol.com) specializes in the development of novel immune-regulatory therapeutics for the treatment of autoimmune diseases on the basis of a recombinant, soluble, non-glycosylated version of the human Fcg receptor IIB and of receptor binding antibodies. Suppremol was recently acquired by Baxter International Inc. (NYSE:BAX) offering an ideal setting for its therapeutic projects.

ANCIENT DNA REVEALS THE GENOMIC FOOTPRINTS OF STONE-AGE EUROPEANS

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Genomic information from ancient human remains is beginning to show its full potential for learning about human prehistory. I review the last few years' dramatic finds about European prehistory based on genomic data from humans that lived many millennia ago and relate it to modern-day patterns of genomic variation. The early times, the upper Paleolithic, appears to contain several population turn-overs followed by more stable populations after the Last Glacial Maximum and during the Mesolithic. Scandinavia is one of the last areas to become ice-free and was colonized by several pioneering groups adapted to high-latitude conditions. Some 11,000 years ago the migrations driving the Neolithic transition start from around Anatolia and reach the north and the west of Europe millennia later. These findings show that culture and lifestyle were major determinants of genomic differentiation and similarity in pre-historic Europe rather than geography as is the case today.

RECENT ADVANCES IN HUMAN APPEARANCE GENETICS AND IMPACT ON FORENSIC DNA PHENOTYPING

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Retrieving appearance details of an unknown trace donor from his/her DNA left behind at a crime scene can be useful information to focus police investigation towards finding unknown perpetrators of crime, who typically cannot be identified via standard DNA profiling. Moreover, establishing appearance information from DNA obtained from human remains can be useful for finding potential relatives in missing person cases where no ante-mortem samples or relatives are available but can also be relevant for anthropological purposes. Over recent years, this notion has led to the establishment and further development of DNA-based prediction of appearance traits such as in the context of Forensic DNA Phenotyping (FDP). Of all externally visible characteristics, genetic knowledge on pigmentation traits is most advanced, which led to the development and forensic validation of suitable genotyping and statistical tools for predicting categorical eye, hair, and skin color from crime scene DNA, with accuracies deemed suitable for practical applications. Recent activities towards understanding the genetic basis of human appearance more comprehensively concern pigmentation traits on more detailed level, various hair traits including head hair structure, head hair loss particularly in men, eye brows, as well as some other externally visible traits. This talk will summarize recent progress in advancing genetic knowledge of human appearance by identifying new genes and predictive DNA variants, and will discuss their impact on Forensic DNA Phenotyping.

FROM GENOMICS TO THERAPEUTICS: UNCOVERING AND MANIPULATING THE CIRCUITRY OF NON-CODING DISEASE VARIANTS AND CANCER

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Perhaps the greatest surprise of human genome-wide association studies (GWAS) is that 90% of disease-associated regions do not affect proteins directly, but instead lie in non-coding regions with putative gene-regulatory roles. This has increased the urgency of understanding the non-coding genome, as a key component of understanding human disease. To address this challenge, we generated maps of genomic control elements across 127 primary human tissues and cell types, and tissue-specific regulatory networks linking these elements to their target genes and their regulators. We have used these maps and circuits to understand how human genetic variation contributes to disease and cancer, providing an unbiased view of disease genetics and sometimes re-shaping our understanding of common disorders. For example, we find evidence that genetic variants contributing to Alzheimer's disease act primarily through immune processes, rather than neuronal processes. We also find that the strongest genetic association with obesity acts via a master switch controlling energy storage vs. energy dissipation in our adipocytes, rather than through the control of appetite in the brain. We also combine genetic information with regulatory annotations and epigenomic variation across patients and healthy controls to discover new disease genes and regions with roles in Alzheimer's disease, heart disease, and prostate cancer. Lastly, we manipulate these circuits by genome editing and gene targeting in human cells and in mice, demonstrating tissue-autonomous therapeutic avenues in Alzheimer's disease, obesity, and cancer. These results provide a roadmap for translating genetic findings into mechanistic insights and ultimately therapeutic treatments for complex disease and cancer.

THE INCREDIBLE BUT TRUE IDENTIFICATION STORY OF KING RICHARD III

King T

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When Turi King was asked to advise and lead on any required genetic analysis for the Grey Friars excavation in Leicester, ostensibly to look for the lost remains of King Richard III, she was told not to worry, her involvement would be minimal as his remains were unlikely to ever be found. Turi will give an overview of the Richard III project from the excavation through to the historic, forensic, osteological, genetic and genealogical analysis leading to the identification of Skeleton 1 from the Grey Friars site being that of King Richard III, whose remains had been missing for over 500 years.

SOLVING DIAGNOSTIC ODYSSEY CASES THROUGH GENOMICS

Klee EW

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The advent of accessible and affordable genome-wide clinical testing has led to an unprecedented advancement in the diagnosis of monogenetic Mendelian disease and improvement in our ability to care for patients with rare and undiagnosed diseases. Many of these patients receive extensive clinical testing without obtaining a genetic diagnosis, enduring a protracted and arduous journey on their diagnostic odyssey. The specific genetic defects identified are often extremely rare, with many representing N=1 situations, however, rare disease is relatively common, with ~25 million people in the United States suffering from some form of rare genetic disorder. Fortunately, the clinical use of exome sequencing has resulted in a genetic diagnosis for >25% of the patients tested. While an extraordinary advancement in diagnosis rates has occured from where the clinical community was just 5 years ago, there still remains ~75% of patients where clinical testing returns an ambiguous or completely empty result. At the Mayo Clinic, we have established a research program focused on further investigation into these cases. Using complementary -omic testing (whole-genome sequence and RNA sequencing), *in silico* protein structure and dynamic modeling, laboratory based functional studies, and extensive world-wide collaboration, additional genetic diagnoses have been made. Here, we report on the process, success, and efforts for systematic improvement of this Undiagnosed Disease Program.

GEOGRAPHIC STRUCTURE OF EPIGENETIC VARIATION

Kobor MS

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Epigenetics is emerging as an important interface between environments and the genome. In human populations, the most studied epigenetic modification is DNA methylation. Primarily occurring in the context of CpG dinucleotides, DNA methylation is tightly linked to gene expression. DNA methylation accompanies tissue differentiation and also demonstrates plasticity across the lifecourse. Variable DNA methylation has also been associated with different human populations, in part due to genetic variation influencing DNA methylation. Highlighting data from several collaborative projects, this presentation will focus on diverse and temporally distinct geographic and population structures that partake in sculpting the human DNA methylome. These include comparative studies of 180 cell lines derived from one African and one European population, DNA methylation profiles for 362 rainforest hunter-gatherers and sedentary farmers in Western Africa, and epigenetic interrogation of 100 seniors living in the Nicoya peninsula in Costa Rica, a region with high longevity. Collectively, these studies suggest that the DNA methylation landscape is related to ethnicity, evolution and temporal changes in lifestyle and habitat, as well as healthy aging in specific regions.

HIGH-THROUGHPUT GLYCOMICS FOR PATIENT STRATIFICATION: WHAT DID WE LEARN FROM THE FIRST 50,000 ANALYSES

Lauc G

University of Zagreb & Genos Glycoscience Research Laboratory, Zagreb, Croatia

The majority of proteins are glycosylated and their glycan parts have numerous structural and functional roles. This essential posttranslational modification is generated by a complex biosynthetic pathway comprising hundreds of glycosyltransferases, glycosidases, transcriptional factors, ion channels and other proteins. Since glycans are created without the genetic template, alternative glycosylation creates an additional layer of protein complexity by combining genetic variability with past and present environmental factors. Individual variability in glycome composition is very large, but glycosylation of an individual protein seems to be under strong genetic influence, with heritability being up to 80% for some glycans. Structural details of the attached glycans are of great physiological significance and many pathological conditions are associated with various types of glycan changes. For example, glycans attached to the Fc part of immunoglobulin G are important modulators of IgG effector functions. Slight modifications in the composition of the IgG glycome significantly tune IgG towards binding to different Fc receptors and can convert IgG from a pro-inflammatory effector into an anti-inflammatory agent.

Since the onset of genome wide association studies, thousands of genetic loci have been associated with different diseases and traits. However, in the last few years it is becoming increasingly clear that variations in a DNA sequence are only a beginning of the understanding of complex human diseases. Genetic polymorphisms have to be put in the context of complex biology of life and a more elaborate approach that combines different ‘omics phenotypes is needed to understand disease mechanisms and perform patient stratification that transcends genomics. Glycomics, as by far the most complex epiproteomic modification, has an immense potential in this respect, which is only beginning to be investigated.

DNA TYPING IN HIGH PROFILE CASES

Lee H

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The value of DNA evidence has been demonstrated in all aspects of criminal investigations, civil litigations, major disasters and national security issues. As science and technology continue to advance, the importance and the value of DNA evidence in the protection of our society will also continue to grow. However, forensic scientists do not usually make the decisions about the extent of DNA evidence involvement in cases however. crime scene investigators, or evidence technicians usually involve in recognition and collection of DNA evidence at the initial investigative stages. In the litigation stages, prosecution and defense attorneys direct the utilization of DNA evidence. In the adjudicative stages, the judges control the admission and legal ruling of DNA evidence. There is no guarantee that these groups will understand the limitation of DNA evidence In addition, the public and media attentions also added additional pressure for Forensic DNA examiner. The examiner bears the burden of legal requirements and scientific limitations in interpretation results and reconstruction of the case. Case example; such as JFK assentation, William smith and Kobe Brown rape trail, in the O. J. Simpson, Jason Williams, Peterson, Jon Benet Ramsey, Vincent Foster, Elizabeth Smart, Chandra Levy Casey Anthony case. And Clinton case will be used to illustrate DNA typing procedures, the value of DNA data bank, the newest developments in DNA technology, and the legal and scientific issues in utilization of DNA evidence in High Profile cases.

MICROBIAL COMMUNITY CHANGE ACCURATELY PREDICTS THE POSTMORTEM INTERVAL

Metcalf J

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Estimating the postmortem interval (PMI) is a critical step in many crime scene investigations. Current methods such as those that rely on insects or chemical decay products are limited because they may only work for a short duration, are specific to particular geographic regions, are not very accurate, or a combination of these factors. Therefore, it is important to develop new tools that complement existing tools to improve accuracy for estimating PMI. From an ecology perspective, a corpse is a rich, ephemeral source of nutrients that catalyzes a cascade of competition between microbes, invertebrates, and scavengers. This competition results in a rapidly changing community of microbes that efficiently consume each other, and corpse-related biochemical waste. Thus, microbial communities are excellent targets for tracking temporal changes during corpse decomposition. However, the feasibility of using microbes for forensic purposes is only just beginning to be rigorously tested. Recently postmortem microbiome studies have shown that amplicon-based next-generation sequencing data can be used to accurately estimate PMI both in model organisms as well as in humans. This talk will describe initial studies demonstrating that bacterial (16S rRNA) and microbial eukaryotic (18S rRNA) data associated with decomposing mice in laboratory experiments and humans in outdoor experiments allow for accurate predictions of PMI. Furthermore, this talk will highlight an on-going large-scale multidisciplinary project in which three anthropological research facilities will place human bodies over multiple seasons to train and test a robust regression model for estimating PMI in humans, and establish error rates for use in the criminal justice system.

TAILOR-MADE NGS FOR ANCIENT DNA ANALYSIS

Meyer M

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Technical improvements made over the past years have greatly improved our ability to recover sequences from highly degraded DNA. One important step forward was the development of a single-stranded DNA library preparation method that converts the two complementary strands of double-stranded DNA fragments separately into library molecules. This method increases the amount of sequence information that can be recovered from ancient DNA by approximately a factor of 10 compared to conventional double-stranded techniques and was used to reconstruct the first high-quality genome sequences from archaic humans. Other advances include the development of decontamination methods for ancient bones and teeth as well as DNA extraction methods optimized for the recovery of extremely short DNA fragments. Combining these methods allowed for the recovery of mitochondrial and nuclear DNA sequences from the ~430,000-old hominins from Sima de los Huesos, Spain, extending the temporal limits of ancient DNA analysis of non-permafrost remains by a few hundred thousand years. Nearly the entire sample preparation workflow has now been automated on liquid handling platforms, enabling high-throughput screening of large numbers of samples with the most sensitive methods. This presentation will describe the obstacles that had to be overcome to enable automation and highlight some of the applications of this technology to research on human evolutionary history.

THE GENETIC BASIS OF HUMAN PHYSIOLOGICAL ADAPTATION

Nielsen R

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As the first anatomically modern humans spread around the globe, they had to adapt to a new and diverse set of environments. Today we can find the traces of this evolutionary process in the genomes of modern humans. In this talk, I will give two examples of human physiological adaptation to the local environment. The first example concerns physiological adaptation to the hypoxic environment of the high-altitude plateau of Tibet. Tibetans harbor genetic variants in two genes, EPAS1 and EGLN1, that affect hemoglobin production. Recently, we have shown that the adaptive EPAS1 haplotype was transferred into humans by introgression from Denisovans. I will discuss recent progress on understanding the process of adaptive introgression in humans and its role in altitude adaptation. The second example is adaptation of the indigenous people of Greenland, the Inuit, to life in the Arctic, including low temperatures and a diet based primarily on fish and marine mammals and rich in ω-3 polyunsaturated fatty acids (PUFAs). Studies of Inuit have been used to argue for the benefits of a high dietary intake of ω-3 PUFAs. We recently performed the first scan of Inuit genomes for signatures of adaptation and find extreme signals in several loci, relating to metabolism of fatty acids, particularly PUFAs. Using association mapping, we show that the selected alleles have strong effects on a number health-related phenotypes, and we replicate the findings in Europeans. Our results show that Inuit have unique physiological adaptions to life in the artic, in particular a diet rich in ω-3 PUFAs.

METABOLIC CONTROL OF TRANSCRIPTIONAL MEMORY

Ordog T

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Whereas epigenetic mechanisms have received most attention as key factors of carcinogenesis and oncotherapy resistance, the same biological processes are also indispensable for mediating gene-environment interactions during physiological adaptation and under pathophysiological conditions including metabolic imbalance. Our central aim is to understand the effects of diabetes on the gastrointestinal (GI) neuromuscular apparatus including interstitial cells of Cajal (ICC), electrical pacemaker and neuromodulator cells of the gut. ICC depletion is central to diabetic gastroparesis and aging-related GI dysfunction. Using various in-vitro and in-vivo approaches including genetic lineage tracing and ablation, we have demonstrated ICC differentiation from local stem/precursor cells (ICC-SC) and age-related ICC transdifferentiation into fibroblast-like cells (FLC), a distinct interstitial cell class possessing a more limited functional repertoire than ICC. Central to these phenotypic processes is a reversible transcriptional switch between KIT and PDGFRA receptor tyrosine kinases driving cell type-specific gene expression patterns. With the aid of integrated transcriptional and multi-parameter epigenomic profiling, creERT2-loxP- and CRISPR-Cas9-mediated genome and epigenome editing, RNA interference, and epigenetic pharmacology, we have identified the super-enhancers underlying the ICC phenotype and their reversible repression by polycomb-mediated mechanisms in ICC-SC and during ICC-to-FLC transition. Studies in diabetic mice have pointed at ICC-SC failure and differentiation block as the main causes of ICC depletion. Our current efforts are directed toward investigating the role of succinate, a mitochondrial metabolite that accumulates in diabetes, in setting up the aberrant ICC-SC epigenomic state. Our results reveal a metabolic-epigenetic regulation of phenotypic transitions in the ICC lineage and may identify novel therapeutic options for diabetic gastroparesis.

USE OF DNA FOR BORDER SECURITY, COUNTERTERRORISM, AND HUMAN RIGHTS

Palmbach T

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Today, millions of people are immigrants, refugees, enslaved in some form of human trafficking, or otherwise susceptible to conflict or acts of terror. There is no simplistic solution to address these issues, as matters of nation state policies, rule of law, poverty, conflict, lack of unified global response, and substantial resource needs are major influencing factors. However, some of these issues can be managed by the use of modern day forensic and biometric methods, and these tools can assist in overall prevention, protection of victims, and identification and prosecution of the offenders.The first step is for a nation state or government agency to assess the problem and needs, and commit to design and implement the use of a modern-day forensic science system with a biometric collection focus.As protocols that utilize any of the various biometric systems are being designed they must assure that all handling protocols are consistent with nation state rule of law as well as international standards for safeguarding personally identifiable information (PII), and that all actions will comply fully with UN international human rights standards and protocols. There are legitimate concerns with the development and proper use of biometric databases, yet the potential value of these systems is significant enough to move forward with carefully constructed and regulated data capture, storage, analysis, and access.

FROM FORENSIC GENETICS TO FORENSIC GENOMICS

Parson W[1,2]

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The introduction of molecular genetic markers and methods was a revolution in forensic biology in the late 1980's. The transition from forensic haemogenetics, which was mainly based on serological methods, to forensic genetics happened through Restriction Fragment Length Polymorphism (RFLP) analysis and PCR-based analysis of Variable Number of Tandem Repeat loci (VNTRs) and multiplex PCR of Single Nucleotide Polymorphisms (SNPs). Soon those markers were replaced by Short Tandem Repeats (STRs) that offered high levels of discrimination coupled with increased sensitivity and robustness for the analysis of heavily degraded biological samples. Harmonized nomenclature for STRs based on repeat-number dependent size categories provided unambiguous genotype assignment and paved the way for National Intelligence DNA Databases that are now of central importance in combating crime. Core STR markers were defined with large overlap between international jurisdictions allowing direct comparison of forensically relevant data between countries. The workhorses for the typical forensic genetic laboratory in the 1990's up to today, were capillary gel electrophoresis instruments that separated PCR amplified DNA fragments by size. Recent technical developments in Massively Parallel (Next Generation) Sequencing offer new perspectives for the way forensic specimens can be investigated. The sheer amount of available sequence information permits larger PCR multiplexes to be developed and many more markers can be tested in a single run. Sequence information increases the differentiation capacity of established STR markers and the ability to resolve components present in mixtures of multiple DNA donors. It allows the simultaneous analysis of ancestry-informative and phenotype-associated SNPs that previously could not be directly coupled to STR analysis. The field of mitochondrial genetics finds new opportunities to produce full mitogenomes from even severely degraded DNA using MPS, which significantly improves the scope of this application in forensics. Current research investigates the analysis of PCR-based megaplexes that combine hundreds of diverse DNA markers to maximize relevant information to solve crime. Recent research demonstrates that epigenetic markers, such as differentially methylated CpG sites, can be analyzed with current MPS instrumentation in a way that e.g. the chronological age of a sample can be estimated. With these developments forensic genetics is entering the new era of forensic genomics, which will provide enormous possibilities for future research and routine applications.

GENETIC ANIMAL MODELS OF CARCINOGENESIS

Saur D

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Maintenance and drug resistance of gastrointestinal cancer subtypes depends on cancer cell intrinsic mechanisms and a stroma that supports tumor growth. Mouse models have provided important insights into the evolution of this highly lethal tumor, but there are no models that allow secondary genetic manipulation of autochthonous tumors, the tumor microenvironment or the metastatic host niche once the tumor has formed. We generated inducible dual-recombinase based cancer models that permit spatial and temporal control of gene expression. This tool provides unparalleled access to the native biology of cancer cells and their hosting stroma, and rigorous genetic validation of candidate therapeutic targets. We performed tumor cell-autonomous and non-autonomous targeting, uncovered hallmarks of human multistep carcinogenesis, validated genetic tumor therapy, and showed that mast cells in the tumor microenvironment, which had been thought to be key oncogenic players, are in fact dispensable for tumor formation.

EPIGENETIC DIFFERENCES FOLLOWING PRENATAL ADVERSITY: THE DUTCH HUNGER WINTER

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The potential of the epigenome to undergo environmentally-induced changes is central to its postulated role in non-malignant human diseases. Animal experiments under controlled conditions have highlighted early gestation as a particular sensitive period of development during which epigenetic differences may be induced with phenotypic consequences. The tragedy of the Dutch Hunger Winter, a severe 6-month famine at the end of WWII, offers a quasi-experimental setting in humans to study the link between the timing of an environmental exposure, adult health and the epigenome. During my talk I will give an overview of published and new results on the influence of prenatal famine exposure on DNA methylation patterns, one of the major epigenetic marks. I will highlight the influence of the exact gestational timing of the exposure on health and the methylome and discuss the link between famine associated DNA methylation patterns and later-life metabolic health. Moreover, I will discuss a novel hypothesis to explain the DNA methylation differences associated with early gestational exposure to an environmental insult.

POPULATION-GENETIC AND FUNCTIONAL STUDIES OF CLASSIC SELECTIVE SWEEPS IN HUMANS

Szpak M[1], Mezzavilla M[1], Ayub Q[1], Chen Y[1], Wardle-Jones H[1], VancollieV[1], Ramirez-Solis R[1], Lelliott C[1], Xue Y[1], **Tyler-Smith C[1]**

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We have developed a method, Fine-Mapping of Adaptive Variation (FineMAV), which combines population differentiation, derived allele frequency and a measure of molecular functionality to prioritize candidate positively-selected variants in humans for functional follow-up. We calibrated and tested FineMAV using eight 'gold standard' examples of experimentally-validated causal variants underlying positive selection, and were able to pick out the known functional allele in all instances. In addition, we evaluated it using simulations and by comparison with a meta-analysis of reported positively-selected genes, and confirmed its low false positive rate and ability to identify positively-selected genes more widely. We then used FineMAV to identify the best candidate variants driving local adaptations in the 1000 Genomes Project Phase 3 SNP dataset including Africans, admixed Americans, Europeans, East and South Asians, and validated a subset of these using available data. As a result, we report many novel examples including rs6048066 in TGM3 associated with curly hair, and rs7547313 in SPTA1 associated with erythrocyte shape and possibly malaria resistance in Africa, as well as two different variants in PRSS53 linked to hair shape in East Asia and South Asia.

NEXT-GENERATION SEQUENCING TECHNOLOGIES AND CHALLENGES

Wieben E[1], Dawson B[3], Burgess D[2], Kocher J-P[1], Aleff R[1], Oliveira J[1], Klee E[1], Jen J[1]

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Next generation sequencing technologies have revolutionized our ability to harvest DNA and RNA sequence information from a variety of samples. Nevertheless, there are still significant challenges in accessing reliable sequence information from some difficult regions of the genome and from some sample types. To improve our ability to predict, prevent and diagnose genetic conditions in our patients, we have been working to improve our ability to harvest meaningful sequence data from clinically-relevant regions of the genome that have traditionally been inaccessible to mainstream sequencing technologies. We have used a variety of sequencing technologies and bioinformatics tools, including targeted capture, single molecule real-time sequencing, and molecular barcoding to access medically relevant sequence information from previously intractable regions of the human genome. Accurate, phased sequence data has been produced from a variety of medically-relevant regions of the genome, including the HTTLPR (depression), CYP2D6 (pharmacogenomics),), β-globin gene cluster locus (hemoglobin disorders), and loci involved in several repeat expansion diseases (neurology and ophthalmology). We have now begun to apply these approaches to some clinical research samples and are working to transition these strategies to the clinical testing laboratory at Mayo. Targeted capture and single molecule sequencing can be used to inform clinical care.

WHAT WE CAN LEARN FROM ANCIENT GENOMICS

Willerslev E

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In the past two decades, ancient DNA research has progressed from the retrieval of small fragments of mitochondrial DNA from a few specimens to large-scale genome studies of ancient human populations, the diseases they carried, and the environment surrounding them. Increasingly, ancient genetic information is providing a unique means to directly test theories in archaeology, anthropology, ecology, and evolutionary biology. Initial results have changed the way we look at long debated topics such as early peopling of the Europe, Asia, and the Americas.

ONCOHISTONE MUTATIONS REPROGRAM CANCER EPIGENOME

Zhang Z

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Somatic mutations on genes encoding histone H3 proteins have been found at high frequency in different tumors. For instance, mutations at the *H3F3A* gene, one of the two genes encoding histone H3 variant H3.3, have been observed in the majority of diffuse intrinsic pontine glioma (DIPG), an aggressive primary brain tumor found exclusively in children. The mutation found in DIPG leads to replacement of lysine 27 of histone H3.3 with methionine (H3.3K27M). In addition, over 95% of chondroblastoma patient samples contain a heterozygous mutation at the *H3F3B* gene (another gene encoding H3.3), replacing lysine 36 of histone H3.3 with methionine. In human genome, in addition to two genes (*H3F3A* and *H3F3B*) encoding H3.3, there are 13 genes encoding canonical histones H3.1/H3.2, which differ from H3.3 by four or five amino acids. Lysine 27 and lysine 36 are conserved among all these histone H3 proteins. Therefore, it was unknown how these mutations, occurring at one allele of 15 genes are linked to tumorigenesis. In this meeting, I will present our recent studies on how these onco-histone mutations reprogram epigenome of progenitor cells and promote tumorigenesis.

EPIGENETIC EDITING USING THE CRISPR/CAS9 SYSTEM AND ITS APPLICATIONS IN PRECISION MEDICINE

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Epigenetic studies relied so far on correlations between epigenetic marks and gene expression pattern. Technologies developed for epigenome editing now enable direct study of functional relevance of precise epigenetic modifications and gene regulation. The reversible nature of epigenetic modifications, including DNA methylation, has been already exploited in cancer therapy for remodelling the aberrant epigenetic landscape. However, this was achieved non-selectively using epigenetic inhibitors. Epigenetic editing at specific loci represents a novel approach that might selectively and heritably alter gene expression. We have repurposed the CRISPR-Cas9 system for targeted DNA methylation and demethylation by fusing the methyltransferase and methyltransferase dioxygenase catalytic domains to catalytically inactive Cas9 protein (dCas9). We validated our tools on the genes that has been previously associated with both IgG glycosylation and inflammatory bowel disease (IBD): the *MGAT3*, *LAMB1, IL6ST* and *BACH2* genes. The dCas9-DNMT3A fusion enabled elevation of methylation level at CpG sites, located within the promoter region of the candidate genes *IL6ST* and *BACH2* up to 60% followed by decrease in their transcript levels. The fusion construct dCas9-TET1 decreased CpG methylation at the promoter region of the candidate gene *MGAT3* up to 50%, followed by increased gene activation. The dCas9-DNMT3 and dCas9-TET1 fusion proteins can be targeted to any 20 bp sequence followed by the NGG trinucleotide by co expression of a guide RNA, with the peak activity throughout a ~35 bp wide region. We also showed that using multiple guide RNAs we could target our tools to multiple sites, which enabled hypermethylation or hypomethylation of a wider genome region. As a follow-up to our basic tools we designed and constructed a comprehensive molecular toolbox harnessing excellent targeting properties of Cas9 in order to deliver domains for activation, repression, CpG methylation/demethylation and histone modifications to promoters or other control elements of targeted genes. The molecular toolbox is highly modular and easily reconfigured for a range of applications in gene regulation and epigenome editing. Co-expression of several sgRNAs enables multiple targeting, while different Cas9 orthologs provide a platform for independent targeting with multiple classes of sgRNAs, each specific for a single ortholog.

YOUNG INVESTIGATOR AWARD ABSTRACTS

Presentation number: AG 1 Abstract number:

RECONSTRUCTING THE POPULATION HISTORY OF LATE NEANDERTALS

Hajdinjak M[1], Fu Q[2], Stenzel U[1], Hübner A[1], Petr M[1], Mafessoni F[1], Grote S[1], Rougier H[3], Crevecoeur I[4], Semal P[5], Soressi M[6,7], Talamo S[7], Hublin J-J[7], Gušić I[8], Kućan Ž[8], Rudan P[8], Golovanova LV[9], Doronichev VB[9], Posth C[10,11], Krause J[10,11], Korlević P[1], Nagel S[1], Nickel B[1], Prüfer K[1], Kelso J[1], Meyer M[1], Pääbo S[1]

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The Middle to Upper Palaeolithic transition in Europe was characterized by major cultural and biological changes which coincided with the arrival of anatomically modern humans and the disappearance of Neandertals. In order to reconstruct the population history of Neandertals from this period, we determined DNA preservation in 78 hominin bones from 21 late Neandertal sites across Eurasia. We were able to recover nuclear genome sequences with coverages ranging from 1.0 to 2.7x from five Neandertal individuals. These originated from the Troisième caverne of Goyet (Belgium), Spy (Belgium), Les Cottés (France), Vindija Cave (Croatia) and Mezmaiskaya Cave (Russia). When compared to two previously determined high coverage Neandertal genomes, all late Neandertals, regardless of their geographical origin, were genetically closer to Vindija 33.19, a >45,000-year-old Neandertal from Croatia, than to the older Altai Neandertal from Siberia. Moreover, the genetic diversity of the nearly contemporaneous late Neandertals was substantially lower than that of humans today, agreeing with the small population size of Neandertals prior to their disappearance. We find that the Neandertal population that contributed genetic material to the ancestors of present-day non-Africans was genetically closer to all late Neandertals than to the Altai Neandertal, indicating that the majority of the gene flow occurred from a Neandertal population that was equidistant to late Neandertals and divergent from the Altai population. The successful recovery of these low coverage Neandertal genomes, as well as the prospect of generating additional Neandertal genomes in the near future, will allow the reconstruction of Neandertal population history at a finer resolution and across a wider geographic and temporal range.

Keywords: Neandertals, ancient DNA, low coverage genomes, population history, admixture

Presentation number: MG 1 Abstract number:

EPIGENETIC DISCRIMINATION OF MONOZYGOTIC TWINS FROM BLOOD AND SALIVA IN THE FORENSIC SCENARIO

Vidaki A[1], López CD[1], Kalamara P[1], Carnero-Montoro E[2], Spector T[2], Bell J[2], Kayser M[1]

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Monozygotic (MZ) twins share the same STR profile, demonstrating a practical problem in forensic casework. Here, we investigated epigenetic MZ twin differentiation from blood and saliva under the forensic scenario comprising the i) discovery of candidate twin-differentially methylated sites (tDMSs) in reference-type DNA via genome-wide analysis, ii) technical validation of candidate tDMSs in reference-type DNA using a sensitive targeted method, and iii) analysis of validated tDMSs in trace-type DNA. Genome-wide methylation analysis in blood DNA from 10 MZ twin pairs identified on average 56 tDMSs per pair with >0.3 twin-to-twin methylation differences. Across all pairs, 67.9% of the top three, twin-specific blood candidate tDMSs generated >0.1 methylation differences in reference blood samples, and from the validated tDMSs 68.4% resulted in >0.1 differences in bloodstains using methylation-specific qPCR. Applying an updated marker selection strategy, we analysed 8 additional tDMSs for an example MZ pair; 87.5% of which were positively validated in both reference- and trace-type blood DNA. Similarly, following the revised selection approach the majority of selected saliva candidate tDMSs generated >0.1 methylation differences in reference saliva samples from the same twin pairs, from which the majority also resulted in >0.1 differences in DNA from cigarette butts. Lastly, we showed that about one third of the identified tDMSs with large twin-to-twin methylation differences were longitudinal for up to 2 years. Overall, our study highlights that the number of tDMSs is crucial as some candidate markers identified in reference DNA analysis were not informative in the trace DNA due to technical and biological reasons. Future studies will need to address the minimal number of tDMSs required for reliable identification of MZ twin individuals as well as assess the effect of various forensic variables that could influence such identification.

Keywords: monozygotic twins, forensic epigenetics, DNA methylation, Illumina 450K array, quantitative PCR

Presentation number: MG 2 Abstract number:

EZH2-DEPENDENT EPIGENETIC REPROGRAMMING CONTROLS A DEVELOPMENTAL SWITCH BETWEEN MODES OF GASTRIC NEUROMUSCULAR REGULATION

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Neuromuscular control of gastrointestinal (GI) functions involves KIT$^+$ interstitial cells of Cajal (ICC), which mediate cholinergic excitatory and nitrergic inhibitory innervation of smooth muscles and electrically pace phasic contractions, and PDGFRA$^+$ "fibroblast-like" cells (FLC), which mediate purinergic inhibition. ICC depletion is central to many GI diseases and aging-related dysfunction. Here, we aimed to identify ICC fates and determine their mechanisms during postnatal development in mice. We hypothesized that following loss of KIT, ICC may upregulate PDGFRA and differentiate toward FLC. Using genetic lineage tracing in *Kit*$^{creERT2/+}$;*R26*$^{mT\text{-}mG}$ mice and conditionally immortalized ICC we demonstrate that during the first 6 months of life, 21% of ICC die and 28% lose their phenotype and function with 56% of these "post-functional ICC" acquiring PDGFRA expression and the more limited functional repertoire of FLC. Integrated transcriptome and epigenome profiling revealed that genes related to ICC identity and function bearing specific patterns of acetylated histone H3 lysine (K) 27 became preferentially silenced during the ICC→FLC transition due mainly to increased occupancy by trimethylated H3K27 (H3K27me3). Single guide RNA-mediated targeting of a construct containing catalytically inactive Cas9 and the H3K27 methyltransferase EZH2 to the *KIT* promoter of GIST882 cells, a human ICC model, resulted in a 2-4-fold increase in H3K27me3 occupancy, a 4.7-fold decrease in *KIT* and a 1.4-fold increase in *PDGFRA* expression. EZH2 inhibition, knock-down or in-vivo genomic deletion in *Kit*$^{creERT2/+}$;*Ezh2*$^{fl/fl}$ mice permitted the recovery of functional, KIT$^+$ ICC even after the phenotypic transition had taken place. These results demonstrate a role for EZH2-mediated epigenetic repression in physiological mammalian transdifferentiation and identify FLC as a reserve from which ICC can potentially be restored by epigenetic reprogramming in common GI disorders where ICC are depleted.

Keywords: Polycomb, *KIT*, PDGFRA, transdifferentiation, epigenome editing

Presentation number: MG 3 Abstract number:

CRISPR/CAS9-BASED MOLECULAR TOOLS FOR EPIGENETIC MANIPULATIONS

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We have repurposed the CRISPR-Cas9 system for targeted DNA methylation and demethylation. De novo DNA methyltransferase DNMT3A was fused to catalytically inactive Cas9 (dCas9) and this fusion enabled silencing of gene expression by methylation of CpG sites located in regulatory regions of the targeted candidate genes - the *BACH2* and *IL6ST*, involved in IgG glycosylation. Targeted gene promoter demethylation was accomplished by an analogous construct comprising dCas9-TET1 fusion and the tool activity was validated on the *MGAT3* and *LAMB1* gene promoters involved in IgG glycosylation, as well. We set out to rigorously characterize the activity of our constructs in a HEK293 model cell line. The dCas9-DNMT3 tool can be targeted to any 20 bp sequence followed by the NGG trinucleotide by co expression of a guide RNA, with the peak activity throughout a ~35 bp wide region. DNA methylation activity was highly specific for the targeted region and heritable across mitotic divisions. We also showed that multiple guide RNAs could target the dCas9-DNMT3A construct to multiple sites, which enabled hypermethylation of a wider genome region. We demonstrated that the candidate gene *IL6ST* decreased expression level following promoter hypermethylation, which served as a proof of the concept of artificial epigenetic silencing by targeted CpG methylation *in vivo*. The TET1-based molecular tool for targeted demethylation showed similar activity pattern to Cas9-DNMT3A fusion: up to 60% demethylation and the peak activity about 30 bp downstream from the binding site. The reversible nature of epigenetic modifications, including DNA methylation, has been already exploited in cancer therapy for remodelling the aberrant epigenetic landscape. However, the classical approach uses epigenetic inhibitors non-selectively. In contrast, epigenetic editing at specific sites could selectively alter the gene expression pattern. Thus, our newly developed constructs represent promising molecular tools for unravelling details of molecular processes in a living cell (such as protein glycosylation), and they have potential as therapeutics upon further development.

Keywords: CRISPR-Cas9 system, DNA methylation, IgG glycosylation

ABSTRACTS OF THE SELECTED ORAL PRESENTATIONS

Presentation number: MG 4 Abstract number:

ECTO-5' -NUCLEOTIDASE CD73 (NT5E), VITAMIN D RECEPTOR AND FGF23 GENE POLYMORPHISMS MAY PLAY A ROLE IN THE DEVELOPMENT OF CALCIFIC UREMIC ARTERIOLOPATHY IN DIALYSIS PATIENTS - DATA FROM THE GERMAN CALCIPHYLAXIS REGISTRY

Rothe H[1], Brandenburg V[2], Haun M[3], Kollerits B[3], Kronenberg F[3], Wanner C[4], Ketteler M[1]

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Calciphylaxis/calcific uremic arteriolopathy affects mainly end-stage kidney disease patients but is also associated with malignant disorders such as myeloma, melanoma and breast cancer. Genetic risk factors of calciphylaxis have never been studied before. We investigated 10 target genes using a tagging SNP approach: the genes encoding CD73/ecto-5'-nucleotidase (purinergic pathway), Matrix Gla protein, Fetuin A, Bone Gla protein,VKORC1 (all related to intrinsic calcification inhibition), calcium-sensing receptor, FGF23, Klotho, vitamin D receptor, stanniocalcin 1 (all related to CKD-MBD). 144 dialysis patients from the German calciphylaxis registry were compared with 370 dialysis patients without history of CUA. Genotyping was performed using iPLEX Gold MassARRAY(Sequenom,San Diego, USA), KASP genotyping chemistry (LGC, Teddington, Middlesex, UK) or sequencing. Statistical analysis comprised logistic regression analysis with adjustment forage and sex. 165 SNPs were finally analyzed and 6 SNPs were associated with higher probability for calciphylaxis (OR>1) in our cohort. Nine SNPs of three genes (CD73, FGF23 and Vitamin D receptor) reached nominal significance ($p< 0.05$), but did not reach statistical significance after correction for multiple testing. Of the CD73 gene, rs4431401 (OR = 1.71, 95%CI 1.08±2.17, $p = 0.023$) and rs9444348 (OR = 1.48, 95% CI 1.11±1.97, $p = 0.008$) were associated with a higher probability for CUA. Of the FGF23 and VDR genes, rs7310492, rs11063118, rs13312747 and rs17882106 were associated with a higher probability for CUA. Polymorphisms in the genes encoding CD73, vitamin D receptor and FGF23 may play a role in calciphylaxis development. Although our study is the largest genetic study on calciphylaxis, it is limited by the low sample sizes. It therefore requires replication in other cohorts if available.

Keywords: calciphylaxis, end-stage kidney disease, CD73, vitamin D receptor, FGF23

Presentation number: AG 2 Abstract number:

RETRIEVING NEANDERTAL AND DENISOVAN DNA FROM LATE AND MIDDLE PLEISTOCENE SEDIMENTS

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As skeletal remains of ancient hominins are rare, evidence of human occupation at Pleistocene sites is often restricted to artefacts and other durable traces of human activity. The incompleteness of the fossil record hinders our understanding of hominin evolution and our ability to assign excavated assemblages to hominin taxa. This could be improved by recovering hominin DNA from sediments even in the absence of osseous remains. We extracted DNA from 85 sediment samples from Middle and Late Pleistocene layers at Caune de l'Arago (France), Chagyrskaya Cave (Russia), Denisova Cave (Russia), El Sidrón (Spain), Les Cottés (France), Trou Al'Wesse (Belgium) and Vindija Cave (Croatia). By targeted enrichment of mitochondrial (mt) DNA, we recovered one Denisovan and eight Neandertal mtDNA genomes from sediments deposited at four sites. We detected Neandertal mtDNA in sediment from the fossil-bearing layers of El Sidrón, Chagyrskaya Cave and Denisova Cave. At the latter site, we also retrieved Denisovan and Neandertal mtDNA from layers devoid of hominin skeletal remains, extending the known record of both groups in the region. DNA from the Late Pleistocene layer at Trou Al'Wesse, where no hominin osseous remains were discovered, provides direct evidence for the occupation of the site by Neandertals. Our work demonstrates the feasibility of detecting molecular traces of ancient hominins in sediments and opens the possibility to infer the presence of hominin groups at sites where no skeletal remains are found.

Keywords: ancient DNA, Neandertal, Denisovan, sediment, Pleistocene

Presentation number: AG 3 Abstract number:

GENETIC PERSPECTIVES ON POPULATION DYNAMICS AND CULTURAL INTERACTIONS IN NORTHWESTERN AMAZONIA

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Comparisons of mitochondrial DNA (mtDNA) and the non-recombining portion of the Y-chromosome (NRY) are useful for contrasting the maternal vs paternal history of populations, which are often influenced differently by cultural practices. Here, we investigate the genetic variation in Native American populations from Northwestern Amazonia (NWA) in Colombia, an area of great anthropological interest given its high diversity in terms of languages, cultural practices, and subsistence strategies. We have sequenced complete mtDNA genomes and ~2 Mb of the NRY in 426 and 284 individuals, respectively, covering the extant ethnolinguistic diversity of NWA. These data reveal high genetic diversity in NWA populations, especially for the mtDNA. However, the percentage of between-population differences is higher for the NRY than for the mtDNA (34 % vs. 12 %), indicating more genetic structure for NRY than for mtDNA. These results suggest that population contact has involved mainly the movement of women among groups, which is supported by the high proportion of shared mtDNA haplotypes. Of particular interest are the groups belonging to the Eastern Tukanoan family (ET), because they practice linguistic exogamy, -a marital system in which men are required to marry women from a different language group-. ET groups share more haplotypes and have higher levels of diversity in the mtDNA, while the levels of NRY diversity are lower than in other groups, suggesting an effect of linguistic exogamy on the patterns of genetic diversity. To conclude, the evidence provided by this study constitutes an important source of information to understand the peopling of NWA and the impact of cultural practices, and provides new insights into a remote and little-studied part of the world.

Keywords: mtDNA, NRY, Native-Americans, language, exogamy

Presentation number: MG 5 Abstract number:

GENOME-WIDE ASSOCIATION ANALYSIS OF FOOD INTOLERANCE IN PATIENTS WITH HASHIMOTO'S THYROIDITIS

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Genetic and environmental factors play a role in development of Hashimoto's thyroiditis (HT). As food intake is considered to be an important environmental factor, the goal of our study was to evaluate intolerance to food, measured by IgG antibodies to food antigens, with HT manifestation. The second goal was to analyse genetic background of identified immune reaction. We measured IgG antibodies to 125 food antigens in 74 HT patients and 245 controls using microarray-based ELISA test and analysed differences in IgG levels between two groups. IgG antibodies that were positively associated with HT were further analysed on genome-wide level in HT patients, using linear regression model adjusted for age and sex. HT patients were genotyped on Illumina HumanOmniExpressExome platform and imputed using 1000 genomes reference panel. Results: We found statistically significant differences in IgG levels between HT cases and controls for 3 food antigens: plum ($P=1{,}70\times10^{-8}$), egg-white (P=0,01) and barley (P=0,04). Genome-wide association analysis identified one genetic variant near *IYD* gene associated with IgG levels specific to plum ($P=2{,}53\times10^{-7}$). *IYD* gene is highly expressed in thyroid gland where it maintains iodine homeostasis. Additionally, we found 11 other suggestively associated loci ($P<10^{-5}$) with IgG levels specific to plum, 7 with egg-white and 4 with barley. Pathway analysis identified "Ion channel transport" as the most prominent pathway for plum intolerance, "Integrin" pathway for egg-white and "Post-translational protein modification" pathway for barley. This is the first study that analysed food intolerance status in HT patients and linked it with genes that are relevant to thyroid function or belong to biologically interesting pathways. Additional analyses are needed to answer the question if identified intolerance to plum, egg-white and barley are side-effects of disease or environmental triggers to HT development.

Keywords: Hashimoto's thyroiditis, genetic and environmental factors, food intolerance, IgG antibody, genome-wide association analysis

Presentation number: MG 6 Abstract number:

EXPLORING GENETIC LANDSCAPE OF NEURAL TUBE DEFECTS USING TARGETED NEXT GENERATION SEQUENCING

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Neural tube defects (NTDs) are caused by failure of neural tube closure (NTC) and ~400 NTD associated genes have been reported in animal models. However, the NTD candidate genes from mouse study cannot adequately support our understanding of the genetic basis underlying the etiology of human NTD. To systematically investigate NTD-associated variants and their function, we used a targeted next generation sequencing (NGS) approach to identify sequence variation of 280 genes which are suspected of being involved in NTDs from multiple biochemical pathways in 343 NTDs and 206 unaffected Chinese population. A significant NTD-risk association of the WNT5A gene was identified at the gene-level analyses. Further, 18 genes with rare loss of function (LoF) variants, including CASP9, MKS1, FUZ, PRMT2, DNAAF1, MARCKSL1, CELSR2, FTCD, APAF1, NFKB1, SHROOM2, SHROOM3, RHO, NUP85, PHACTR4, NF1 and SHMT1, were identified. And the rare deleterious protein altering variants (DPAVs) of the above genes being diversed to three signal pathways accounted for up to 47% of NTDs that we analyzed. Protein-protein interactions analyses also showed that those 18 genes which were significantly enriched in cytoskeleton signaling and apoptosis pathways are both interacted with the third pathway, Planar Cell Polarity (PCP) pathway. Three DPAVs (p.N594fs; p.Y321C; p.R414Q) of SHROOM3 were further evaluated in demonstration for their role on NTC via in vivo and in vitro functional analyses. Our results suggested that genes with rare DPAVs in cytoskeleton and apoptosis pathways may play a major contribution to the etiology of human NTDs through interaction with PCP genetic network. Our study also provided direct evidence that rare DPAVs of SHROOM3 contributes to the etiology of human NTDs through SHROOM3-DVL2 and SHROOM3-ROCK1 interactions.

Keywords: Neural tube defects, targeted next-generation sequencing, deleterious protein altering variants (DPAV), loss of function (LoF), PCP (Planar Cell Polarity)

Presentation number: MG 7 Abstract number:

DEVELOPMENT OF A NOVEL WHOLE GENOME SEQUENCING METHOD FOR NON-INVASIVE PRENATAL DETECTION OF FETAL SUBMICROSCOPIC CHROMOSOME ANOMALIES

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Fetal copy number variations (CNVs) associated with clinically significant chromosome disease syndromes, occur in approximately 1% of all pregnancies. To expand the clinical utility of non-invasive prenatal testing (NIPT) beyond common chromosomal aneuploidies, we aim to determine the reliability and accuracy of a novel PCR free NIPT pipeline specifically designed to detect and delineate fetal CNVs. Plasma DNA libraries were generated in a single step using a combination of DNA modifying enzymes without PCR amplification and then directly subjected to massively parallel sequencing. Approximately 12-14 million uniquely and perfectly mapped 36bp reads were used for data analysis. An analysis pipeline based on PCA and HMM was used to identify CNVs. Results: For clinical validation, we analysed six archived plasma samples with known fetal CNVs previously detected by SNP array analysis of amniocyte DNA. Three samples had single CNVs and three samples had terminal chromosome CNVs associated with unbalanced translocations. In a blinded analysis, all CNVs with sizes above 1Mb in the six samples were correctly identified. The analysis algorithm was then applied to 3513 sequential NIPT samples. Fetal CNVs from 7 samples were identified and verified by invasive prenatal diagnosis, along with 22 maternal CNVs. For fetal CNVs, the combined positive predictive value was 91.4% and the false negative rate was zero. Our optimised PCR free whole genome sequencing NIPT pipeline using relatively deep sequencing was reliable and accurate for detection of a range of fetal and maternal CNVs varying in size and genomic location.

Keywords: whole genome sequencing, non-invasive prenatal testing, submicroscopic chromosome anomalies, copy number variations, cell free DNA

Presentation number: FG 1 Abstract number:

MICROHAPLOTYPES ARE READY FOR IMPLEMENTATION IN CASEWORK

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The discovery phase for microhaplotypes (MHs) potentially useful in forensics has documented over 180 multiallelic loci appropriate for genotyping by massively parallel sequencing (MPS). Most loci range from 20 bp to 200 bp in extent involving 2 to 5 SNPs defining 3 to 6 and more alleles. All have been characterized for haplotype frequencies in 83 different population samples; data on 130 loci have been published (FSIG 29 (2017) 29–37). Over 60 of those have subsequently been characterized in at least 10 additional populations. The implementation phase using MPS sequencing for more than 75 of these loci documents that microhaplotypes contribute excellent information on 1) individualization (RMP), 2) mixtures, 3) biological relationships, 4) biogeographic ancestry. Some phenotype information also exists, but not currently sufficient for valid inference.1) The top 28 MHs for the 83-population global average effective number of alleles (Ae) predict a RMP of at least 1.5E-23. The many additional loci can decrease this probability by several orders of magnitude.2) Synthetic mixtures and forensic type samples have been genotyped using the Ion S5TM with a multiplex MPS assay that included MHs, STRs and Individual Identification SNPs, in parallel with conventional capillary electrophoresis (CE) analysis of STRs. Results showed that MHs outperformed STR typing done by either MPS or CE. MHs detected the presence of minor contributors in mixtures down to a 40:1 ratio and in forensic like samples, where STR analysis simply indicated the possible presence of a minor contributor, MHs clearly detected the presence of a minor component and generated a profile suitable for comparison. 3) The multiple alleles clearly allow relationship estimation with likelihood ration statistics dependent on the frequencies of the relevant alleles. 4) Finally, the full set of MH loci genotyped on 83 populations allow distinction of a minimum of six major biogeographic regions. The subset of MH loci type genotyped on over 90 populations allow distinction of eight regions globally.We conclude that microhaplotypes are the next generation of forensic markers appropriate for most forensic applications.

Keywords: massively parallel sequencing, mixtures, RMP, ancestry

Presentation number: MG 8 Abstract number:

CHROMOSOMAL MICROARRAY IN CLINICAL DIAGNOSTICS - A STUDY OF 337 PATIENTS WITH CONGENITAL ANOMALIES AND DEVELOPMENTAL DELAYS/INTELLECTUAL DISABILITY

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Chromosomal microarray (CMA) technique is used as the first-tier test to detect genomic imbalances in patients with congenital and developmental abnormalities including dysmorphia, intellectual disability (ID), developmental delay (DD), autism spectrum disorders (ASD) and congenital anomalies (CA). The aim of the study was to the determine 1. the diagnostic yield and 2. criteria which could help to classify and interpret the CNVs detected. Here we present the results of the CMA analysis of copy number variations (CNVs) in 337 patients with DD/ ID with or without dysmorphism, ASD and/or CA. Of these, in 30 subjects chromosomal imbalances have previously been detected by chromosome karyotyping, multiplex ligation-dependent probe amplification or fluorescence in situ hybridization. In 73 patients, clinically relevant variants were detected and/or better characterized, 61 microdeletions and 30 microduplications. Most of them were > 1 Mb. In 19 patients multiple imbalances were found. The pathogenic CNVs were unevenly distributed throughout the genome with clusters in 8p23, 15q11.2, 22q11.21, and 18q22.1q23. Variants of unknown clinical significance (VOUS) were discovered in 35 subjects, 17 microdeletions and 25 microduplications. In the majority of cases (61,9%), VOUS were <500 kb. Deletions and de novo imbalances were much more frequent in pathogenic CNV than in VOUS category. With high diagnostic yield (43/307,14%; excluding patients previously detected by other methods) CMA has proven to be a valuable test, establishing the diagnosis in a high proportion of patients. Criteria which help classify and interpret CNVs include size (we recommend using a 300 kb as a cutoff for clinically relevant CNV), mode of inheritance, and genotype-phenotype correlation. Our results have shown that Agilent ISCA v2 Human Genome 8x60 K oligonucleotide microarray format has provided reasonable resolution for clinical use, particularly in the ISCA regions associated with well-established syndromes.

Keywords: chromosomal microarray, CNV, developmental delay, intellectual disability, congenital anomalies

Presentation number: FG 2 Abstract number:

GENOTYPING HORSE EPITHELIAL CELLS FROM FECAL MATTER BY ISOLATION OF PCR PRODUCTS (IPCRp) METHOD

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Obtaining genotype out of horse epithelial cells from fecal matter can benefit the forensic investigations involving human and non-human DNA. Correctly selecting appropriate DNA extraction and amplification methods could increase the probability of obtaining genotype.The DNA from the horse fecal matter was extracted by modified Qiagen DNA Stool Mini Kit protocol. Following the extraction, the DNA genotypes from fecal samples were obtained by the most powerful PCR amplification method, the IPCRp. The IPCRp-based multiplex kit amplified biotin-labeled strands were captured on streptavidin-coated plates, where everything but the dye-labeled target sequence was washed, eliminating all the background noise, released, and run on a genotyping instrument in a single-strand configuration. The IPCRp-based multiplex kit (6 loci) revealed equine DNA full genotype profiles (appearance of all six loci) when sampled from fresh feces in 87% of the samples and partial genotype profile (appearance of one to five loci) in 13% of the samples, for a total of 100% genotyping success rate. Previously, Bellemain et al., Lucchini et al., and Bhagavatula and Singh, reported 73%, 53%, and 21%, success rate in obtaining partial genotypes from fresh fecal samples, respectively. These results indicate that the IPCRp amplification method, coupled with the Qiagen DNA Stool Mini Kit extraction, are the best choice of methods that can maximize the likelihood of obtaining horse DNA genotypes from fecal samples.

Keywords: IPCRp, PCR, DNA extraction, fecal matter DNA, horse genotyping

Presentation number: MG 9 Abstract number:

INVESTIGATION OF SMOKING-RELATED DNA METHYLATION CHANGES IN KOREAN ADULTS

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Cigarette smoke is one of the most potent environmental modifiers of DNA methylation. Exposure-related DNA methylation changes are considered useful for predicting an individual's life style. Several previous studies reported that cigarette smoking was related to the decrease in DNA methylation of the AHRR (Aryl-Hydrocarbon Receptor Repressor) gene, and that the subsequent increase in AHRR expression could play a role in the elimination of the harmful environmental chemicals contained in cigarette smoke. In the present study, we analyzed blood and saliva samples from 21 Korean males using HumanMethylation450 BeadChip array: Among the 21 males, 7 were current smokers, 7 were former smokers, and 7 had never smoked. Because the array replicated cigarette smoking-related DNA methylation changes in the AHRR gene, we confirmed this further with the samples obtained from 284 individuals using a duplex methylation SNaPshot reaction for the analysis of two CpGs (cg05575921 and cg23576855) in the AHRR gene. Strong negative correlation between pack-years and DNA methylation percentage of the two CpG markers were found both from blood and saliva samples. Age, sex, BMI, age when smoking began, alcohol consumption, exercise, the smoking status of housemates, disease state, period of abstinence from smoking, and the smoking period of ex-smoker have no correlation with DNA methylation change in the two CpG sites of the AHRR gene. To test the smoking predictability, we performed ROC analysis and estimated AUC values. The two CpG markers in the AHRR gene showed considerable sensitivity and specificity. Our results suggested that the AHRR DNA methylation will be a good molecular marker for life style prediction from body fluid samples at crime scenes.

Keywords: forensic, DNA methylation, cigarette smoking, blood, saliva

ABSTRACTS OF POSTER PRESENTATIONS

FORENSIC GENETICS

DNA ANALYSIS IN VICTIMS OF MASS DISASTERS

Presentation number: FG 3 Abstract number:

RE-WRITING THE HISTORY GENETIC IDENTIFICATION OF HOSTAGES KILLED BY A NAZI FIRING SQUAD IN THE DETENTION CENTER IN BIALYSTOK, POLAND

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Almost 6 million people died in Poland during the Nazi occupation and about 570 thousand during the Soviet occupation. But the end of the war was not the end of the trauma. Historians estimate that at least 30 thousand people were killed during the Stalinist regime in Poland. The exact number is unknown, because both the executions and the burials were kept secret. In 2012 the Institute of National Remembrance started to search for hidden burials of victims of communism. Many exhumations were carried out under the project. One of them took place in Białystok, eastern Poland. According to information gathered by local historians, a detention centre in the heart of city was the place of secret burials of victims of the communist regime. But the place had a longer story. When the Nazi occupation started in 1941 it went under the control of the Gestapo. During the exhumation work a burial pit with the remains of 24 victims was found. It's characteristics supported the hypothesis that these people were shot on the spot, in a mass execution during the Nazi occupation. Historians knew of only one such execution, but its victims - according to the available records - were supposed to have been exhumed at the end of the war. During the exhumation works historians were contacted by a man whose grandfather was executed in the 1940s. At this stage of the study it was not yet clear whether this kinship may have been related to the discussed mass grave. When genetic profiling of the remains began, the first match was found between the Y-STRs of the said man and one of the skeletons removed from the investigated grave. It was a starting point for the collection of reference material for other victims.Findings from historical research have necessitated the revision of the existing historic records. Exhumation works and the discovery of the discussed mass grave put in question the events of 1944, which would have been impossible without the field work. The first identifications confirmed the doubts of historians, since both the results of genetic profiling and the conducted anthropological analysis revealed that at the end of the war a mistake was made, and bodies other than those suspected had been exhumed. Having established this fact, the mass grave created at that time should be investigated to reveal the identity of the remains uncovered then.

Keywords: mass grave; war grave; HID; DVI; identification

Presentation number: FG 4 Abstract number:

APPLICATION OF THE NGM-DETECT KIT FOR IMPROVED FORENSIC ANALYSIS

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A new generation of STR amplification kits with improved sensitivity and additional genetic markers is emerging on the market, meeting the growing demands of forensic genetic laboratories. The new kits are designed particularly for analyzing difficult traces, with a high DNA degradation index, the presence of inhibitors and a low level of DNA. The amplification process of these samples is problematic and can make genetic identification very challenging or even impossible. In the newest product of ThermoFisher Scientific company – the NGM-Detect Kit – modifications including changing the primers' sequences and shortening DNA fragments of STR markers are introduced. Quality control markers (IQCS, IQCL) used to detect PCR correctness and the presence of DNA degradation or inhibitors in the sample are an additional feature. The purpose of this study was to compare the analysis results obtained with STR kits used in our laboratory (NGM, GlobalFiler) with profiles generated using the new NGM-Detect kit. In the study different kinds of typical casework samples including: blood, semen, saliva, and bones were analyzed according to the manufacturer's instructions. The results indicate that the NGM-Detect kit is particularly useful for the analysis of challenging samples – for which incomplete profiles are generated with the NGM and GlobalFiler kits. The increased number of positively typed alleles enables achievement of better statistical parameters and, as a result, more reliable genetic identification. We conclude that the NGM-Detect kit can be suggested as a good supplement for STR analysis.

Keywords: STR, NGM-Detect kit, DNA degradation, inhibition

Presentation number: FG 5 Abstract number:

MASSIVELY PARALLEL SEQUENCING AND ITS APPLICATION IN HUMAN IDENTIFICATION FROM SKELETAL REMAINS

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Identification of human remains is one of the most demanding endeavours of forensic laboratories. The introduction of massively parallel sequencing (MPS) to laboratory practice seems to be a promising alternative to PCR-CE STR typing technology for compromised DNA samples analysis. An internal validation step is essential before routine use of new sequencing technology for casework. The aim of this study was to validate the ForenSeq DNA Signature Prep Kit on MiSeq FGx apparatus (Illumina) for genetic identification of bone material. The influence of the DNA degradation index (DI) on phenotyping results and bio-geographical ancestry prediction was ascertained. The robustness of the new technology was determined and compared with that of the GlobalFiler and Y-FilerPlus kits (ThermoFisher Scientific) routinely used in practice. Casework samples consisted of 71 bone and tooth samples at different stages of decomposition. Estimation of the degradation index (DI) was performed with Quantifiler Trio. MPS was conducted with a ForenSeq DNA Signature Prep Kit on MiSeq FGx apparatus. Validation shows that DNA samples with mild degradation (DI:1,5-4) produce reliable results for 80-100% markers. Samples with a higher DI (DI>4) deliver solid results for iSNP loci (more than 80% of typed loci), while STR data shows noticeable allele dropouts. Phenotype prediction indicates a dependence on DI level but in some, even severely degraded samples, results were obtained. Comparison to the PCR-CE STR results shows concordance in shared markers in most of the samples. In a few cases, a marker observed as a homozygote showed differences in MPS in the sequence of the same allele, which is an advantage in human identification studies.

Keywords: MPS, human identification, skeletal remains

Presentation number: FG 6 Abstract number:

POLISH GENETIC DATABASE OF TOTALITARIANISMS VICTIMS-MASSIVE IDENTIFICATION OF VICTIMS OF TOTALITARIAN SYSTEMS AND HOLOCAUST IN POLAND (O)

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We would like to present results of 5 years of research conducted within the project of the Polish Genetic Database of Totalitarianisms Victims. The project was created in 2012 for the purpose of collecting of the reference material from living relatives of the victims. As part of the project, over 800 human remains discovered in nearly a dozen sites all over Poland are being analyzed. To this day, more than 70 victims were identified and for about 400 of analyzed remains, we already completed the genetic study. Initial research was focused on identifying victims of the communist system in Poland, but it soon became clear, that the project can also be used for the victims of Nazis. Therefore currently within the project we are studying remains of victims of communist crimes - found in Warsaw, communist and German Nazi crimes – in Białystok and victims of Holocaust, whose remains were found in both Sobibor Concentration Camp. In our study we use a set of autosomal STR markers, STRs located on sex chromosomes, we also analyze sequences of mtDNA's. In addition, we implemented the next generation sequencing technology into our project. In human remains analysis, apart from the personal identification itself, haplotyping, based on the analysis of haploid markers - Y-chromosomal STRs and mtDNA sequence - is becoming more and more essential. It sometimes occurs that at one site we find victims of different regimes. Haplotyping of those victims buried in mass graves helps to determine the origin of the crime.

Keywords: HID, Holocaust, Nazi, Concentration Camp

Presentation number: FG 7 Abstract number:

OPTIMIZING AND AUTOMATING THE DNA EXTRACTION OF COMPROMISED BONE SAMPLES

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Disasters with large victim numbers such as the MH17 airplane crash in the Ukraine in 2014 reveal the demand for an automated method to profile a large number of compromised bone samples. An automated protocol requires that: 1) bone powder is liquefied by the use of a decalcification buffer so that DNA-binding magnetic beads can be used to extract the DNA; 2) volumes are small so that liquid handling can be performed on compact instruments; 3) buffer compositions and temperature incubations are optimized in order to prevent flocculation and gelatin formation. Various optimization steps were explored and addressed. Optimal results are obtained when 0.1 grams of bone powder are very finely ground and extracted through the recently launched Promega bone extraction kit on the Maxwell FSC extraction robot. We are currently validating this extraction system and protocol for casework implementation in our laboratory using a range of compromised bone samples.

Keywords: Mass disaster, Compromised bone samples, DNA bone extraction, Automated DNA extraction

Presentation number: FG 8 Abstract number:

SEARCHING FOR AND IDENTIFYING VICTIMS OF WARS AND TOTALITARIAN CRIMES

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The case presented in this paper is an example that modern methods applied for human personal identification backed up by extensive archival research are effective in case of the World War II victims. Together with civilizational progress more and more attention was being paid to the fate of single persons, which led for instance to attempts of identification of soldiers killed in action during then numerous armed conflicts. Apart from the wounded also killed soldiers were currently evacuated from the battlefield for the sake of their personal identification. Such activities were of a huge psychological importance for the soldiers in active front-line service as well as for the rest of the community that, in most cases, did not support the idea of the armed conflict. In spite of the fact, that the last war was raging over the area of the West Pomerania more than 70 years ago, the problem of killed and missing during those events persons still exists. Intensification of building industry and road-building has led recently to skyrocketing of World War Two burial places discoveries. In identification research the key role is attributed to DNA analysis, both STR (short tandem repeats) and mitochondrial DNA (mtDNA) methods. DNA analysis with the use of STR markers are widely used recently for personal identification both in ordinary criminal cases and mass-disasters victims' identification. In case of totalitarian systems and war conflicts victims, especially World War Two, such studies are conducted only incidentally. It results from many factors. Isolation of DNA of proper quality and quantity for DNA analysis with the use of STR markers causes much difficulties. Also a great number of victims remains' discoveries as well as collecting the samples of comparative genetic material is a huge problem. The scale of problem in Poland as well as in many other European countries is enormous, even though more than 70 years elapsed since the war was ended.

Keywords: DNA, HID, Victims, Wars, totalitarian

FORENSIC AND COMPARATIVE GENETICS

Presentation number: FG 9 Abstract number:

APPLICATION OF MASSIVE PARALLEL SEQUENCING (MPS) IN OLD MISSING PERSONS CASES

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Massive Parallel Sequencing (MPS) technology revolutionized the field of forensic genetics. In this study, old missing persons cases, involving DNA samples exposed to harsh conditions, containing highly degraded DNA, were reinvestigated using MPS technology, demonstrating advantages of MPS over standard capillary electrophoresis technology. Highly degraded samples from the old police archives were sequenced using Illumina MiSeq FGx sequencing platform and ForenSeq kit according to manufacturer's recommendations. Quantity of DNA used in amplification and library preparation workflow was increased to 1.2 ng. Data were analyzed using ForenSeq Universal Analysis Software and custom based workflow. Examination of heavily degraded real-casework samples using the MPS method was more effective than using standard capillary electrophoresis and GlobalFiler Human identification kit. An average number of extra genotyped markers was 24 in case of STRs (including autosomal, Y and X-STRs) and 78 in case of identity SNPs. High-throughput sequencing of STR markers provides extra information in forensic human identification testing comparing to simple length analysis using CE. Additional STR sequence variants increase the power of discrimination and may allow more unambiguous human identification in many cases. Moreover, MPS technology enables simpler and more relevant deconvolution of DNA mixtures. Finally, due to shortened amplicon sizes in MPS HID kits, analysis of degraded DNA becomes much more effective. Clear and single nomenclature is needed for additional sequence STR variants to make the data easy to interpret and compare.

Keywords: Massive Parallel Sequencing, old missing persons cases, degraded samples, genetic identification, cold cases

Presentation number: FG 10 Abstract number:

IDENTIFICATION OF AN UNKNOWN CORPSE BY DNA SIBLING ANALYSIS

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In forensic genetics, identification of human bodies found in an advanced decomposing state represents one of the most difficult parts. In our study , we present the identification of an unknown corpse found in an advanced decomposed state using as reference samples, biological samples provided by two alleged biological brothers. To obtain an increased reliability of the test, we used autosomal and Y-STR markers. Dura mater was obtained during the autopsy examination from the unknown victim. From buccal swabs of the two alleged biological brothers we isolated DNA as reference samples. For DNA amplification we used the multiplex PCR kit AmpFlSTR Identifiler Plus Kit for autosomal STR markers and AmpFlSTR Y-filer PCR Amplification Kit for the Y-STR markers. Further, we separated the DNA products on an ABI 3500 genetic analyzer. Gene Mapper ID-X version 1.4 software was used to visualize the DNA fragments. Data interpretation was done by GenoProof-3 - Kinship Examination. We obtained genetic profiles for the 3 men on autosomal and Y -STR markers and could establish a biological sibling relationship between them as full siblings.
Based on the obtained genetic information the unknown corpse could be identified as a full sibling of the males reference samples.

Keywords: unknown corpse; DNA; genetic profile; PCR; STR

Presentation number: FG 11 Abstract number:

INVESTIGATING THE USE OF RNA-SEQ FOR FORENSIC BODY FLUID IDENTIFICATION

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Cell and tissue specific messenger RNA markers are used for identification of forensically relevant body fluids, which can offer important insight into the circumstances of a crime. With the increasing interest in Next Generation Sequencing (NGS) in forensic science, we can now produce large amounts of sequencing data which allows for analysis of RNA that is not possible using current methods like RT-PCR. However, forensic RNA samples present many challenges including environmental degradation, minimal starting material, and the potential effects of crime scene sampling methods. By sequencing a variety of fresh and aged body fluids on both the MiSeq FGx and HiSeq 2500 systems, we are evaluating the use of NGS and subsequent bioinformatics analyses for both body fluid identification and biomarker discovery. We have also investigated the effect of chemical fingermark enhancement reagents on downstream RNA analysis. We found haem-reactive reagents were detrimental to RNA-seq data quality, particularly when combined with H2O sampling methods, while protein dye reagents did not differ from control samples. With this research we aim to answer some of the questions around making the move from PCR to NGS for forensic body fluid identification.

Keywords: RNA-Seq, NGS, body fluid identification, forensic, gene expression

Presentation number: FG 12 Abstract number:

AGE-RELATED DNA METHYLATION IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS DIFFERS FROM HEALTHY INDIVIDUALS: DISEASE RELATED EFFECTS PREDOMINATE OVER CORRELATION WITH CALENDAR AGE

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Recently we found that DNA methylation of a set of CpGs located in ELOVL2, C1orf132, TRIM59, KLF14, and FHL2 analyzed in blood can accurately predict calendar age in healthy people. To evaluate the effect of chronic lymphocytic leukemia (CLL), the most common adult leukemia in Western hemisphere, on the age-related methylation signature of human blood. Using pyrosequencing we studied methylation of 32 CpGs in the abovementioned genes in 41 CLL patients and 91 controls; DNA was isolated from the whole blood. The mean calendar age of the patients (64.7y) and controls (63.3) was similar (p=0.41, t-test). Conversely, age predicted from methylation was statistically significantly higher among patients (75.5y) than controls (60.9y, p<1.8x10-12, t test). Among controls the mean age predicted from methylation was 60.9y, i.e. 2.4y less than the mean calendar age (63.3y) - a difference with borderline statistical significance (p=0.049, t-test). Conversely, among patients the mean age predicted from methylation was 75.5y which was significantly higher (by 10.7 y) than the mean calendar age in this group (64.7y, p=0.0001, t-test). Among controls there was a strong correlation between calendar age and age predicted from methylation (Spearman r=0.78, p= 6.1x10-20, n=91). Among patients this correlation had marginal statistical significance (Spearman r=0.38, p=0.013, n=41). Analysis of clinical parameters showed a number of effects including a positive correlation between methylation of C5 at ELOVL1 and RAI stage (Pcorrected <0.003). In the presence of CLL calendar age cannot be reliably predicted by analysis of CpG methylation proposed for healthy people.

Keywords: methylation, leukemia, DNA, age, model

Presentation number: FG 13 Abstract number:

EVALUATION OF EFFECTIVENESS OF NGM DETECT AMPLIFICATION KIT FOR THE ANALYSIS OF HIGHLY DEGRADED BONE MATERIAL

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Many research groups lead studies on improvement of DNA profiling from degraded bone material. When working with degraded DNA in human identification cases, obtaining a full STR profile is one of the most challenging tasks faced by forensic genetics. When it comes to the degraded DNA, the amplification rate decreases with the increasing length of a marker. Thus far, in the Department of Forensic Genetics of the Pomeranian Medical University in Szczecin, the most successful results were obtained with the use of GlobalFiler amplification kit. Despite many of the kit's advantages, drop-outs were still observed in degraded samples, especially in the longest marker included – SE33 – which could reach the length of up to 500 bp. Recently, a new kit was introduced, namely NGM Detect in which markers were notably shortened, in case of the longest one even by 30%. For the evaluation of the effectiveness of NGM Detect, samples from the time of Second World War were chosen. Used remains were found in a mass grave during an exhumation which took place in Białystok (Poland) under the project of The Polish Genetic Database of Totalitarianisms Victims. For the purpose of the study, 54 tooth samples were tested and the amplification rate was compared between two mentioned kits. DNA concentration in the majority of the samples didn't reach the 0,01 ng/μL level and the degradation index indicated high degradation in all of them. Difference in amplification rate was observed in all the autosomal STRs, with the biggest significance noticed for SE33 marker.

Keywords: forensic genetics, STR profile, human identification,degraded DNA, drop-outs

Presentation number: FG 14 Abstract number:

NETWORK ANALYSIS ON Y CHROMOSOME HAPLOGROUPS IN WESTERN BALKAN POPULATIONS

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The region of Western Balkans has been inhabited since the Paleolithic era and was the route of farming from the Middle East to Europe during the Neolithic era (7th millennium BC). In the present study, Y-STR data from worldwide populations have been used to construct median-joining networks. The study was performed using Whit Athey's Haplogroup Predictor, Y Utility and Network 4 software packages to construct networks, perform clustering of closely related Y chromosomes and calculate time estimates between individual nodes. The results of the study imply that geographically close populations cluster together on both worldwide and European level. It was observed that an elevated number of study populations and individual haplogroups increases the possibility that individuals of different racial and ethnic background cluster within the same or neighboring clades of network. An example is the case of the Nigerian population clustering closely with the populations from the Western Balkans. Subsequent time estimates performed based on the mutation frequency between the ancestral node and its descendant nodes revealed that I2a is the oldest haplogroup in the major area of the Balkan Peninsula (estimated separation time from its ancestral state: 4858 years), followed by haplogroups E1b1b (4088 years) and R1a (3910 years). This study is based on data collected from a single database and, therefore, gives approximations of the relative time distance between the nodes. Our results are nonetheless in accordance with previously published papers investigating the frequency of Y haplogroups based on Y-SNP variant frequencies, indicating that Western Balkan countries are mainly represented by I2a subclade (average for six countries 35.93%), followed by the other two haplogroups (average for six countries 23.16% and 10.62% regarding R1a and E1b1b, respectively).

Keywords: Western Balkans, Y chromosome, Y haplogroup, median-joining network analysis, time estimation

Presentation number: FG 15 Abstract number:

STRAF: STR ANALYSIS FOR FORENSICS - A TOOL FOR THE EVALUATION OF POPULATION DATA

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Population datasets of good quality are crucial for forensic genetics. A variety of powerful programs are available to statistically assess such data. However, they were mostly developed by labs engaged in fundamental research on population genetics and were not tailored for the forensic genetics community. Their handling usually requires a substantial amount of experience and more than one program has to be used to check all the parameters that are forensically relevant. We present here an easy to use online tool for the statistical assessment of STR population data in forensic genetics. STRAF allows for classical statistics applied in population genetics, such as F statistics or control for Hardy-Weinberg equilibrium. Furthermore it provides forensically relevant parameters such as match probability or power of discrimination for the selected STR loci. The dataset can be analyzed at once for all loci, based on a very simple input file format, also accepting “point alleles” (e.g. TH01 = 9.3). Results, such as frequency tables, can be downloaded and displayed graphically online. We also provide the possibility to perform a principal component analysis on the data.

Keywords: population data, statistics, STR, software, forensic genetics

Presentation number: FG 16 Abstract number:

GENETIC FINGERPRINTING: THE EXPERIENCE OF THE GENETIC TYPING CORE FACILITY IN INSTITUT PASTEUR IN TUNIS

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Genetic fingerprinting or DNA profiling is a DNA typing method used to characterize individuals based on their DNA profiles. This technique was first reported in 1984 by Sir Alec Jeffreys at the University of Leicester in England. Since, it has been applied in many fields such as parental testing and forensic science. In Tunisia, the use of DNA typing for parental testing began in 1998, according to the Law No. 98-75 on the patronymic naming of children born out marriage as well as in case of marital conflict. In our laboratory, DNA profiling is performed only in the frame of judicial context. DNA typing is currently used to include or exclude paternity or to confirm the identity of living persons. Furthermore, we have been requested to determine genetic fingerprinting in forensic in order to contribute to the resolution of some criminal cases and to identify victims of terrorist attacks both in Tunisia and Lybia. We performed also DNA profiling for prenatal diagnosis, to check maternal foetal contamination. The analysis of the typology of the requisitions showed that the highest request concerns those related to marital conflicts. Our methodology consists to extract DNA from blood samples for living persons and tissue biopsy, bone and/or theeth samples for post-mortem human remains. The genetic profiling is based mainly on the genotyping of 16 nuclear STR (Short Tandem Repeat markers) including the amelogenin gene. In some cases we added the genotyping of Y chromosome STR and mitochondrial DNA sequencing. We have developed in Tunisia a rapid and effective molecular protocol for human body identification from teeth and we are working on the development of other cost-effective methods.

Keywords: DNA profiling, parental testing, forensic, nuclear STR, Y chromosome STR

Presentation number: FG 17 Abstract number:

VALIDATION OF THE SUREID® 21G HUMAN STR IDENTIFICATION KIT AND CONCORDANCE STUDY OF THE NEW GENERATION MULTIPLEX STR KITS

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Short tandem repeats, also reffered as microsatellites, are polymorphic genetic markers constisting of short repetitve sequences which are 2-7 base pairs long and repeated certain number of times at the defined locus. STRs are most commonly used markers in forensic identification because they are proved to be most ideal markers for that purpose. With the expansion of usage of STR markers in forensic identification, commercial multiplex STR kits appeard on the market. They are designed to analyze more autosomal STR loci in one PCR reaction. Purpose of this study is to validate The SureID® 21G Human STR Identification Kit which was produced by HEALTH BioMed Company from China and to do the concordance study which compares this commercial kit with the kits that are already used in forensic analayses in Bosnia and Herzegovina. The SureID® 21G Human STR Identification Kit is a 5-dye, short tandem repeat (STR) multiplex assay that amplifies 20 autosomal STR loci and the sex determining marker. During the research study, 105 DNA profiles form individuals living in Bosnia and Herzegovina, were analyzed. Detected loci, intra-locus balance, inter-locus balance, stutter analysis and concordance study of this one and other kits used in BiH were examined during the research. According to the results that were obtained, it can be concluded that this kit is suitable for forensic identification in Bosnia and Herzegovina, as well as the other kits used in previous analysis in this region. This study is the first validation of the SureID® 21G Human STR Identification Kit in BiH and region.

Keywords: STR, Multiplex STR, Validation, Concordance

Presentation number: FG 18 Abstract number:

COMPARISON OF POWERPLEX® Y23 AND RAPIDLY MUTATING Y-STR MARKERS BY PHYLOGENETIC ANALYSIS

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The importance of accurate and precise typing and statistical analysis of Y-STR-based data led to the development of several commercial kits, such as the PowerPlex® Y23 (PPY23) System. In 2012, a novel approach in Y-STR analysis has been launched and a panel of 167 markers has been analyzed. Of those, 13 Y-STR loci with exceptionally high mutation rates (>10-2 per locus per generation) were selected to form the first set of the so-called rapidly mutating (RM) Y-STR markers. When compared to previously manufactured commercial kits with haplotype diversity (HD) rates of 0.99995 for Yfiler (17 loci) and 0.999995 for PPY23 (23 loci), RM markers had an improved performance with HD value of 0.999997. In order to test the hypothesis that the minor difference in HD values between PPY23 and RM locus panels of 0.000002 makes the latter set advantageous in phylogenetic analyses, separate phylogenetic trees consisting of worldwide human populations were constructed using these two sets of markers in POPTREE2. Better resolution and more meaningful regional sub clustering of populations has been observed in the RM-based phylogenetic tree despite the fact that PPY23 contains 10 Y-STR loci more than the rapidly mutating Y-STR system. The conclusion of the present study is that a difference of 0.000002 in discrimination capacity corroborates an improved resolution in phylogenetic analyses.

Keywords: Y-STR, PowerPlexÂ® Y23, Rapidly Mutating Y-STR, Comparison, Phylogenetic Analysis

Presentation number: FG 19 Abstract number:

DNA TAPE FOR STUDIES ON SEXUAL OFFENCES

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In case of sexual offences typical traces include: saliva, sperm, hair and blood. The prosecution of sexual offences other than rape, associated with the sexual breach of bodily integrity, is associated with the necessity to employ the analysis of touch traces, which are secured in such crime cases. During instances when the perpetrator does not leave any traces of sperm, blood, hair or saliva in or on the body of the victim, the victims clothes or the crime scene, it is necessary to search for residual epithelial cells on the victims clothes or in places which did not have direct contact with the external environment. Such places include: the inner surface of upper parts of pants, skirts, underpants and bra cup padding. The presence of suspect material in such places may be a crucial clue, since the transfer of the genetic material on such surfaces is associated with sexual offences.Several types of glue tapes are used in order to collect touch traces from porous surfaces, which facilitate the gathering of biological material from the surface. The most notable issue in the dissemination of this method of collecting evidence materials is to ensure that the tape is prepared without any contamination. We introduce a validation of collecting touch samples from the surface of clothes using the DNA TAPE®. The tape was prepared in the form of single-use bars with a working are of 15x60 mm, which were laser cut in order to allow for simple and safe collection of evidence material from clothes. The results of comparative analyses indicate that the proposed method if far more efficient compared to cutting fragments of clothes. The validation methods indicate that in 80% of cases unequivocal genetic profiles of the users are obtained from used clothes. Even in case of a short contact between the perpetrator and the victims clothes, unequivocal genetic profiles are obtained, including the range of autosomal STR loci as well as Y-STR markers.

Keywords: sexual offences, touch trace, DNA TAPEÂ®, genotyping

Presentation number: FG 20 Abstract number:

12 X-CHROMOSOME STR MARKERS IN THE POPULATION OF SOUTH CROATIA

Korolija M[1], Ozretić P[2], Crnjac J[3], Merkaš S[1], Račić I[1], Rožić S[1], Sukser V[1], Barbarić L[1], Popović M[4], Mršić G[1], Špoljarić D[4]

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Short tandem repeat (STR) markers have been used in forensic identification and kinship testing for decades. Routinely used autosomal STRs provide sufficient information for personal identification. However, forensic casework samples are usually of low quantity, contaminated and degraded, yielding partial and/or mixed profiles. X-STR profiling can in that sense assist identification in forensic investigations as an additional source of information. Moreover, because of its unique pattern of inheritance, X-STR typing has been proved useful in kinship testing. Therefore we assessed applicability of Investigator® Argus X-12 Kit for routine forensic work involving Croatian population. 12 X-STR loci belonging to four linkage groups were co-amplified and analyzed by capillary electrophoresis in 298 female and 301 male DNA samples of unrelated donors. Computations based on allele and haplotype frequencies were performed. Forensic parameters, linkage disequilibrium and genetic distance from ten non-Croatian populations have been determined. In female samples, Hardy-Weinberg equilibrium was confirmed for all X-STR markers. DXS10135 was the most, while DXS8378 was the least informative marker. In both male and female samples, combined power of discrimination exceeded 0.999999999. Linkage group 1 was the most informative. Significant genetic distance from south Croatia population was confirmed for Greenlandic, and three non-European populations. In conclusion, Investigator® Argus X-12 Kit can be used in forensic casework for both identification and familial testing in the population of south Croatia.

Keywords: X-STR, InvestigatorÂ® Argus X-12, Population study, South Croatia, Forensics

Presentation number: FG 21 Abstract number:

ABERRANT X-CHROMOSOME STR PROFILES DETECTED IN THE CROATIAN SAMPLE POOL

Korolija M[1], Ozretić P[2], Crnjac J[3], Merkaš S[1], Račić I[1], Sukser V[1], Rožić S[1], Barbarić L[1], Popović M[4], Mršić G[1], Špoljarić D[4]

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Analysis of X-chromosome short tandem repeat (STR) markers has proven its forensic value as both a supporting method to traditional autosomal STR profiling, and a method of choice in solving a number of complex kinship cases. As the first step towards implementation of X-STR typing into routine forensic practice of the Croatian Forensic Science Centre “Ivan Vucetic”, we performed a population study using Investigator® Argus X-12 Kit. Approximately 1000 of both male and female blood samples collected at 1:1 sex ratio during routine forensic work were analyzed by capillary electrophoresis. All samples containing either off-ladder alleles, allele dropouts or exhibiting uncommon profiles were re-extracted and re-analyzed for confirmation. Although aberrant profiles cannot be included in calculation of the population forensic parameters, awareness of their potential to add complexity to actual forensic investigation is important. Here we present 7 profiles exhibiting biallelic and triallelic patterns in males and females respectively at the DXS10079 locus, and one triallelic pattern found in one female profile at DXS1034. In addition, we show 3 female profiles indicating XXX condition (Triple X syndrome) and 1 male profile indicating XXY condition (Klinefelter syndrome). Our findings identify DXS10079 locus as a mutational hotspot in Croatian population. They also provide improved estimations of X chromosome aneuploidies in general population directly from genetic data, given that affected persons often stay undiagnosed due to mild symptoms in certain cases.

Keywords: X-STR, InvestigatorÂ® Argus X-12, Croatian population, DXS10079, Forensics

Presentation number: FG 22 Abstract number:

COMPARATIVE MOLECULAR GENETIC ANALYSIS OF THE ISOLATED BOSNIAN-HERZEGOVINIAN AND SLOVENIAN HUMAN POPULATIONS

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There are local human communities in Selska Valley which are more or less isolated. In this case, isolation have been influenced mostly by geographic and cultural factors. Therefore, the population of Selska Valley is very suitable for molecular-genetics studies of its population structures. Anthropological studies of Selska Valley are performed in 1993 Vidovic (2005) has analyzed a genetic structure of isolated populations which is based on the distribution of the surnames in this area, using Isonomy method as one of the indirect genetic methods. Fifteen autosomal STR loci have been studied in geographically close, but still isolated, populations of villages located in the valley area. The first goal of this study was to identify the possible differences between the populations from two groups of villages: the lowland villages (Bukovica, Sevlje, Dolenja Vas, Selca, Zelezniki and Zali log) and the mountain villages (Podlonk, Prtovc, Spodnje Danje, Zgornja Sorica and Spodnja Sorica). Even though there have been different isolation levels and openness among these villages, in genetic terms they, especially those in the mountain area, may be considered inland islands. The DNA has been obtained from 86 individuals, and the allele frequencies and genetic diversity have been compared among these two sample groups. In addition, all of the fifteen STR loci have been used in a comparative population analysis between the Selska Valley and the Bosnian mountain area. Although the sample sizes are relatively small, the observed variation within any of these small isolated populations is high and comparable to less isolated groups. Even though the populations are geographically isolated, the STR data are similar among the populations. Selska Valley and its village populations certainly represents a distinct isolate, and therefore studies of this type could significantly contribute to a better understanding of the populations and isolates in general.

Keywords: Short Tandem Repeat (STR), Isolated population, Heterozygosity, Inland Islands, Genetic distance

Presentation number: FG 23 Abstract number:

COMPARISON OF SOUTHWESTERN US HISPANIC POPULATIONS TO MEXICAN HISPANIC POPULATIONS USING IMMUNOGLOBULIN HAPLOTYPES

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The heavy chain immunoglobulin (IGH) haplotypes were the earliest ancestry informative markers (AIMs), the Kappa light chain (KM) haplotypes are less so. Historically in the Southwestern US, individuals identified as Hispanic have originated in Mexico. The question arises as to variation among the SW US Hispanics and their relationship to Mexican populations. To investigate this data on IGH and KM haplotypes were used.

IGH and KM haplotypes from 1,265 HHANES samples from 17 locations (SW US) and 1,400 Parentage samples, were compared to 1,517 Mexican Indians (7 tribes) and 1,408 Mexican urban samples (9 locations) using F_{wr}, F_{rt} and F_{st} statistics.

No significant variation was found among the 17 US samples divided by state for immunoglobulin haplotypes. There was highly significant variation between Mexican tribal and urban samples (Frt=0.0321, , χ^2=8.254, p=0.004; Fst=0.056, χ^2=8.254, p=0.0001) for IGH haplotypes, while only total Fst was significant for KM haplotypes (Fst= 0.037, χ^2=9.576, p=0.0019) after Bonferonni correction. Comparing US to Mexican (tribal and urban) indicated significant variation (Frt= 0.046, χ^2=8.449, p=0.0036; Fst=0.064, χ^2=11.589, p=0.0006) for IGH haplotypes after Bonferroni correction. The primary difference was the amount of European admixture, increasing from Mexican Indians, Urbans and US populations (0.057, 0.246, 0.375, Fst=0.098, χ^2=19.340, p=0.000), with African haplotypes also increasing (0.014, 0.035 and 0.045), but not significantly.

These results indicate that SW US Hispanics are a relatively homogeneous group. Representing immigrants with more European and African gene flow than populations resident in Mexico. However, individual SW US Hispanics have wide variation in individual admixture.

FORENSIC DNA DATABASES

Presentation number: FG 24 Abstract number:

ALLELE FREQUENCIES AND GENETIC PARAMETERS FOR 15 SHORT TANDEM REPEAT LOCI IN THE POPULATION OF BOSNIA AND HERZEGOVINA

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Allele frequencies of the 15 polymorphic STR loci (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, FGA) for Bosnian and Herzegovinian population were established and statistical forensic parameters were calculated. In order to expand national population data with allele frequencies and statistical data for fifteen STR loci, 1000 unrelated individuals born in Bosnia and Herzegovina volontarly participate in the study. Qiagen DaeasyTM Tissue Kit was used for DNA extraction from buccal swabs. Genomic DNA amplification was performed using PowerPlex® 16 System which enables amplification and detection of 15 STR and amelogenin. For PCR amplification GeneAmp PCR System 9700 (Applied Biosystems) was used. The capillary electrophoresis of amplified products was carried in an ABI 310 Genetic Analyzer while numerical allele designations were determined using GeneMapper®ID software v.3.2. Microsoft Excel workbook template—PowerStats was used for calculating allele frequencies, matching probability (MP), power of discrimination (PD), power of exclusion (PE) and typical paternity index (PI). Powermarker v.3.25 was used for calculation of number of alleles (AN), deviation from Hardy–Weinberg equilibrium, observed and expected heterozygosity (Ho and He) and polymorphism information content (PIC). Exact test of population differentiation was estimated using Arlequin v.3.5.1.2. After Bonferroni's correction, statistical significance for deviation from Hardy–Weinberg equilibrium was considered as $P<0.01$, while for population differentiation test $P<0.001$. Number of effective alleles (AE) was estimated by $1/\sum pi2$, where p is allele frequency for particular locus. Ratio of effective and detected allele numbers and its statistical significance were also calculated. No statistically significant deviation ($P \square 0.05$) from HWE was found for analyzed loci, except for D8S1179 locus, which was not significant after applying the Bonferroni's correction ($P \square 0.01$). Heterozygosity excess has been detected for D3S1358, D21S11, D18S51, D16S539, vWA, TPOX loci. Total of 160 alleles were detected, among which 32 are considered as rare alleles (frequency $\square 0.005$). The highest number of alleles was detected for PentaE (18) and the lowest for TH01 (7).

Keywords: STR markers, allele frequencies, forensic parameters, DNA database B&H population

Presentation number: FG 25 Abstract number:

DEVELOPING FORENSIC DNA DATABASES BY 18 STR FOR THE REPUBLIC OF BELARUS

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The goal of our study is developing forensic DNA databases by 18 STR for the capital of the Republic of Belarus – Minsk and the Republic of Belarus taking in consideration historical ethnographic regions (Poozerye, Ponemanye, Podneprovye, Center, Western and Eastern Polessye). Data on variability of 18 STR: population dataset from 11 populations (N = 1040), paternity testing dataset (N = 2550), dataset of genotypes from a criminal registration database (N = 8756) were studied. In the total dataset, anonymous data on genotypes by 18 autosomal STR loci in 12346 representatives from 118 administrative regions of the Republic of Belarus were included. Also, data on variability of 18 STR in Minsk population (N=473), including ethnically pure Belorussian (N=218), were used. Samples from population of the Republic of Belarus were characterized by 18 STR (vWA, TH01, TPOX, CSF1PO,D5S818, D7S820, D13S317, D16S539, F13B, D18S51, D8S1179, D21S11, FGA, PentaE, PentaD, D2S1338, D19S433, D3S1758): multiplex PCR was performed using "PowerPlex® 16 System" and "AmpFlSTR Identifiler™", capillary electrophoresis was performed in "ABI Prism 3130 XL Genetic Analyzer" ("Applied Biosystem", USA). Statistical treatment was performed using the software packages: Arlequin ver. 3.000, GDA 1d16c, and FSTAT. Questionnaire data were collected for the population dataset and for the population of Minsk. The absence of significant differences between the datasets studied and the absence of inner subdivision of the datasets was demonstrated that allowed us to unite the datasets into unique reference database. The absence of inner subdivision by historical ethnographic regions of the Republic of Belarus was, also, demonstrated. The reference DNA database was characterized by parameters of informative significance: MP=7,089342x10-22, PE=0,999961, and PD=99,9999999999999999 % for 18 STR. Comparison with other Slavic populations is made. The necessity of using the original reference DNA database for forensic expertise in Belarus was demonstrated. Developing regional DNA databases in the residence of minorities of non-Slavic origin is recommended. For Minsk, as a megalopolis with special genetic demographic processes, using original DNA database is proposed.

Keywords: reference DNA database, 18 STR, Belarus, Minsk

FORENSIC DNA PHENOTYPING

Presentation number: FG 26 Abstract number:

CHARACTERIZING GENETIC INTERACTIONS UNDERLYING HUMAN HEAD HAIR SHAPE

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The epistatic effects are thought to play an important role in the etiology of many complex traits. It seems that head hair shape in humans provides a good model for studies on epistasis due to the complex network of known biological interactions between the proteins responsible for molding and strengthening the hair structure. Nevertheless, genetic basis of human head hair shape is still not well established. To date, several studies have been performed which revealed few genes (TCHH, WNT10A, FRAS1, PRSS53, EDAR, GATA3) associated with hair shape in humans and explaining only a small proportion of the total variation in this distinguishing trait. It seems that proper identification of genetic interactions may be essential to explain the remaining fraction of the overall heritability. Furthermore, elucidating the genetic underpinnings of human hair morphology may improve DNA-based prediction of hair morphology for forensic and anthropology purposes. Here, we analyzed main effects and interactions between 58 SNP candidates for hair shape in a total of 635 individuals from Poland. Candidate SNPs were selected from literature based on their potential role in human hair morphogenesis and growth, expression pattern in the hair follicle, involvement in protein-protein interactions and relationship with pathological condition of human hair structure. Genotypic data were gathered using massively parallel sequencing provided by the Ion AmpliSeqTM technology and Ion PGMTM system. Statistical interactions were studied using several computational approaches, including PLINK 1.9, BlocBuster, and MDR 3.0.2 software. As a result, 14 out of 58 SNPs were found to be associated with hair morphology in the studied population. Our study revealed several significant epistatic effects including interaction between P2RY5 and EBF1 confirmed with MDR and logistic regression and explaining additional 2.3% of the total variation of human hair shape. This research was supported by the grant from the National Science Centre in Poland no 2014/15/D/NZ8/00282.

Keywords: head hair shape, genetic interactions, next generation sequencing, forensic DNA phenotyping, single nucleotide polymorphisms

Presentation number: FG 27 Abstract number:

SEEKING GENES UNDERLYING HUMAN HAIR SHAPE VARIATION AND RELEVANCE FOR DNA-BASED PREDICTION

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Human head hair shape shows striking variation and is largely heritable. Eight genes have been previously identified with involvement in hair shape globally, which however cannot explain the total phenotype variation observed and also not the high heritability of up to 95% estimated in twin studies. Improving genetic knowledge of hair shape variation may facilitate its future use in DNA-based prediction such as in Forensic DNA Phenotyping. A meta-analysis of three European genome-wide association studies in a total of 16,763 individuals identified 8 novel loci and confirmed 4 previously known ones. A prediction model including 51 SNPs from 47 genes reached a cross-validated AUC value of 0.71 in 10,607 Europeans for straight versus non-straight hair prediction. An extended set of 90 candidate SNPs was further validated in a set of 2,118 independent individuals of European origin collected by 10 participants of the EUROFORGEN-NoE Consortium and one additional partner from the US (SW). Genotypic data were established using two massively parallel sequencing technologies Ion Torrent PGM (Thermo Fisher Scientific) and MiSeq (Illumina) or mass-spectrometric analysis using Sequenom EpiTYPER, depending on the participant. Prediction modelling involving 90 SNPs achieved a cross-validated AUC value of 0.67 when applied to EUROFORGEN-NoE samples. A previous model based on 3 SNPs from 3 genes obtained for 528 Polish samples gained an AUC of 0.62. Difference in model-based prediction accuracies may be explained by the DNA predictors, the phenotype accuracy, the genetic heterogeneity of the tested individuals, their age, the sample size and other parameters. Overall, our study substantially improves genetic knowledge of hair shape variation, and provides increased accuracy of DNA-based prediction of this externally visible characteristic. This research was supported by several projects including grants from the European Union Seventh Framework Programme no. 285487 (EUROFORGEN-NoE) and from the National Science Centre in Poland no 2014/15/D/NZ8/00282.

Keywords: Human hair shape, genome-wide association study, single nucleotide polymorphism, next generation sequencing, forensic dna phenotyping

Presentation number: FG 28 Abstract number:

DNA METHYLATION-BASED AGE PREDICTION FROM SALIVA: HIGH AGE PREDICTABILITY BY COMBINATION OF 7 CPG MARKERS

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DNA methylation is rising as one of the most promising age-predictive markers. Many DNA methylation-based age predictive models have been developed based on DNA methylation patterns from blood. However, a few studies have attempted to predict age from saliva, which is frequently found at crime scenes. In this study, we generated genome-wide DNA methylation profiles of saliva from 54 males and identified 6 CpG markers on the SST, CGNA3, KLF14, TSSK6, TBR1, and SLC12A5 genes that showed a high correlation between methylation and age. With 226 bisulfite converted saliva DNA samples, we investigated DNA methylation at 6 age-associated CpGs and a cell type-specific CpG from the PTPN7 gene using methylation SNaPshot method. Then an age-predictive model was constructed with age information and the methylation profile from the 113 training samples. The model showed a high correlation between predicted and chronological age of more than 90%, and the mean absolute deviation from chronological age (MAD) of 3.13 years. Subsequently, the validation set composed of the rest 113 samples presented a 95.2 % correlation between predicted and chronological age and 3.15 years of MAD. In addition, as the developed model is based on the multiplex methylation SNaPshot, it might be easily integrated into the forensic laboratory workflow.

Keywords: DNA methylation, age prediction, saliva, HumanMethylation450 Beadchip, SNaPshot

Presentation number: FG 29 Abstract number:

IMPROVING ANCESTRY DISTINCTIONS AMONG SOUTHWEST ASIAN POPULATIONS

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The Kidd Lab panel of 55 AISNPs can provide up to 10 statistically relevant biogeographic groupings of populations globally. After applying worldwide screening of the current AISNPs panels, a second-tier panel would be useful for increasing the accuracy for within-regional differentiation. Because recent advances in massively parallel sequencing (MPS) methods allow us to sequence a much higher number of SNPs, we are now searching for additional SNPs that will provide refined discrimination among a biogeographic subset of the global pattern; Southwest Asia and the nearby Mediterranean region (SWA) is our current target for such a “second tier” panel. We selected the potentially the best SNPs from various sources: our own laboratory database (>4600 SNPs), refining AISNPs panels (Kidd 55 and Seldin 128 SNP panels), and also choosing from published papers that studied European and SW Asian populations. Rosenberg’s Informativeness, Fst, and allele frequency heatmap matrixes are used to determine the best SNPs for the region. A total 2607 individuals from 40 different populations, were used in the refinement processes and analyses included five new populations (Turkey, Turkish Cypriots, Greece, Iran and Palestinian Arabs), as well as the Kidd Lab and 1000 Genome Phase3 populations exclusively from Eastern Africa to Central South Asia. The final selection of AISNPs includes potentially the best SNPs from this combination of the SNPs. The PCA and Structure (K=4) indicate differentiation between SWA and Northern Europeans. The final Joint 87 AISNPs provide the basis for building an improved, optimized panel of AISNPs that provides additional information on differences among populations in this part of the world. We are currently planning to test this panel with additional populations from the area and also improve the panel with new SNPs or microhaplotypes.

Keywords: Biogeographic ancestry, AISNPs, Southwest Asia

Presentation number: FG 30 Abstract number:

THE USE OF PHYLOGENETIC ANALYSIS IN CASES OF HUMAN TRAFFICKING

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Forensic research in cases of human trafficking are especially challenging, since the reference material originating from victims is rarely available. We describe a case of suspicion of participation in human trafficking which included a phylogenetic analysis of results of genetic polymorphism in the range of HV1 and HV2 regions of mtDNA and Y-STR loci. Hand towels and suspenders were collected from the house belonging to people suspected of trafficking Vietnamese, Albanian and former USSR citizens from the area of a major city in Poland to Western Europe. Traces of faeces were found on the hand towels, whereas the suspenders contained traces of blood. Based on the analysis of autosomal STR markers, the traces originating from the owner of the house were discarded and biological material belonging to two males and one female was identified. Polymorphism typing in the range of HV1 and HV2 mtDNA as well as Y-STR loci was conducted in case of traces originating from males using the Y-Filer kit. The genetic material belonging to the female was subjected to typing in the range of mtDNA. Based on the results of the analyses, the maternal as well as fraternal kinship between these people was discarded. The results of polymorphism typing in the range of mtDNA were subjected to further analysis using the HaploGrep application. Based on the Y-STR typing, the haplogroup was established using Haplogrup Predictor whereas the geographical origin was determined using the yhrd.org database. It was established that the female belonged to mtDNA haplogroup M7b1a+, which occurs in Easters Asia. In case the material originating from the two males, the first was identified as the member of haplogroup N in the range of Y chromosome and mtDNA haplogroup N, whereas the second belonged to haplogroup I2a, which occurs mainly in the area of south-eastern Europe, and mtDNA haplogoup T2, which occurs in Southern Europe and the Caucasus. The presented results show that the use of phylogenetic analysis in standard forensic studies, which is realized in the range of Y-STR loci and/or mtDNA may provide valuable data regarding the probable geographical origin of people, whose traces were secured during the course of cases associated with human trafficking.

Keywords: human, trafficking, phylogenetic, mtDNA, Y-STR

Presentation number: FG 31 Abstract number:

PREDICTIVE DNA ANALYSIS BY ON-ARRAY MINISEQUENCING IN DNA IMAGING TECHNOLOGY

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Predictive DNA Analysis have been included as standard forensic analyses. Several SNP markers linked with phenotypic traits were have already been described. The most well-known markers include those associated with the iris pigmentation, hair and skin colour as well as blood types in the AB0 system. Predictive analysis is characterized by a certain detection value, however, in contrast to typical analysis of polymorphic genetic markers, it does not possess an evidential value and is therefore rarely used. Due to this fact there is a need to develop simple, comprehensive methods of analysis for predictive markers. The method includes a multiplex PCR amplification of 24 SNP loci associated with pigmentation and 5 associated with the AB0 group system: N29insA, rs11547464, rs885479, rs1805008, rs1805005, rs1805006, rs1805007, rs1805009, Y152OCH, rs2228479, rs1110400, rs28777, rs16891982, rs12821256, rs4959270, rs12203592, rs1042602, rs1800407, rs2402130, rs12913832, rs2378249, rs12896399, rs1393350, rs683, AB0-SNP1, AB0-SNP2, AB0-SNP3, AB0-SNP4, AB0-SNP5. The fragments are amplified in a single PCR reaction, purified and then subjected to cyclic on-array mini-sequencing with the use of dideoxynucleotides labelled with different fluorescent dyes. The purified PCR products are subjected to cyclic SBE reaction of oligonucleotides immobilized on array in a Mastercycler Nexus Flat (Eppendorf) thermocycler, then rinsed two times in deionized water in a High Throughput Wash Station (ArrayIt), dried by centrifugation in a Microarray High Speed Centrifuge and analysed using a GenePix 4300A (Molecular Devices) scanner at 4 different ranges of excitation and cut-off lengths for specific dideoxynucleotides. Due to the use of cyclic array mini-sequencing the sensitivity and specificity of the reaction is notably enhanced. The quantification level is at 0.01 ng/µl. The use of array divided into 4 sub-arrays dedicated to the analysis of positive and negative control as well as two analyses of target sample allowed to conduct the typing of 29 predictive markers during a single analysis with ensured control of highest test quality.

Keywords: Predictive, DNA, analysis, on-array, DNA-Imaging

Presentation number: FG 32 Abstract number:

THE BODY CALENDAR: CHRONOLOGICAL AGE DETERMINATION USING DNA METHYLATION MARKERS AND MASSIVE PARALLEL SEQUENCING

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Over the last few years it became clear that additional information is hidden within epigenetic modifications, and that especially DNA methylation (DNAm) could provide useful evidence to the criminal justice system. Within this project, specific changes in DNAm levels upon age progression at selected loci were used to develop an objective scientific tool to determine the chronological age of an (unknown) individual. This information can be used to narrow down the list of suspects during criminal investigations or to determine the age of a person in other legal contexts such as human trafficking. Raw data from publicly available DNAm profiles (450K BeadChip arrays) were quality-selected and normalized to obtain one dataset for further investigation. After preselection of markers, a random forest regression model was applied to discover the most 15 promising age-correlated markers. These markers as well as an age-independent marker for control purposes were selected to develop an amplicon based massive parallel sequencing (MPS) approach. DNA of whole blood from 208 individuals in the age range of 18-69 years was extracted, sodium bisulfite converted, and the loci of interest amplified and sequenced using MPS. This analysis was used to train and optimize the machine learning algorithm to predict age. Finally, 13 age-dependent markers were included and resulted in a mean RMSE of 4.1 years (repeated 5-fold CV) and MAD of 3.3 years. Additional 104 blood samples were analyzed for independent model testing, confirming the results of the cross-validation by getting an RMSE of 4 years and MAD of 3.2 years. We confirmed some of the markers that were selected for age prediction in previous forensic studies (ELOVL2, TRIM59, F5, KLF14) and we added further age prediction markers (e.g. HOXC4, RPA2, ZYG11A) which are not yet included in assays. We also investigated whether the inclusion of CpG sites in close proximity of the CpG site of interest can improve the model and if the appearance of SNPs within the amplified locus could explain the deviation of the predicted age compared to the chronological age in some cases. In conclusion, we demonstrate that MPS is an accurate and efficient tool to determine DNAm levels of specific CpG sites, and that in combination with machine learning it is a useful tool for chronological age determination.

Keywords: DNA phenotyping, Age prediction, DNA methylation, massive parallel sequencing, machine learning

Presentation number: FG 33 Abstract number:

PREDICTIVE ACCURACY OF THE HIRISPLEX-S SYSTEM IN A GROUP OF MINORS WITH RECORDED AGE RELATED HAIR DARKENING

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HIrisPlex-S provides the first complete system for simultaneous prediction of eye, hair and skin colour developed especially for use in forensic and anthropological investigations. The method relies on genotyping of 41 SNPs in 16 genes and interpretation of the obtained genotypes with three multinomial prediction models. Implementation of any new method in forensic genetics should be accompanied with appropriate validation study. Validation of predictive DNA analysis tools should involve the reliability of genotyping assay and the accuracy of prediction model since both steps may affect the final outcome of analysis. In this study we addressed the problem of prediction accuracy of the models implemented in the HIrisPlex-S system with a special focus on a potential role of age and gender. 480 minors from Poland (aged 8 – 18 years) were investigated using high-throughput DNA sequencing provided by the Ion AmpliSeqTM technology and Ion PGMTM system. Pigmentation phenotype was assessed by a combination of quantitative measurements (skin and hair color) and qualitative assessment (eye and hair color). The information about gender and potential age related changes in hair colour were taken into account. Prediction accuracy of the HIrisPlex-S system was evaluated for various phenotype categories and compared with results obtained for adults. Prediction performance was also compared between children with age related hair darkening and those without this phenomenon.

Keywords: HIris-Plex-S, hair darkening, next generation sequencing, DNA phenotyping, single nucleotide polymorphisms

Presentation number: FG 34 Abstract number:

PREDICTION OF PIGMENTATION TRAITS FROM BONE SAMPLES USING HIRISPLEX-S AND ION-TORRENT SEQUENCING TECHNOLOGY

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Identification of human remains is an important part of forensic investigations. DNA methods based on STR and mitochondrial DNA analyses have been well established but they may not have practical application when reference material is not available. In such cases predictive DNA analysis can provide investigative leads used to identify an unknown individual. Ancestry, age and some appearance traits can be predicted from DNA but analysis of skeletal remains may be very challenging. The recently announced HIrisPlex-S system enables simultaneous prediction of eye, hair and skin colour by analysis of 41 SNP predictors.In this study we used Ion AmpliSeqTM technology and Ion PGMTM system to analyze the HIrisPlex-S markers in 80 bone samples collected at various postmortem ages from 20 to 60 years. The study involved WWII samples, from individuals of an unknown identity, included in the Polish Genetic Database of Totalitarianisms Victims operated by the Pomeranian Medical University in Szczecin. We verified the usefulness of massively parallel sequencing technology as a method for analyzing multiple markers in problematic material such as degraded bone samples. Performance of the method was assessed by comparing its efficiency with the outcome of STR analyses. In some cases, prediction reliability of the HIrisPlex-S models was verified by analysis of available data about phenotypic features of the investigated individuals.

Keywords: HIrisPlex-S, next generation sequencing, DNA phenotyping, pigmentation, single nucleotide polymorphisms

GENETIC ANALYSIS OF FORENSIC NON-HUMAN MATERIAL

Presentation number: FG 35 Abstract number:

OPERATION TIGER´S EYE: DNA TESTING OF TRADITIONAL CHINESE MEDICINE ARTIFACTS IN CZECH REPUBLIC

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Traditional Chinese medicine (TCM) has been practiced for thousands of years, but only within the last few decades has its use become more widespread outside of Asia. Concerns continue to be raised about the efficacy, legality, and safety of many popular complementary alternative medicines, including TCMs. Ingredients of some TCMs are known to include derivatives of endangered, trade-restricted species of plants and animals, and therefore contravene the Convention on International Trade in Endangered Species (CITES) legislation. Some recent textbooks of TCM still recommend formulas containing various animal tissues such as tiger bones, antelope, buffalo or rhino horns, deer antlers, testicles and os penis of the dog, bear or snake bile. Usually, animal tissues are combined with medical herbs. The authors will present the DNA based species identification results obtained from various seized materials during the operation Tiger´s Eye run by Czech law enforcement agencies. Some of the artefacts submitted for DNA analysis did not yield any animal DNA or no amplifiable DNA at all. The methods employed for the analyses comprised sequencing of animal mtDNA genes coi and cytb. However Sanger sequencing would not be appropriate method for a complex mixtures of biological material, where more different animal or even plant species are used for the preparation of the particular TMC. DNA analysis of complex mixtures would thus require the use of Massive parallel sequencing (MPS).

Keywords: species identification, CITES, forensic DNA, COI, cytochrome b

Presentation number: FG 36 Abstract number:

MOLECULAR BIOLOGICAL INVESTIGATION OF CASES OF POACHING ON ROE DEER

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Detection of animal species or individuum from samples of traces, tissues or hair are possible by molecular biological methods therefore these are used to assess facts in judicial procedures. Four different cases of suspicion of poaching on roe deer were investigated. In the first case, we investigated the samples of traces of blood, in the second and third case samples of frozen meat and in the last case samples of hair of unknown origin. We have examined the species of the animal that the samples belong to determine wether all the samples were from the same animal, and the gender of the animal. DNA from samples were isolated using QIAamp DNA Investigator kit according to different protocol of manufacturer for different types of samples (QIAamp DNA Investigator Handbook). Subfamily Cervinae was detected by the genotypisation of cytochrome b gene. Species within the genus Cervinae were detected using genotypisation of microsatellite NVHRT73. Sample identity was determined by the profile of six microsatellites characteristic for roe deer. Gender of animal was detected using results of SRY gene fragment amplification. Based on the results of individualization, speciation and gender determination species, all the cases were succesfully concluded.

Keywords: forensics, species, identification, gender, roe deer

ANTHROPOLOGICAL GENETICS

ANALYSIS OF ANCIENT DNA

Presentation number: AG 4 Abstract number:

DNA ANALYSIS OF SKELETAL REMAINS FROM WELL-KNOWN BOSNIAN-HERZEGOVINIAN MEDIEVAL NECROPOLIS REVEALED IMPORTANT HISTORICAL PERSON

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Bosnia and Herzegovina has a rich cultural heritage with many important archeological sites. Recently, archaeological excavations of medieval necropolis in Kopošići near Ilijaš (Bosnia and Herzegovina) were carried out. From historical sources and inscriptions from one of the stećak tombstone, it is known that there were buried members of the high nobility of Bosnia - a great prince Bosnian, Batić Mirković from the court of King Tvrtko II. It was assumed that his father - Prince Mirko Radojević, a faithful companion of the most powerful Bosnian ruler King Tvrtko I Kotromanić (1377-1391), and thus more significant and prominent personality, could have been buried there. Archaeological excavations revealed well preserved skeletal remains of five persons. Two bone samples and seven teeth were sent to DNA analysis in the Institute for Genetic Engineering and Biotechnology, University of Sarajevo. After washing teeth and bones, samples were ground to a fine powder for DNA extraction. DNA was isolated from bone and teeth samples using an optimized phenol/chloroform DNA extraction procedure. All samples required a pre-extraction decalcification with EDTA and additional post-extraction DNA purification using filter columns. PowerPlex® Fusion and PowerPlex® Y23 System were used for amplification of 22 autosomal STRs, single Y and amelogenin as well as 23 YSTR loci. PCR amplification was done within GeneAmp PCR System 9700 (Applied Biosystems). The capillary electrophoresis of PCR products were carried with in ABI 310 Genetic Analyzer. Y haplogroups were predicted based on detected Y-STR haplotypes. In this study we obtained four useful male DNA profiles. Comparison of profiles revealed that two of analyzed remains are in father-son relationship. From the historical sources it was known that one sample belongs to Batić Mirković. Knowing that, we could conclude that the other DNA profile was of Mirko Radojević, father of Batić Mirković. This discovery, along with obtained genetic information, has great archeological and historical significance and brings new insights in cultural heritage of Bosnia and Herzegovina.

Keywords: Ancient DNA, STR markers, archaeological discovery, tombstone stećci, medieval necropolis in Kopošići

Presentation number: AG 5 Abstract number:

CHANGE OF IL10 A-1082G FREQUENCY OVER TIME IN POLISH POPULATION

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Infectious diseases constitute one of the strongest agents driving human natural selection and the course of host-pathogen co-evolution is mirrored in the reciprocal changes in their genomes. IL10 A-1082G SNP, which is associated with both susceptibility to mycobacterial diseases and protection from excessive inflammatory and autoimmune response, was previously proposed to remain under balancing selection, although no conclusive data are available. Here, we investigated the allele fluctuations of IL10 A-1082G over time in order to test which evolutionary forces could have shaped its present-day incidence. The frequency of IL10 A-1082G was assessed in six Polish populations dated to 1st - 17th century by RFLP and Sanger sequencing analysis. The strength of evolutionary forces was estimated by F statistics, the TAFT analysis and forward simulation approach. The distribution of the polymorphism in 5 out of 6 historic groups differed significantly when compared to the modern Polish population. The TAFT analysis suggests that the increased frequency of IL10 A-1082G in modern Poles as compared to the historic populations is the result of non-stochastic mechanisms. The obtained results of forward simulation approach suggest that s = 0.018 would be a plausible power of selection to drive the observed change in allele frequencies. Allele conferring susceptibility to mycobacterial diseases was less prevalent in historic groups than in modern Polish population and the increase of incidence of immunosuppressive variant over time could result from heterozygote advantage. However, low level of heterozygosity in all studied groups indicates the probable inbreeding of historic populations which could bias the results of authentic allele frequency. Further research on allele frequency from older samples and the analysis of longer aDNA fragments would unarguably provide more accurate data on the strength of natural selection acting on this locus or reveal additional forces which shaped the present-day incidence of IL10 A-1082G. This work was supported by grant no. 2013/11/N/NZ7/00380 from the National Science Centre, Poland.

Keywords: ancient DNA, natural selection, genetic drift, innate immune response

Presentation number: AG 6 Abstract number:

GENETIC ORIGIN OF THE INDIVIDUALS BURIED IN POLISH MEDIEVAL CEMETERY INFERRED FROM ADNA ANALYSIS OF MATERNAL LINEAGE

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The important scientific issues, which are studied within historical archaeology and classical archeology concern the origin of the ancient human populations, migration routes, but also the formation and development of interpopulation relations. The contribution of immigrants to the analyzed local population and their effect on the gene pool of autochthonous population has been found difficult to evaluate with classical morphological methods (anthropometric). The burial of fourteen individuals in the studied Kałdus cemetery had the form of a chamber grave, which is typical for Scandinavian culture in the medieval period. The presence of chamber graves cannot be interpreted as absolute proof that the individual (in the biological sense) was allochthonous from foreign lands. These individuals could be autochthons who only adopted a different burial rite. To verify this, we analyzed the HVR I of mitochondrial genome from individuals buried in very richly furnished chamber graves in the medieval cemetery in Kałdus. The obtained results for the mtDNA do not corroborate the Scandinavian origin of the analyzed individuals. We determine typical European haplogroups such as H, T and U. Moreover, we did not find haplogroup I, which is the one typical of populations that historically inhabited the north of Europe.This work was supported by grant no. 2012/07/D/HS3/03822 from the National Science Centre Poland.

Keywords: mtDNA, chamber grave, haplogroup, ancientDNA, migration

Presentation number: AG 7 Abstract number:

EXTENSIVE FARMING IN ESTONIA STARTED THROUGH A SEX-BIASED MIGRATION FROM THE STEPPE

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The transition from hunting and gathering to farming in Europe was brought upon by arrival of new people carrying novel material culture and genetic ancestry. The exact nature and scale of the transition – both material and genetic – varied in different parts of Europe. Farming-based economies appear relatively late in Northeast Europe and the extent to which they involve change in genetic ancestry is not fully understood due to the lack of relevant ancient DNA data. Here we present the results from new low coverage whole genome shotgun sequence data from five hunter-gatherers and five first farmers of Estonia whose remains date to 4,500 to 6,300 years before present. We find evidence of significant differences between the two groups in the composition of autosomal as well as mtDNA, X and Y chromosome ancestries. We find that Estonian hunter-gatherers of Comb Ceramic Culture are closest to Eastern hunter-gatherers, which is in contrast to earlier hunter-gatherers from the Baltics who are close to Western hunter-gatherers. The Estonian first farmers of Corded Ware Culture show high similarity in their autosomes with European hunter-gatherers, Steppe Eneolithic and Bronze Age populations, and European Late Neolithic/Bronze Age populations while their X chromosomes are in addition equally closely related to European and Anatolian/Levantine early farmers. These findings suggest that the shift to intensive cultivation and animal husbandry in Estonia was triggered by the arrival of new people with predominantly Steppe ancestry, but whose ancestors had undergone sex-specific admixture with early farmers with Anatolian ancestry.

Keywords: aDNA, population genetics, population history

Presentation number: AG 8 Abstract number:

DETERMINATION OF GENETIC RELATIONSHIP THROUGH OF THE Y HROMOSOME BETWEEN THE POPULATION OF THE PETNJICA MUNICIPALITY (MONTENEGRO) AND TRACKING THE MOVEMENTS OF THE ANCESTORS

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Montenegro is not marked as ethnic crossroads. However, demographic studies show trends of changes in the population structure of the last few hundred years. At the beginning of this century, molecular-genetic diversity of Montenegro population became the focus of modern research. DNA genealogical tests are becoming more and more popular for the last ten years or so. Y-STR markers were initially used in examination of isolated as well as complete human population of modern Montenegro. Many studies shows that in mountain population of the Montenegro we is not expected more ten six to eight Y haplogroups. In this study we determination genetic diversity in Petnjica Municipality (Montenegro). Same haplogroup typically differ from each other by mutations. Those dates can be calculated rather reliably in each particular case, which in turn provides with a genealogical pattern of bearers of presented haplotypes (softver developed by FamilyTreeDNA). Those dates can be calculated rather reliably in each particular case, which in turn provides with a genealogical pattern of bearers of presented haplotypes. In best cases DNA genealogy data can be compared with documented genealogy data, obtained from archives, or with family legends, etc. However, documented genealogy has its severe limitations, and typically does not spread deeper than several hundred years, more often a hundred or two hundred years. DNA genealogy can easily go into thousands of years ago. This experience may and ought to be used as solid foundation for further efforts in examination of notable genetic diversity in human populations in the whole country.

Keywords: Y hromosom, common, ancestor, haplogroup, matches

Presentation number: AG 9 Abstract number:

TRACING THE ORIGIN OF ANCIENT POLYNESIAN HUMAN GENOMES ACROSS THE PACIFIC

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In 2010, mitochondrial DNA extracted from two ancient skulls found in southern Brazil belonged to the haplogroup B4a1a1a, exclusively found in Polynesia. Radiocarbon analyses indicated that these individuals most probably died before the 19th century, prior to any registered transport of Polynesian people to South America by European vessels. Further genome-wide analyses showed a complete Polynesian ancestry for both samples, with Cook Islands as the closest source population. However, scarcity of genotyping data from modern Polynesian populations posed a major limitation for inferring a more specific place of origin for said skulls. Here, we re-analyze these ancient DNA samples using an extended reference panel that comprises over 475 genotyped samples from 18 different locations across the Pacific Ocean. With this data we explore the genetic affinities of the Botocudo skulls at a finer scale to potentially pinpoint their genetic origin, and we demonstrate the importance of assembling diverse genetic reference panels to shed light on the evolutionary past of human remains devoid of archeological context.

Keywords: Ancient DNA, anthropological genetics, population genetics

Presentation number: AG 10 Abstract number:

CROATIAN LEGAL FRAMEWORK FOR SAMPLING AND ANALYSIS OF ANCIENT SKELETAL REMAINS: PRESENT STATE AND FUTURE CONSIDERATIONS

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Increased number of scientific institutions that curate and study ancient skeletal remains, along with the development of novel scientific methods calls for clearly defined regulations and guidelines for procedures regarding human remains from the archaeological context. It requires regulations and recommendations for excavation, curation, and scientific research on the material, especially in cases of aDNA analysis and other methods that inevitably lead to permanent loss of valuable materials. Additionally, this topic raises numerous legal and ethical issues that must be answered and included in regulations. Therefore, the aim of this study was to analyze current Croatian legislation pertinent to the issue, and by analysis and best practices of comparative legislation to introduce the proposals for the improvement of the legal framework in Croatia. We analyzed Croatian laws and subordinate regulations, as well as practice that deals with the issue in the various context, summarized the findings and compared them to the well-defined national regulations from other countries. The results of the study showed that although there are regulations on archaeological excavation and preservation of cultural goods term “human skeletal remains” is nowhere mentioned. So it can only be assumed that the human remains should be treated the same way as other findings and artifacts. Also, in laws that cover procedures with human tissues, this issue is not included. Thus it is not clear how old the remains should be to be considered “ancient” and which conditions should be met for sampling and scientific research. Therefore, we propose definitions of important terms in this context and offer solutions that could be fundamental for the development of legal framework as well as guidelines for procedures with human skeletal remains. We also aim to raise awareness of this issue and to show the importance of preservation of osteological material as invaluable part of cultural heritage for present studies and future generations.

Keywords: legal framework, skeletal remains, sampling, destructive methods, Croatia

Presentation number: AG 11 Abstract number:

SWEDISH DELUGE: HISTORICAL DISCOVERY IN THE EYES OF FORENSIC GENETICS

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For a long time, historians had at their disposal both non-scientific (descriptive, providentialistic, synchronistic) and scientific (e.g. philological, evolutional, sociological, genealogical) methods that were burdened with a large margin of error. Since the era of genetic analysis begun, new tools for anthropologists, historians and archaeologists arose: first haplotyping, both by Y chromosome and mitochondrial DNA analysis, followed by the most recent one - bioancestry analysis based on SNP markers distributed throughout the genome. One of the most memorable historical events that took place in early modern period in Poland, was undoubtedly the Swedish Deluge, that occurred during the Polish-Swedish Wars. The Department of Forensic Genetics of the Pomeranian Medical University in Szczecin received biological material from 15 individuals which were believed to be the skeletonized remains of Swedish soldiers from the XVII century. For the purpose of the ongoing study, DNA was isolated from the material with both phenol-chloroform and magnetic particles method. Autosomal and Y chromosomal STRs were amplified and Y-STR haplogroups of the individuals were estimated by Whit-Athey's algorithm and YHRD database, where possible. Mitochondrial DNA HV1 and HV2 fragments were sequenced and based on those results, mitochondrial haplogroups were estimated by the EMPOP database. As of this day, the results obtained during the study confirmed the assumption about Northern-European origin of the individuals. In the next phase of the study, we plan to use the technology of Massive Parallel Sequencing, which will allow for bioancestry analysis based on autosomal chromosomes' SNP markers.

Keywords: haplogrouping, STR, aDNA, mitochondrial DNA, Y-STR

Presentation number: AG 12 Abstract number:

COMPARISON OF DENTAL CALCULUS MICROBIOME FROM TWO MEDIEVAL POPULATIONS IN PILSEN

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Dental calculus becomes a valuable material of choice for retrospective studies aimed to investigate health status and dietary habits of past populations. In a course of this project, we aim to compare human remains from two late medieval to early modern cemeteries in Pilsen, Czech Republic. A cemetery near the spital Church of St. Mary Magdalene was, according the written sources, the least popular among all the city burial sites and was used mostly for spital inmates and poor and not local dead. Contrary to that, cemetery of the Church of St. Bartholomew was one of the favorite cemeteries among the rich burghers. These two cemeteries thus provide us a chance to study individuals from both distinct sides of the sociologically and economically stratified medieval population within the single city. Besides osteological and paleopathological analyses of the human remains, we collected dental calculus for the microbial DNA analysis. Dental calculus from teeth found freely and without any context in the burial sites was used for method development. Used methods and obtained results will be discussed.

Keywords: Dental calculus, ancient DNA, medieval, microbiome

Presentation number: AG 13 Abstract number:

DNA IDENTIFICATION OF 10TH CENTURY FEMALE SKELETON FROM A PRAGUE CASTLE APPARENTLY BELONGING TO A MEMBER OF RULING PRZEMYSLIDS DYNASTY

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The start of dynasty dates back to the 9th century when Przemyslids ruled a territory around Prague, populated by the Czech tribe of the Western Slavs. The first historically-documented Przemyslid Duke was Borivoj I (†888-890). The aim of this work is to present the results of DNA identification of 10th century female skeleton from a Prague Castle apparently belonging to a member of ruling Przemyslids Dynasty. The skeleton was buried next to the skeletal remains that can be assigned to Boleslav I (†972) or Boleslav II (†999). DNA analysis yielded partial autosomal (MiniFiler, Thermo Fisher Scientific, USA) and X-STR profile (Argus X-12, Qiagen, Germany) and mtDNA haplotype (HVR1 16017-16569, HVR2 001-577). DNA typing data did not match to any results obtained so far from the Prague castle burials. Therefore we performed a comparison of mtDNA haplotype with publicly available databases as well as with mtDNA haplotypes of other European dynasties.

Keywords: ancient DNA, STR typing, mtDNA, sequencing, Przemyslids

Presentation number: AG 14 Abstract number:

GENOMIC AND ISOTOPIC STUDIES SHED LIGHT INTO THE MOBILITY OF MEDIEVAL INDIVIDUALS FROM CENTRAL EUROPE

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Genomic analyses and strontium isotope measurements are a powerful tool for tracing the ancestry and the mobility of past human populations. We used them in a pilot study of medieval skeletons excavated at Detkovice – Za zahradama archaeological site. The cemetery consisted of human graves dated to 11th c. AD, located in the Olomouc County (Prostejov district), Central Moravia, Czech Republic. Archaeological artefacts, in particular coins allowed for detail dating of the burials and suggested the presence of not only local individuals but also possibly migrants from Hungary. In order to determine the genetic identity and ancestry of population from Detkovice we conducted ancient genomic studies for 11 individuals. Blunt-end genomic libraries were built for each individual and sequenced using Illumina HiSeq system. Acquired genomic data was used for statistical analyses including PCA, statistics D and f3. Moreover, to determine the geographic origins of 6 individuals their tooth enamel samples were analysed for strontium isotope ratio (87Sr/86Sr) by the thermal ionization mass spectrometry (TIMS). Obtained results, both genomic and isotopic indicate high mobility of particular individuals and mixed genetic affiliation to neighbouring ancient and present-day populations.

Keywords: ancient DNA, nuclear genomes, strontium isotopes, Middle Ages, mobility

Presentation number: AG 15 Abstract number:

MICROBIOME OF 1000-2000 YEAR OLD HUMAN REMAINS

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The advances in the NGS sequencing have revolutionized the field of paleogenomics. Technological and methodological improvements allowed for the reconstruction of genomes of long-dead organisms. Typically, the studies of ancient DNA (aDNA) isolated from human or animal remains are focused on the endogenous DNA only. However, most reads in those NGS datasets do not originate from the studied individual, but from microorganisms that colonized a sample postmortem. We employed metagenomics analysis to study comprehensively microbial composition of ancient human remains. 166 samples dated to 1-1200AD from seven archaeological sites in Central Europe and of different storage conditions were analyzed. The majority of identified microbes were ubiquitous environmental species that most likely colonized the host remains not long ago. In line with those results we showed that there is no correlation between specific prokaryotic taxa and temporal or geographical origin of the samples. Instead we noticed that the microbial profile is unique for an individual. Bacteria and archaea species characteristic for human oral and gut flora, as well as potential pathogens, were identified in two-thirds of the samples. The genetic material of those human associated bacterial species revealed a typical for aDNA damage pattern at a degree comparable with endogenous human aDNA. Our work maximizes the amount of information that can be obtained from ancient samples. We evaluated the impact of micro-environments on microbial composition and propose a workflow which permits to determine the source and taxa of exogenous contamination (human-related/soil prokaryotes) and to estimate the potential age of microbial aDNA. Based on the collected data we were able to distinguish between human associated, pathogenic or non-pathogenic bacteria, and microbial contamination from the surrounding environment. This further improves authentication process of potential ancient pathogens in particular.

Keywords: microbiome, ancient DNA, NGS, metagenomics, ancient Pahogens

Presentation number: AG 16 Abstract number:

Y-CHROMOSOME HAPLOGROUP ASSIGNMENT THROUGH NEXT GENERATION SEQUENCING OF ENRICHED ANCIENT DNA LIBRARIES

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The Y-chromosome is passed down only from father to son and it contains the longest non-recombining portion of DNA in the human genome. This feature makes it a perfect object for studying patterns of human migrations and genealogical reconstruction. Here, we present the analysis of Y-chromosome obtained from seventeen, not yet reported, ancient male samples excavated from different burial sites in Poland: Kowalewko (Roman Iron Age), Maslomecz (Roman Iron Age), Legowo (early Middle Ages) and Niemcza (early Middle Ages). We used a custom-design approach for target enrichment of over 5K Y-chromosome SNPs which were selected from the International Society of Genetic Genealogy (ISOGG, https://www.isogg.org) database. Enriched libraries were sequenced on Illumina Genome Analyzer IIx. Raw reads were mapped with BWA against the hg19 reference genome. The Genome Analysis Tool Kit (GATK) was utilized to recalibrate base quality scores and call genotypes. To determine haplogroups we used sites that overlap 9.99-Mb of the non-recombining region of the Y-chromosome as proposed by Poznik et al. (Science, 2013) where alleles were observed in the derived state and MAPQ ≥ 30. We successfully assigned haplogroups to sixteen individuals. Eight belonged to haplogroup I1 (I-M253). Three of them belonged to the sub-branch I1a3a1a1a (I-L1237) and one to I1a2a (I-Z59). I1 is the most common haplogroup in present day Scandinavia, and it is found in all places invaded by ancient Germanic tribes and Vikings. Four samples belonged to haplogroup G2a (G-P15) which is spread uniformly throughout Europe. Other individuals were assigned to I2a2 (I-M436), R1a (R-M420), R1b1 (R-L278), E1b1 (E-P2). The next portion of samples is under investigation. With this study we hope to shed new light into the genetic structure of populations inhabiting lands of contemporary Poland during the Roman Iron Age and the Middle Ages.

Keywords: Y-chromosome, haplogroups, ancient DNA, NGS, target library enrichment

Presentation number: AG 17 Abstract number:

MTDNA VARIABILITY IN IRON AGE CENTRAL/NORTH EUROPE: INSIGHTS FROM NEXT GENERATION SEQUENCING

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Genetic variation of mitochondrial DNA (mtDNA) of Central Europe populations was shaped by multiple events of admixture and replacement. Processes leading to and stemming from the post Early Bronze Age (EBA) genetic shift remain unclear. Here, we make the first attempt to fill the post-EBA gap in our understanding of the process of the Central European genetic landscape formation. To this end, we analyzed a Wielbark culture population (60 individuals) from Kowalewko (Great Poland). Analysis of intrapopulation diversity, performed for individuals with successfully assembled a full mtDNA sequence, suggests that they do not represent a small isolated population (genetic diversity was similar to these observed for extant European populations). Next, we treated this population as the OVIA group: population living between Oder and Vistula rivers in Iron Age) and determined genetic composition of OVIA group and its affinities to other ancient and contemporary populations. The mtDNA haplogroup profile of OVIA showed the highest similarity to the Corded Ware Culture population. Unexpectedly, we noted chronological changes in U5a/U5b haplogroups frequencies, U5a domination in the Early Neolithic-EBA period, U5b in the Iron Age, and again U5a in CEM. Analyses of genetic distances (determined based on the mtDNA hyper variable sequence I (HVS-I)) showed lowest Fst values for OVIA-BEC and next for OVIA-SSIA. With respect to CEM, lowest Fst values were reported for SSIA-CEM and OVIA-CEM. Although, OVIA and SSIA were located close each other, their distances to preceding Late Neolithic (LN) and EBA populations were different. Best optimization of AMOVA parameters was found when both OVIA and SSIA were grouped with LN/EBA populations. Interestingly, HVS-I haplotypes common in all LN/EBA populations were significantly more frequent in OVIA than in SSIA. Lastly, we noted that women from Kowalewko had mtDNA genetic profile more closely resembling Early-Middle Neolithic populations than men. Obtained results revealed that Iron Age Central/North Europe population had a complex, mosaic genetic structure.

Keywords: aDNA, Iron Age, population genetics, mitochondrial DNA, human history

Presentation number: AG 18 Abstract number:

SSDNA2.0: SINGLE-STRANDED LIBRARY PREPARATION FROM HIGHLY DEGRADED AND DAMAGED DNA USING T4 DNA LIGASE

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If DNA is preserved in ancient biological material, it is usually present in small quantities only. Generation of genome-wide sequence data thus relies on highly efficient sample preparation techniques. It has been shown that losses of molecules during DNA library preparation can be minimized by converting the complementary strands of DNA fragments separately into library molecules. We present an improved method for single-stranded library preparation, ssDNA2.0, which is based on highly efficient end-to-end ligation of single-stranded DNA to adapter oligonucleotides using T4 DNA ligase in combination with a splinter oligonucleotide. ssDNA2.0 is less expensive than the previous approach based on CircLigase, an RNA ligase, and more robust to varying concentrations of input DNA. More importantly, the protocol does not include extended incubation steps at high temperature and is therefore compatible with automation on open liquid handling platforms. An in-depth comparison of single- and double-stranded library preparation methods on a diverse set of samples shows that single-stranded library preparation increases the yield of informative sequences from ancient DNA by more than a factor of 10 on average. When applied to DNA isolated from tissues that had been stored in formalin for many years, single-stranded library preparation yields up to 2,000 times more library molecules, allowing whole genome sequencing from samples that produce virtually no useful data with double-stranded approaches.

Keywords: sequencing library preparation, degraded DNA, ancient DNA, FFPE, single-stranded ligation

Presentation number: AG 19 Abstract number:

INVESTIGATING KINSHIP IN NEOLITHIC TIMES - EXAMPLES, METHODS AND PERSPECTIVES

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Ancient DNA (aDNA) analyses are currently one of the most popular tools used in the bioarchaeology and their development lead to many spectacular discoveries. However, until recently most of the studies focused on large scale models, addressing the problems of intercultural relations and migrations. The problems of kinship and social structure of ancient populations were rarely addressed by aDNA researchers. Major reason for such state was difficulty in obtaining sufficient amount of overlapping genomic data from all of the putative relatives. However, recent developments help us to increase the yields of available genomic information, and at the same time to analyze the kinship using also the low coverage sequencing data usually available for prehistoric individuals. Here we take a closer look at some of recently developed methods and present their potential applications in kinship studies. We discuss our ongoing project, namely potential of newly developed methods to test and ultimately reconstruct different degrees of kinship between individuals buried under the floors of one of the households in Neolithic Catalhoyuk in Central Anatolia, with the use of low coverage shotgun sequencing data. We also present the results from kinship studies of Neolithic individuals from Krusza Zamkowa archaeological site from Central Poland, where we used capture based enrichment technique. In result, we have obtained four complete mitochondrial genomes and excluded maternal and first degree kinship among analyzed individuals.

Keywords: ancient DNA, kinship, Neolithic

MOLECULAR ANTHROPOLOGY

Presentation number: AG 20 Abstract number:

GENETIC AND LINGUISTIC DIVERSITY IN GLOBAL DATABASES: TRACKING DOWN MATCHES AND MISMATCHES

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Anthropological research has often investigated the congruence of linguistic and genetic histories to study population origins, diversification and contact. Linguistics can help to inform the interpretation of genetic results and to label samples by ethnolinguistic affiliation. Broad databases of quantitative linguistic data have recently been assembled with the scope of employing new computational methods with increased analytical power. To integrate these resources into genetic analysis, and provide demographic and genealogical information to non-geneticists, we propose a standardized panel representative of worldwide genetic population diversity. The database comprises published genetic data for autosomal, Y chromosome and mtDNA, each population sample being associated to a code linking it to existing linguistic and cultural databases. Our first study addresses mismatches between linguistic and genetic variation, often disregarded as exceptional cases. We focus on instances of high genetic distance among speakers of related languages (which may point to language shifts) and cases of close genetic distance between speakers of unrelated languages (relevant to the formation of language boundaries), evaluating the incidence of these mismatches in each language family. With this resource, we aim to develop a realistic understanding of the complex mechanisms behind cultural transmission. The change of cultural features through time not only impacts our ability to trace human prehistory, but also influences the definition of "population" as the unit of research. Our approach promotes deeper understanding of the human history behind molecular data, with implications for population genetics and forensics.

Keywords: database, STR, population genetics, human diversity, linguistics

Presentation number: AG 21 Abstract number:

THE GENETIC HISTORY OF THE INDONESIAN PYGMIES OF FLORES

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Modern human pygmy populations are distributed globally, and their short stature is hypothesized to represent one aspect of a complex eco-geographic adaptation to rainforest or island environments. Although numerous genetic studies have been conducted on pygmies in Africa and Southeast Asia, to date, there have been no genome-scale analyses of the pygmy population living on the island of Flores, Indonesia. Intriguingly, this population lives in a village near the cave where remains of a small-bodied human species, Homo floresiensis, were recently found. Here, we describe whole-genome sequences (>40x) from 10 Flores pygmy individuals, as well as genome-wide SNP data from 32 individuals. The Flores genomes harbor on average 48 Mb and 4.4 Mb of Neandertal and Denisovan sequence, respectively. Height-associated loci identified in European populations are significantly differentiated in the Flores pygmies, who possess an excess of height-decreasing alleles and a deficiency of height-increasing alleles. This result is consistent with a hypothesis of polygenic selection acting on standing variation for reduced stature in Flores. Finally, we identify a strong signature of recent positive selection encompassing the FADS gene cluster on chromosome 11, encoding for fatty acid desaturase that regulate the metabolism of long-chain polyunsaturated fatty acids (LC-PUFA).

Flores individuals are nearly fixed for an ancestral haplotype that is predicted to confer reduced capacity to synthesize LC-PUFA from plant-based precursors. Our results add to emerging evidence that the FADS region has been a recurrent target of selection in diverse human populations, possibly in response to changing diets.

Keywords: Indonesia, Neandertal, archaic admixture, fatty acid desaturases, height

Presentation number: AG 22 Abstract number:

MASSIVELY PARALLEL SEQUENCING OF HIGHLY FRAGMENTED SKELETAL MATERIAL IN FORENSIC CASEWORK

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Identification of skeletal remains that have been discovered after an extended period of time is becoming an increasingly important part of the fields of anthropology and forensic science. Examples of this are the missions of the International Commission on Missing Persons and the Armed Forces DNA Identification Laboratory, as well as the role of the Medical Examiner's system. Skeletal remains have typically been exposed to environmental insults resulting in increased DNA fragmentation, in some cases to less than 150 base pairs (bps) in length. Current human identification methods use a combination of mitochondrial DNA and short tandem repeat (STR) analysis, which target segments of DNA ranging from 100 to 500 bps in length. Due to the highly fragmented nature of the DNA recovered from more degraded skeletal remains identification is not always possible with these techniques. Single nucleotide polymorphism (SNP) analysis targets shorter regions of DNA (40 to 70 bps) and is ideal for these types of samples. Since current methods of DNA extraction in forensics have been optimized for recovery of larger fragment sizes, it would be desirable to use methods optimized for recovery of the relatively abundant smaller fragments for SNP typing. Two sets of experiments were performed to evaluate specific parameters for recovery optimization utilizing sheared, pristine DNA and sheared DNA combined with demineralized animal bone. In both cases the efficiency of different binding buffers and columns was evaluated in order to identify an optimized protocol. To demonstrate the successful application of this protocol seventy human skeletal remains from the 7th to 18th century recovered in Croatia were extracted and the mitochondrial control region subsequently sequenced using the Promega 10-plex kit. Haplotypes were generated, haplogroups inferred, and comparisons made to anthropological findings. The sensitivity and robustness of the Illumina ForenSeq system of STR and SNP loci was assessed and the results indicated that for material of an average size of 150 bp and input amounts of 125 pg to 1 ng the ForenSeq system is robust enough to provide identification confirmation. However, for material with an average size of 100 bp, a combination of SNPs and STRs are necessary in order to provide significant identification information.

Keywords: ancient DNA, human identification, MPS

Presentation number: AG 23 Abstract number:

AN INVESTIGATION ON THE PATERNAL LINEAGES OF THE NORTHERN IRAQI TURKMENS

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Today, Iraq is home to numerous ethnic groups, each with their distinct lingual and cultural traditions. Among these ethnic groups, the Iraqi Turkmens (Arabic: تركمان العراق, Turkish: Irak Türkmenleri, Irak Türkleri) are a Turkish speaking ethnic group that constitute the second largest minority in Iraq. In this study, the paternal lineages of a Northern Iraqi Turkmen population sample (n=102) were investigated using the LifeTechnologies AmpFlSTR® Yfiler® kit. The Northern Iraqi Turkmen Y-STR dataset was found to have 74 unique haplotypes among 102 samples analysed, and the discrimination capacity and haplotype diversity observed were 72.55% and 0.99592, respectively. Next, an allele frequency-based phylogenetic analysis of the Northern Iraqi Turkmen 17-loci Y-STR dataset along with those from neighbouring and distant populations was carried out. As expected, the Northern Iraqi Turkmen population was found to cluster most closely with the Iraqi population within a Middle Eastern cluster, which included other Turkish and Arabic populations at the next level. Once published, results from this study would constitute the first Y-chromosomal dataset on Iraqi Turkmens in the literature. The Northern Iraqi Turkmen Y-STR dataset is expected to have immediate forensic applications, such as missing person's investigations. Furthermore, the new Y-STR dataset would also contribute to a better understanding of the population genetics of the Near East in general because despite the historical importance of this geography, unfortunately still very little data exists on the populations therein.

Keywords: Northern Iraqi Turkmens, Middle East, Yfiler, Forensic parameters, Phylogenetic analysis

Presentation number: AG 24 Abstract number:

IMPACT OF NON-LTR RETROTRANSPOSONS IN THE DIFFERENTIATION AND EVOLUTION OF ANATOMICALLY MODERN HUMANS

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Transposable Elements (TEs) are biologically important components of eukaryote genomes and their activity generated at least 46 % of the human genome. In Anatomically Modern Humans (AMH), only some TE subfamilies of the non LTR-retrotransposon sub-class have recently been active. These elements often contain internal promoters, Transcription Factor Binding Sites and polyadenylation signals. They can drive adjacent gene expression, produce alternative transcripts for existing genes and contribute to the generation of new genes and pseudogenes. These characteristics make them one of the primary sources for genomic mutations and variability. This study is aimed at the identification of the role of such retrotransposons in the differentiation and evolution of the genus Homo, by comparing insertions in the genome of AMH (HS37-hg19) with those of our closest extinct relatives, Neanderthals and Denisovans (whose genomes were recently made available). We developed an in silico methodology for identifying species-specific insertions with ancient DNA data, which led to the identification and confirmation of 321 AMH-specific insertions (plus hundreds more that are polymorphic between the three species). The corresponding genomic loci have been characterized according to different approaches: variability in human populations, genetic and epigenetic features, selective pressures and regulatory/expressional alteration inference. The results hereby presented suggest that the activity of non-LTR retrotransposons throughout Homo evolution might have played an important role in AMH differentiation, generating new variability and influencing recent selective events. Further research on this topic can help Anthropologists and Evolutionary Biologists to better understand functional evolution of the AMH genome, also opening new possibilities for studies in genomics and evolutionary dynamics.

Keywords: Retrotransposons, Denisova, Neanderthal, Species-specific insertions, Genomic Variability

Presentation number: AG 25 Abstract number:

THE GATEWAY INTO REMOTE OCEANIA: NEW INSIGHTS FROM GENOME-WIDE DATA

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The widely accepted two-wave scenario of human settlement of Oceania involves the first out-of-Africa migration ca 50,000 ya and the last pre-European dispersal of people, known as the Austronesian expansion, which reached the coasts of Papua New Guinea by 3,000 ya. Earlier genetic studies provided evidence for the extensive sex-biased admixture between the incoming and the indigenous populations, which occurred prior to the expansion into the Remote Oceania. Some archeological, linguistic and genetic evidence however paint a more complicated picture of settlement, indicating that the two-wave scenario is incomplete. To study regional variation in Oceania in more detail, we have compiled a large genome-wide dataset of populations sampled across the Solomon Islands archipelago (SI). The dataset also includes samples from Australia, Papua New Guinea, Bismarck Archipelago (BA), Polynesia, as well as populations from East Asia and Taiwan. With this dataset we are able to show that the initial dispersal of people from BA into Remote Oceania occurred in a "leapfrog" fashion, completely by-passing the Solomons, and that the colonization of SI proceeded in a bi-directional manner. Our results also support a divergence between western and eastern Solomons, in agreement with the sharp linguistic divide, known as the Tryon-Hackman line. We also report prolonged gene flow from Near Oceania to Western Polynesia, not seen further east, and substantial post-Austronesian gene flow across the Solomons.

Keywords: Solomon Islands, Oceania, Austronesian expansion, genome-wide data, admixture

Presentation number: AG 26 Abstract number:

GENETIC CONTRIBUTION TO HUMAN SKIN PIGMENTATION IN SELECTED AFRICAN ANCESTRY POPULATIONS

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Human skin pigmentation is one of the most variable and complex phenotypic features determined by the interaction of genetic and environmental factors. Several selective components have been proposed to be implicated in the evolution of dark pigmentation such as protection against the harmful effects of UV radiation exposure; protection against vitamin B9 photolysis and maintenance of vitamin D homeostasis. Up to date more than 50 genes seem to contribute to skin color but very few candidate-gene studies have been carried out in African ancestry populations. Herein, we evaluated the association between variants in skin color associated loci SLC45A2 (rs12913832), OCA2 (rs16891982), HERC2 (rs12913832), SLC24A5 (rs1426654), BEND7/PRPF18 intergenic region (rs6602666) with skin colors, in 200 healthy people from selected African origin populations: Tuareg from Libia; Ethiopian Amhara and Oromo; Fon, Dendi, Bariba and Berba communities from Benin and Afroecuadorians. Meaningful results validate the association of variants in two previously known skin pigmentation genes (SLC24A5 and SLC45A2) and some interesting new data could be pointed out for the other novel variants that could be evaluated as Ancestry Informative Markers (AIMs). The results support the use of phenotypic inference by molecular information as an auxiliary tool in the personal identification through the use of bio-geographical ancestry information and outwardly visible characteristics such as skin tone.

Keywords: Pigmentation, African Population, Personal Identification, Ancestry Informative Markers, Dark Skin

Presentation number: AG 27 Abstract number:

GENETIC DIVERSITY WITHIN AND AMONG THE TRIBAL GROUPS OF TUVINIANS FROM THE Y-CHROMOSOMAL PERSPECTIVE

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Tuva is located in southern Siberia at the crossroads of movements of many ethnic and tribal groups. More than 25 tribal groups are recognized in Tuva. This study aims reveal the genetic structure of Tuvan population and the differentiation of the two Tuvan tribal groups. We genotyped 546 DNA samples from unrelated healthy male donors from Republic Tuva by 40 Y-SNPs using the TaqMan probes (Applied Biosystems). More than half of the Y–chromosomal pool was represented by North Eurasian haplogroups Q(M242), N2a(P43), and N3(M178). West Eurasian haplogroups (R1a1a(x458), R1b(M343), J2(M172)) accounted for 19%, and the East Eurasian haplogroups (C3(M217), C3c(M48), O1a(M119), O3(M122)), similarly, covered 21% of the variation. In Tuvans we identified one of the highest values of intra-population gene diversity (80%) among Siberian ethnic groups.The Y - gene pool of the northeast Tuvan tribal group kol (Katangsky variant of the Baikal anthropological type) consists of 90% of North Eurasian haplogroups Q(M242), N2a(P43), N3(M178). TheY - gene pool of the central Tuvan tribal group oyun (Central Asian anthropological type) is contrasting, as it is characterized by the maximum range of West Eurasian haplogroups R1a1a(x458), R1b(M343), J2(M172) (~65%).The cluster analysis with other populations of Central Asia, the analysis of molecular variance (FST=0.13), and analysis of genetic and geographic distances (r=0.8) between the Tuvan tribal groups were computed from haplogroup frequencies. Our results revealed the differentiation of the two Tuvan tribal groups.

Keywords: Y-chromosome, Y-SNP, Y-haplogroup, Tuvan tribal group

Presentation number: AG 28 Abstract number:

WORLD WAR II WAR CRIMES- ROLE OF ATTORNEY GENERAL AND COURTS IN MASS GRAVE CASES

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When it comes to war crimes committed during World War II perpetrators are usually unknown and is therefore necessary to take extra precautions to ensure the traces of the crime and objects that can be used to determine facts when a tomb containing the remains of that time is found. Most often as an emergency evidentiary action the Attorney General with the help of the police conducts crime scene investigation. This action also requires the engagement of legal medicine expert appointed by the court.In Splitsko - dalmatinska county most interesting is the case of war crimes from World War II, where mass grave was found in the cemetery area of town Makarska. Mass grave was containing the skeletal remains of 28 male individuals aged 20-40 years buried in 14 trenches. Along with skeletal remains, items such as shell cases, remains of military boots, artificial (plastic) eye, button, glasses in a metal box, plastic bottle with a label in 1944 and carved name, pieces of belts, metal buckles, teeth, pieces wire were also found. Evidence of single or even multiple gunshot wounds was visible on skulls and other skeletal remains. Some remains were found to be disabled during their life because they had amputated lower limbs.Total of 108 samples were taken for DNA identification from which 74 were from skeletal remains and 33 from living relatives. DNA were isolated using organic extraction and using Chelex®100. Amplification of DNA was done using AmpFlSTR Yfiler PCR Amplification kit (Applied Biosystems, 2005.) for male relatives and AmpFISTR Identifier PCR Amplification kit version 2.0 (Applied Biosystems, 2002) for female relatives. Amplified fragments were visualized by capillary electrophoresis using ABI Prism 310 (Applied Biosystems) and processed by GeneMapper® ID software version 3.2. Full DNA profile was obtained for 14 and partial DNA profile for 20 out of 74 analyzed bone samples. Final result was two full matches with living male relatives with probability of finding the same person in the general population of 1: 5.15 x 10^{11} and 1: 5.33 x 1024 respectively.

Keywords: Mass graves, World War II, DNA analysis

Presentation number: AG 29 Abstract number:

COPY NUMBER VARIATION ON THE HUMAN Y CHROMOSOME

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Copy number variation (CNV) on the human Y chromosome is of great interest medically and forensically, as well as in population-genetic studies. We have previously investigated and described human Y chromosome CNVs in ~1,244 men from the 1000 Genomes Project, and identified more than 100 CNVs. We have subsequently followed up some particularly interesting examples using fibre-FISH, breakpoint-PCR and 10X synthetic long read sequencing. Here, we highlight two examples. One of these involves the P8 palindrome which contains the two VCY genes. We found that at least eight different rearrangements, including an entire deletion of one arm (including one copy of VCY) so that the structure in no longer palindromic, duplications of flanking sequence that consequently extend the palindrome arm length, and, in the most extreme cases, duplications that create a completely new palindrome, physically separate from but near the original P8, with a different structure (four copies of VCY). Interestingly, although all of these events appear recently in the Y phylogeny, some are shared between more than one individual suggesting that they are consistent with normal fertility. The second example is a CNV that partially overlaps with the gene TTTY22 in the IR3 repeat, and varies in number from zero to three. The mechanism underlying variation between zero and two copies of this CNV is gene conversion, while the third copy results from a large tandem duplication. All copy numbers are found in multiple individuals, again implying that they are consistent with normal fertility. Our work underlines the importance of experimental validation in CNV studies, as the variants we observe were not fully characterized by the analysis of sequencing data alone. Most importantly, our results confirm and reinforce the view of palindromes as rearrangement hotspots, revealing drastic remodelling including de novo palindrome formation in this genomic region, with each new structure most likely arising as a single mutational event. In addition we highlight the importance of gene conversion as an under-appreciated mechanism of generating copy number variation.

Keywords: copy number variation, Y chromosome, palindrome, gene conversion

MEDICAL GENETICS

BEST PRACTICES IN TRANSLATIONAL & PERSONALIZED MEDICINE

10th ISABS Conference on Forensic and Anthropologic Genetics
and Mayo Clinic Lectures in Individualized Medicine
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Presentation number: MG 10 Abstract number:

PHARMACOGENETICS STUDY TO PREDICT THE ROLE OF THE POLYMORPHIC CYTOCHROME P450 2D6 (CYP2D6) IN OPIOIDS TREATMENT OF THE CHRONIC LOW-BACK PAIN

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Chronic LBP (CLBP) is the second leading cause of disability worldwide being a major welfare and economic problem. Although opioids are recognized as an essential tool in CLBP, it is still unclear how to identify opioid responders and onset of side effects (such as constipation, respiratory depression, excessive sedation, tolerance and hyperalgesia) that frequently occur, together with risk of drug addiction, affecting patients' adherence. The CYP2D6 gene is highly polymorphic across the human population. Understanding opioid metabolism and disposition is essential for assessing risk of toxicity, providing additional information on therapeutic failure. A cohort of 224 Caucasian patients, male and female older than 18 years old, with CLBP (enrolled in FP7 Project, named PainOmics, GA n.602736) was tested correlating codeine and oxycodone side effects to SNPs involved in pharmacokinetics of CYP2D6 in order to identify the patients with low compliance (for side effects and no efficacy). We detected, using xTAG assay Kit on the Luminex® 200™ technology, a panel of single nucleotide variants, including gene rearrangements associated with the deletion (*5) and duplication genotypes. The test determines the diplotype of each sample and detects the complexity of CYP2D6 nucleotide variants across different populations analyzed through PONENT 3.1. The variants revealed are responsible of the variable response to the same therapy and identify each patient into one of four classes of metabolizers. Data show the significant associations both for haplotype distribution, which for that genotype with a p-values of 0.018 omnibus. Haplotypes *6 and *9 can predict a reduced enzyme's activity to determining the therapeutic failure while *2N exhibits the risk of toxicity in painful patients. Furthermore, we detected the genotypes *1/*11, *4/*6, and *41/* 2N as involved although their frequency is more low. Predicting individual opioids' effectiveness and safety through CYP profile variants to perform genotype/phenotype correlations useful to stratify inter-individual variability of opioid response, could be a new biomarker to identify the precision treatment to preventing the adverse effects, increasing benefit and saving money of the Healthcare System.

Keywords: CYP2D6, pharmacogenetics, opioids, Chronic Low-Back Pain, personalized medicine

Presentation number: MG 11 Abstract number:

GENETIC PARTICULARITIES OF VIOLENT BEHAVIOR IN SCHIZOPHRENIA

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Violent behavior can occur both in onset and evolution of schizophrenia, causing severe criminal implications related with forensic psychiatry. Violence in schizophrenia may be associated with two levels of genetic vulnerability and specific clinical symptoms. Primary level is correlated with persistent positive psychotic symptoms (5.1%, Mojtabai 2006) and early onset preceded by conduct disorders (2.5%, Hodgin 2007). Potential genetic alterations are: allelic changes of catechol-O-methyltransferase - met158 associated with dysfunction at the synaptic dopamine receptors (Pooley 2007); GRM3 polymorphism may impact mGluR3 and NMDA receptor functions (Krystal 2010); GABA transmission deficiency resulting from alteration of parvalbumin interneurons and synchronization of neuronal activity in gamma frequency band. Gamma oscillations may be a potential neurophysiological marker for violent behaviors (Burgos 2010); GAD67 and Neuregulin1/erbB4 deficiencies in parvalbumin interneurons. The second level includes: 1. Alcohol addiction. Dual pathology is a marker for violent behavior. Potential genetic markers are: low levels of MAO-A on platelets; D3 receptor gene polymorphism in the accumbens nucleus and limbic structures decreases the cortico-limbic connectivity (endophenotypal risk indicator). 2. Extrapyramidal symptoms induced by antipsychotics. Dystonia correlated with torsion dystonia gene (DYT1) on chromosome 9q34; hyperkinetic movement disorders (akathisia) correlated with genetic alterations like GCH1, TH and PARK2 (Jankovic 2009). These symptoms decrease compliance and adherence to treatment, another risk factor for violent behavior is discontinuation. Association of data from medical literature highlights the potential predictive genetic markers of aggressiveness in schizophrenia. Their correlation with clinical symptoms allows for prevention of violent behavior with forensic implications.

Keywords: Violent behavior, gene alteration, schizophrenia, receptor, risk factor

Presentation number: MG 12 Abstract number:

INTEGRATED GENOMIC, TRANSCRIPTOMIC, AND EPIGENOMIC ANALYSIS OF RELAPSED LYMPHOMAS IN INDIVIDUALIZED MEDICINE CLINIC PATIENTS

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Lymphoma is the sixth most common newly diagnosed cancer, and remains an important cause of morbidity and mortality. Epigenetic alterations involving covalent modifications to the DNA and histones play an important role in lymphoma pathogenesis and many of them can be targeted pharmacologically. Indeed, treatment with histone deacetylase inhibitors is now FDA-approved for relapsed T-cell lymphoma and is also active in various B-cell non-Hodgkin lymphomas (NHL). However, only ~30% of patients respond to this treatment. A critical barrier to the more effective use of epigenetic drugs is lack of understanding of the epigenomic constitution of responding and nonresponding lesions. Here, we aimed to implement, in the setting of an individualized medicine clinic, integrated multi-omic profiling of tumor samples from patients with suspected relapsed lymphomas with the ultimate goal of identifying epigenetic states that can be effectively targeted in a patient-specific manner. Excisional biopsies obtained from 10 patients with relapsed NHL were subjected to genomic, transcriptomic, and epigenomic assays: FoundationONE Heme assay, mRNA-sequencing, reduced representation bisulfite sequencing (RRBS), Tet-assisted RRBS, and chromatin immunoprecipitation-sequencing targeting 6 histone marks. Control cells were isolated from tonsils. Integrated bioinformatic analysis was performed using pipelines developed in-house. Epigenomic profiling functionally validated 18 genomic and 17 transcriptomic alterations affecting pharmacologically targetable epigenetic regulator genes, whereas 6 genomic and 3 transcriptomic changes had no detectable impact on the epigenome. Ten epigenomic changes were not predicted by genomic or transcriptomic findings. We conclude that multi-omic approaches have a strong potential to improve the precision of individualized lymphoma therapy. The predictive value of our findings needs to be tested in cohorts of patients with known response to epigenetic therapy.

Keywords: Precision lymphoma therapy, HDAC inhibitor, EZH2 inhibitor, BET inhibitor, DNA hypomethylating agents

Presentation number: MG 13 Abstract number:

A PERSONALIZED MEDICINE PROSPECTIVE IN THE EVALUATION OF PEDIATRIC PRIMARY IMMUNODEFICIENCIES - A CASE STUDY OF COMMON VARIABLE IMMUNODEFICIENCY

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Immune disorders called primary immunodeficiencies (PID) manifest as a spectrum of distinct clinical phenotypes that require differential diagnosis based on immunologic assays and genetic tests. In view of personalized medicine, the next generation sequencing (NGS) technology is revolutionizing the field of PID in terms of new disorder identification, diagnosis, and clarification of the genotype-phenotype correlation. We aim to establish NGS in the routine translational medicine practice for the PID evaluation and disease management. An 18 year-old male patient diagnosed with common variable immunodeficiency (CVID), group Ib (Freiburg classification), presenting with recurrent respiratory tract infections, i.e. chronic rhinosinusitis and nonresolving pneumonia, as well as low serum IgG (1.5 g/L), has been followed up at our hospital and subjected to intravenous immunoglobulin replacement therapy, immunophenotyping of peripheral blood using the Navios flow cytometer (Beckman Coulter, USA) and the targeted NGS analysis using the TrueSeq Custom Amplicon panel followed by sequencing on the MiniSeq system (Illumina, USA) to screen for the variants of the genes involved in predominantly antibody deficiency. Consecutive immunophenotyping demonstrated a severe lack of B lymphocytes, i.e. the memory B cells, and an occasional decrease of the NK cell population, which has been reported in severe forms of CVID (Ebbo, EBioMedicine, 2016). The targeted NGS analysis identified a novel variant of the complement component receptor 2 (CD2; CD21) gene with the in silico prediction of pathogenic effect; the mutations in the CD21 gene have been associated with common variable immunodeficiency 7, and susceptibility to systemic lupus erythematosus 9. We report a pediatric CVID case with a novel missense substitution in the CD21 gene detected by the targeted NGS analysis of the predominantly antibody deficiency panel, with the likely pathogenic in silico prediction of the identified variant and its potential association with autoimmunity - a typical noninfectious manifestation of CVID. We implement the NGS-based personal genomics as a new diagnostic and predictive method for the early intervention of pediatric PID according to the patient-tailored profile.

Keywords: primary immunodeficiency, next generation sequencing, personalized medicine

Presentation number: MG 14 Abstract number:

AN INTERDISCIPLINARY PERSONALIZED MEDICINE APPROACH IN DIAGNOSIS AND TREATMENT OF PATIENTS WITH OSTEOGENESIS IMPERFECTA TYPE 3

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The goal of St. Catherine Hospital is to develop new solutions for personalized medicine by collaborating with scientists worldwide to provide the most appropriate diagnosis and treatment for the individual needs of our patients. Osteogenesis Imperfecta (OI) is characterized by fractures with minimal or absent trauma, representing a continuum ranging from perinatal lethality to individuals with severe skeletal deformities, to nearly asymptomatic individuals with a mild predisposition to fractures. Diagnosis of OI is an interdisciplinary task based on family and/or patient's history of fractures combined with characteristic physical findings. Radiographic examination reveals fractures of varying ages and stages of healing, wormian bones, and osteopenia. As there is no definitive test for OI, molecular genetic testing by next generation sequencing of COL1A1 and COL1A2-genes and up to 14 other genes is essential to confirm the genetic background. We report 3 cases of multiple bone fractures in early childhood, severe deformities, short stature and spinal curvature (case 1: male 10yr, case 2:6yr and case 3: 10yr female 12yr). Based on the anamnesis, pattern of fractures and bone deformities the suspected clinical diagnosis was progressively deforming OI (OI type3). A multi-gene panel comprising COL1A1 and COL1A2 as well as 14 other OI causing genes was employed for molecular diagnostic. In all three cases the disease causing heterozygous mutations were identified (case1: COL1A1 c.3226G>A (p.Gly1076Ser), case 2: COL1A2 c.982G>A (p.328Ser), case3: COL1A1 c.2235+1G>A splice mutation). The mutations have been published before and were attributed to OI type3. Before admitted to our hospital, patients had received standard treatment which includes care of broken bones, pain medication as well as treatment with bisphosponates. As an example of our personalized medicine approach we describe the diagnosis and treatment of case 3: After the detection of a COL1A1/COL1A2 gene mutation, a series of surgical treatments were performed using the Fassier-Duval intramedullary telescope nail to shape, stabilize and strengthen both femora and lower legs with a very good final result. Subsequent

initiation of rehabilitation procedures enables the ambulation of the child. A significant improvement of quality of life for the patient was achieved.

Keywords:

Presentation number: MG 15 Abstract number:

THE STRUCTURE OF QUESTIONNAIRES FOR NUTRITIONAL HABITS OF THE ADOLESCENT POPULATION

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Data on the nutritional habits of adolescents is an important indicator of their current health status. Comprehension of the quality of their nutritional habits may lead to constructing future nutritional guidelines that would improve these habits and the health status of individuals in general. Consummation of nutritively poor foodstuff during a long period of time that turns into an everyday habit may lead to disease occurrence even in adulthood (chronical degenerative diseases). The goal is to educate adolescents on proper nutrition based on data collected through nutritional habits questionnaires. The structure of the questionnaire must be determined in advance. The examinee should be asked clear, concise questions that are appropriate for their age and their hitherto acquired knowledge on nutrition. Local nutritional habits and cultural, traditional and religious diversity must be respected. Depending on the research goals, the questionnaire should be designed taking into account the content and timeframe. For example, for adolescent nutritional habits research, the following content design is recommended: general information, familiarity with different foodstuff groups and questions about the hitherto acquired knowledge about nutrition and the desire for further education. Each set of questions contains a certain number of particles and this overview will be about the structure of 43 particles from the Questionnaire on nutritional habits of adolescents. Following are the questions on health habits and influence of environmental factors on their own health, with the purpose of obtaining results with data on nutritional habits of adolescents. Based on the analysis of the structure of questions on nutritional habits of adolescents it will be possible to conclude on undertaking educational interventions during design of new questionnaires related to obtaining qualitative data on the nutritional habits of adolescents.

Keywords: questionnaire, nutritional habits, adolescents, structure

Presentation number: MG 16 Abstract number:

MATERNAL HOMOCISTEINE LEVEL IN RELATION TO NEWBORNS` ANTHROPOMETRY - A PRELIMINARY REPORT FROM THE CRIBS STUDY

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Homocysteine is an amino acid involved in several key metabolic processes. In the general population hyperhomocysteinaemia is an indicator of vascular disease, while in normal pregnancy homocysteine blood levels falls. The objective of this study was to examine associations of homocisteine in pregnant women with possible predisposing factors (older age, smoking status, physical inactivity, hypertension), pregnancy outcomes (preterm birth, small/normal/large for gestational age) and newborns` anthropometry (measures z-standardized according to WHO). Fasting homocisteine concentration, taken between 22nd and 26th week of gestation, were determined in 116 women, participants in the CRoatian Islands` Birth Cohort Study (CRIBS). Homocisteine below than referent range (3.4-20.4 μmol/L) was determined in 19 women (16.4%) while its concentration in others was within the referent range. None of the investigated predisposing factors was significantly associated with predicting homocisteine level, nor were pregnancy outcomes. Newborns of mothers with homocisteine <3.2 μmol/L had significantly higher BMI-for-age (p=0.028) and weight-for-length (p=0.038), in comparison with mothers whose homocisteine concentrations were within normal range. Other investigated anthropometric measures also showed a tendency of inverse correleation with homocisteine levels, although borderly significant.In the CRIBS sample, lowered concentration of homocisteine in pregnancy indicates a possibility of increased neonatal size.

Keywords: CRIBS, pregnancy, homocisteine, biomarker, newborns` anthropometry

Presentation number: MG 17 Abstract number:

LIPOPROTEINE A IN NORMAL PREGNANCY - A PRELIMINARY REPORT FROM THE CRIBS STUDY

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Concentration of lipoprotein (a), Lp(a), an important part of the clinical biomarker profile for atherosclerosis, in blood plasma varies both between and among populations. The elevated Lp(a) level in pregnancy was recognized as a risk factor for thrombosis and/or pre-eclampsia, but the referent values for uneventful pregnancies are yet undefined. The objective of this study was to test the association of fasting Lp(a) levels, taken between 22nd and 26th week of gestation, with possible predisposing factors (older age, smoking status, physical inactivity, hypertension, gestational diabetes), pregnancy outcomes (preterm birth, small/normal/large for gestational age) and newborns` anthropometric measures in 113 women (age range 19.8-41.7 yrs, mean 30.6±4.6 yrs) from the CRoatian Islands` Birth Cohort Study (CRIBS). In these women, with no history of chronic disease prior to pregnancy, Lp(a) levels varied largely, from 1.0 mg/dl to 127.3 mg/dl (median 13.1 mg/dl), but most of the women (88.5%) had Lp(a) <50 mg/dL, a cutoff which indicates moderate to high risk of CVD in general population. The results of multiple logistic regression analyses showed significance of older age (>35 yrs), smoking statuts (both current and ex-smokers) and hypertension in predicting Lp(a)≥50 mg/dL (p=0.017, Cox & Snell R2=0.162, Nagelkerke R2=0.333). Nor logistic, neither linear regression models used for the estimation of adjusted associations between Lp(a) and birth weight, length and head circumference, all z-standardized according to WHO, showed significant

associations. In the CRIBS sample, elevated Lp(a) levels were not found to be a predictive biomarker for the pregnancy outcome and neonatal size.

Keywords: CRIBS, pregnancy, newborns` anthropometry, lipoprotein a, biomarker

CELL THERAPY

Presentation number: MG 18 Abstract number:

A YEAR FOLLOW-UP STUDY OF AD MSC THERAPY IN PATIENTS WITH KNEE OSTEOARTHRITIS

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The goal of this study was to investigate therapeutic effect of the adipose derived mesenchymal stem cells (Ad-MSC) therapy in patients with different stage of knee osteoarthritis. Patients and Methods 16 patients (30 knees) with knee OA who fulfill the inclusion criteria (radiological Kellgren and Lawrence grade II-IV; onset of symptoms for more than 6 months in the painful knee; age 40-85 were enrolled in the study. Surgical lipoaspiration followed by intra-articular injection of final product containing derivate of micronized fatty tissue and intact vascular/stromal architecture with pericytes and mesenchymal stem cells (Ad-MSC) into affected knees were performed in all patients. Plasma blood collection and synovial fluid aspirates of IgG glycom, the visual analogue scale (VAS) and the knee function by using Knee Injury and Osteoarthritis Outcome Score (KOOS) were analysed at the baseline and during the time of procedure, 3, 6 and 12 months after. MRI and Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) were performed in all patients during the observed time. dGEMRIC was used to measure glycosaminoglycans's (GAGs) content in several areas of interest: lateral and medial patellar facets, lateral and medial femoral condyles, femoral trochlea and lateral and medial tibial condyles. Measuring T1 relaxation time of cartilage shows that contents of cartilage glycosaminoglycans significantly increased in specific areas of the knee joint consequently reflecting changes in the mechanical axis of lower extremities. There were no significant changes in the glycan profile over the examined period, signaling that inflammation process stabilised after Ad-MSC application. Our current research indicates that the use of adipose derived mesenchymal stromal/stem cells (Ad-MSC) therapy in patients with OI resulted in increased GAGs content deposition in hyaline cartilage measured by dGEMRIC MRI. Those results are in line with secondary outcome measurements including quantitative MRI analysis, KOOS and VAS. To the best of our knowledge, this is the first report describing direct influence of Ad-MSC to GAGs production in patients with OI.

Keywords: adipose derived mesenchymal stromal/stem cells Ad-MSC, glycosaminoglycans (GAGs), delayed gadolinium-enhanced MRI of cartilage (dGEMRIC)

Presentation number: MG 19 Abstract number:

EXPANSION OF CD4+ T CELLS BY USING ANTI-CD3/28 COATED BEADS AND A CLOSED-CULTURE SYSTEM FOR CD4+ T CELL TRANSFUSION IN HIV-INFECTED PATIENTS

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In vitro expansion of CD4+ T cells from human immunodeficiency virus (HIV) infected patients by anti-CD3/28 coated beads can eliminate the viruses and transfusion of autologous anti-CD3/28 expanded CD4+ T cells showed an increase in patients' CD4 counts. In this study, a closed-culture system for in vitro CD4+ T cell expansion was developed by using a commercially available GMP-grade culture bag and anti-CD3/28 activation. Cryopreserved CD4+ T cells from HIV-infected patients with CD4 count over 500 cells/μL were stimulated with anti-CD3/28 coated beads and were expanded in GMP-grade culture bags for three weeks. Cell number, cell viability and phenotypic characters were determined by trypan blue exclusion and flow cytometry. At the end of a 3-week culture, cryopreserved CD4+ T cells from the patients were well expanded with a fold expansion at 415-fold and a percentage of cell viability at 85%. Phenotypic characterization showed that 96% of expanded cells were CD4+ T cells. The results demonstrated that a closed-culture system using culture bags and anti-CD3/28 coated beads can be used to obtain a large number of expanded CD4+ T cells with good reproducibility, suggesting an efficient cell expansion protocol for CD4+ T cell transfusion in HIV-infected patients.

Keywords: HIV, CD4+ T cells, anti-CD3/28 coated beads, cell expansion, transfusion

Presentation number: MG 20 Abstract number:

COMBINATION OF DIFFERENT TECHNIQUES FOR GENETIC STABILITY ANALYSIS OF CELL BASED MEDICINAL PRODUCTS. RELEVANCE OF ARRAY-CGH

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Cell based medicinal products are promising therapeutics. Cell cultivations and related manipulations may cause genomic rearrangements possibly resulting in genetic instability of cells and potential risk for patients. Therefore, the proof of genetic stability is required before human use. Autologous cultivated chondrocytes intended for re-implantation into cartilage defects were tested by combination of two whole-genomic screening techniques. G-banding karyotype analysis (G-banding) was performed at passage 1 and 3, 30 metaphase cells were assessed. Array-based comparative genomic hybridization (aCGH) testing with detection sensitivity of more than 100 kBp was performed only at passage 3 (amount of DNA was not sufficient at passage 1). Detected copy-number variants were proved by peripheral blood analysis. Chondrocytes from 5 patients were evaluated between 6/2016 – 11/2016. All patients signed informed consent with genetic testing. G-banding did not detect any chromosomal imbalances. aCGH detected 5 submicroscopic chromosomal rearrangements (4 gains and 1 loss of genetic material) in 3 patients. The peripheral blood DNA quantitative PCR analyses proved that these chromosomal alterations were present in others patients´ cells and are not related to the cell cultivation process. Array-CGH is an appropriate complementary method to G-banding karyotyping technique. The methods detect different kinds of genomic abnormalities: karyotyping detects major chromosomal abnormalities including balanced changes, aCGH is able to detect minor numeric changes. Based on our results we recommend usage of both methods simultaneously for genetic stability testing of cell based medicinal products.

Keywords: genetic stability testing; chondrocytes; aCGH; G-banding; cell based medicinal product

EPIGENETICS AND EPIGENOMICS IN HEALTH AND DISEASE

Presentation number: MG21 Abstract number:

HYPOXIA-INDUCIBLE FACTOR 1 ALPHA (HIF1A) STIMULATES NEURONAL NITRIC OXIDE SYNTHASE (NOS1) TRANSCRIPTION BY BINDING TO MULTIPLE ENHANCERS AND PROMOTERS

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Reduced NOS1 has been linked to gastroparesis. However, pharmacological stimulation of NO signaling did not improve gastroparesis. *Nos1* transcription is a potential alternative therapeutic target, but its mechanisms are unclear. Non-neuronal Nos isoforms are transactivated by HIF1A and aryl hydrocarbon receptor nuclear translocator (ARNT). We tested the hypothesis that *Nos1* is also regulated by HIF1A and ARNT. Stomachs were from diabetic NOD and matched nondiabetic mice. N1E115 mouse neuroblastoma cells and IM-FEN mouse fetal enteric neurons were cultured under normoxia (21% O_2) and hypoxia (4% O_2). Gene expression was assessed by Affymetrix microarrays, real-time RT-PCR, stranded mRNA and total stranded RNA sequencing. Proteins were analyzed by Western blots. Genome-wide associations of HIF1A, ARNT, acetylated histone H3 lysine 27 (H3K27ac), mono- and trimethylated H3K4 (H3K4me1/3) and H3K36me3 were studied by chromatin immunoprecipitation-sequencing. In diabetic NOD mice, gastric expression of *Nos1* and 6 of the 28 canonical HIF1A target genes was reduced. In both N1E115 and IM-FEN cells, hypoxia robustly increased HIF1A and NOS1 protein and *Nos1* mRNA. In normoxic N1E115 cells, *Nos1*-001 encoding full-length NOS1 was the dominant transcript driven by an $H3K4me3^{hi}me1^{lo}H3K27ac^{+}$ promoter located 28 kb upstream of the ATG in exon 2 (ATG-28). Two small H3K4me3 peaks associated with strong H3K4me1 and H3K27ac peaks were found at ATG-76 and ATG-86, with the latter giving rise to weak expression of *Nos1*-005 encoding a catalytically inactive protein. All 3 H3K4me3 peaks were preceded by $H3K4me1^{+}H3K27ac^{+}$ enhancers, which coincided with HIF1A and ARNT peaks. Hypoxia stimulated both *Nos1*-001 and *Nos1*-005, and enlarged all HIF1A, ARNT, H3K27ac, H3K4me1 and H3K36me3 peaks. H3K4me3 occupancy increased at ATG-28 and ATG-86 but not in the inactive promoter at ATG-76. We conclude that hypoxia activates *Nos1* transcription via HIF1A/ARNT binding to specific enhancers and promoters.

Keywords: ARNT/HIF1B, H3K27ac, H3K4me1, H3K4me3, gastroparesis

Presentation number: MG 22 Abstract number:

SUCCINATE ACCUMULATION EPIGENETICALLY REPRESSES KIT EXPRESSION AND REDUCES INTERSTITIAL CELLS OF CAJAL (ICC)

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Loss of ICC, gastrointestinal electrical pacemaker and neuromodulator cells, from reduced KIT receptor tyrosine kinase expression is the most common cellular change in diabetic gastroparesis. Loss of subunit A, B, C or D of the mitochondrial succinate (SU) dehydrogenase (SDH) complex leads to SU-mediated inhibition of enzymes that remove methyl marks from histones and DNA. SU is elevated in diabetes. Therefore, we hypothesized that SU accumulation from SDH inhibition epigenetically represses KIT transcription leading to ICC loss. Sdhc was genetically inactivated globally in $Tg^{CAG\text{-}creERT2}$;$Sdhc^{fl/-}$ and $R26^{M2rtTA/+}$;$Tg^{tetO\text{-}cre}$;$Sdhc^{fl/fl}$ mice and in a Kit-specific manner in $Kit^{creERT2/+}$;$Sdhc^{fl/fl}$ mice. In KIT^+ GIST-T1 cells used as a human ICC model, *SDHB* was knocked down by small interfering RNAs (si*SDHB*). SU and 2-ketoglutarate (2KG) were measured by GC/MS in gastric muscles of diabetic and nondiabetic patients. Mouse ICC were studied by flow cytometry, immunohistochemistry and Western blots. Genome-wide distribution of histone marks and the ICC transcription factor ETV1 was analyzed by chromatin immunoprecipitation-sequencing (ChIP-seq). DNA methylation (5mC ; repressive) and hydroxymethylation (5hmC ; activating) were studied by dot blot, reduced representation bisulfite sequencing (RRBS: 5mC+5hmC) and TET-assisted RRBS (TA-RRBS: 5hmC). Genomic inactivation of *Sdhc* reduced KIT^+ANO1^+ ICC and ETV1 protein. In GIST-T1 cells, si*SDHB* reduced KIT and ETV1. SU/2KG ratios were elevated in diabetic patients and in response to si*SDHB* in GIST-T1 cells. In GIST-T1 cells, *SDHB* knockdown upregulated the repressive histone marks H3K27me3 and H3K9me2/3. By ChIP-seq we detected increased H3K27me3 occupancy of the KIT transcription start site. 5mC/5hmC ratios were increased globally and in ICC-specific, ETV1-binding super-enhancers in the *PDGFRA/KIT* locus. We conclude that repression of KIT and loss of ICC in diabetes may arise from SDH inhibition and consequent SU accumulation.

Keywords: gastroparesis, DNA methylation, DNA hydroxymethylation, H3K27me3, H3K9me2/3

Presentation number: MG 23 Abstract number:

EZH2 REGULATES DIFFERENTIATION AND SELF-RENEWAL OF STEM CELLS IN THE GASTROINTESTINAL MUSCULATURE

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Interstitial cells of Cajal (ICC) are electrical pacemaker and neuromodulator cells of the gastrointestinal (GI) tract. ICC decline with age and in GI disorders. ICC can differentiate from $KIT^{low}CD34^{+}$ ICC stem cells (ICC-SC) under the influence of KIT receptor tyrosine kinase signaling. Here, we studied the role of ICC-SC in ICC maintenance in vivo and the mechanisms of their differentiation and self-renewal. In *Kit*$^{creERT2/+}$;LSL-*R26*^{DTA} mice, following genetic ablation of ICC sparing 58% of ICC-SC, ICC numbers increased from 2 to 18% over 60 days. Affymetrix microarray analysis showed that relative to ICC, cultured ICC-SC expressed 124 pluripotency genes, downregulated 483 key ICC genes and overexpressed many polycomb group genes including *Ezh2*, *Suz12*, *Rbbp4*, *Pcgf5*, *Bhc1*, *Bap1* and *Mtf2*. Chromatin immunoprecipitation-sequencing showed trimethylated histone H3 lysine 27 (H3K27me3) occupancy of *Kit* upstream regulatory regions, which was replaced by acetylated H3K27 forming a super-enhancer in differentiated ICC. CRISPR-Cas9-mediated deletion of this super-enhancer in GIST-T1 cells, a human ICC model, reduced *KIT* expression (by 48%) and clonogenicity. In ICC-SC, EZH2 inhibition reduced H3K27me3 binding at the *Kit* locus and increased KIT expression. Stable expression of constitutively active KIT^{K641E} in cultured ICC-SC increased key ICC genes including *Ano1*, *Trpc1* and *Itpr2*. Genomic deletion of *Ezh2* in *Kit*$^{creERT2/+}$;*Ezh2*$^{fl/fl}$ mice between 84-151 days of age reduced ICC-SC frequency to 52±8% and the ICC-SC/ICC ratio to 55±9% 60-days post-induction, signifying differentiation toward ICC. In contrast, deleting *Ezh2* in mice aged 46-105 days led to dramatic ICC depletion. EZH2 inhibition in ICC-SC in vitro caused cell cycle arrest. We conclude that ICC-SC are critical for ICC maintenance. KIT signaling and EZH2-mediated epigenetic repression jointly regulate ICC-SC differentiation and self-renewal. Manipulation of these mechanisms may help restore ICC in GI disorders and aging.

Keywords: POLYCOMB, KIT, CD34, EPIGENOME EDITING, DIPTHERIA TOXIN A

Presentation number: MG 24 Abstract number:

CHROMATIN REGULATION BY HISTONE DEACETYLASE SENSITIZES COLON CANCER CELLS TO PHOTODYNAMIC THERAPY

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Hypericin-mediated photodynamic therapy (HY-PDT) is alternative minimally invasive anticancer treatment although cancer cells may acquire resistance. Therefore, combination treatments may be necessary to enhance HY-PDT efficacy. Histone deacetylase inhibitors (HDACis) are used in combination treatments due to their non-genotoxic properties and epigenetic potential to sensitize cells to external stimuli. This study investigates therapeutic effects of HDACis in combination with visible light-mediated PDT, in colorectal cancer cells that are known to be resistance to HY-PDT. Two chemical groups of HDACis were tested in combination with HY-PDT: hydroxamic acids, Saha and Trichostatin A, and short-chain fatty acids, valproic acid and sodium phenylbutyrate (NaPB), as inhibitors of all-class versus nuclear HDACs, respectively. The selected HDACis manifest a favorable clinical toxicity profile and showed similar potencies and mechanisms in intra-group comparisons but different biological effects in inter-group analyses. HDACi combination with HY-PDT significantly attenuated cancer cell resistance to treatment and caused two HDACi groups to become similarly potent. However, the short-chain fatty acids, in combination with HY-PDT, showed increased selectivity towards inhibition of HDACs versus other key epigenetic enzymes, and NaBP induced the strongest expression of the otherwise silenced tumor suppressor cdkn1a, a hallmark gene for HDACi-mediated chromatin modulation. Epigenetic regulation of cdkn1a by NaPB was associated with histone acetylation at enhancer and promoter elements rather than histone or DNA methylation at those or other regulatory regions of this gene. Moreover, NaPB, compared to the other HDACis, caused milder effects on global histone acetylation, suggesting more specific effect on cdkn1a chromatin architecture relative to global chromatin structure. Our results show that HDACis potentiate antitumor efficacy of HY-PDT in colorectal cancer cells, overcoming their resistance to this drug and epigenetically reactivating expression of cdnk1a. Besides their therapeutic potential, HY and these HDACis are non-genotoxic constituents of dietary agents, hence, represent interesting targets for investigating mechanisms of dietary-based cancer prevention.

Keywords: Histone deacetylase inhibitors, photodynamic therapy, colorectal cancer, cdkn1a, chromatin regulation

Presentation number: MG 25 Abstract number:

LEISURE TIME PHYSICAL ACTIVITY AND GLYCEMIC CONTROL IN TYPE 1 AND TYPE 2 DIABETES

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Diabetes mellitus has become a universal health problem with a rising prevalence worldwide. Guidelines for the management of diabetes consist of various self-management strategies, including physical activity and diet. Physical activity has been shown to improve outcomes such as insulin sensitivity, glycaemic control and glucose tolerance. The purpose of this study was to investigate the association between leisure-time physical activity (LTPA), glycemic control and hypoglycemic incidents in type 1 and type 2 diabetes. This cross-sectional study was conducted in collaboration with the primary health care physicians in the area of Osijek-Baranja County. The study involved 53 type 1 and 143 type 2 diabetic patients, with an average age of 42.8 years. LTPA was assessed by a validated 12-month questionnaire. Outcome measures were HbA1c and hypoglycemic incidents. The slightest decrease in HbA1c (1.7%) was found in patients whose physical activity was significantly reduced in the past ten years. In patients whose physical activity was significantly increased, we found the biggest decrease in the level of HbA1c (4.0%). Hypoglycemic events associated with LTPA were significantly more frequent in type 2 diabetic patients than those with type 1 diabetes (Ficher's exact test, p=0.024). Low levels of LTPA were associated with a poor glycemic control. Patients with type 1 diabetes were more physically active, with more vigorous exercises. Furthermore, the level of LTPA was not associated with an increase in hypoglycemic episodes in type 1 diabetic patients. This could be explained by better education of type 1 patients resulting in adequate adjustment of therapy and carbohydrate intake.

Keywords: Diabetes mellitus type 1, diabetes mellitus type 2, leisure-time physical activity, HbA1c, hypoglycemic incidents

GENE THERAPY

Presentation number: MG 26 Abstract number:

IN SITU GENETIC CORRECTION OF F8 INTRON 22 INVERSION IN HEMOPHILIA A PATIENT-SPECIFIC IPSCS USING TALENS/TALENICASES

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Current gene therapy approaches are not applicable for haemophilia A (HA), mainly owing to the big FVIII coding sequence that far exceeds the viral packaging capacity. Here we aim to develop an efficient approach for in situ correction of a large F8 mutation via editing a small sequence. Nearly half of severe HA cases are caused by a 600kb inversion of F8 intron 22 (Inv22) that splits the 186-kb F8 into two parts with opposite transcription directions. The inverted 5′ part preserves the first 22 exons that are driven by the intrinsic F8 promoter, leading to a truncated F8 transcript due to the lack of the last 627bp coding sequence of exons 23-26. In situ genetic correction of Inv22 in patient-specific iPSCs was performed by using TALENs/TALENicases. The 627bp sequence plus a polyA signal was precisely targeted at the junction of exon 22 and intron 22 via HDR with high targeting efficiencies of 62.5% and 52.9%. The gene-corrected iPSCs retained a normal karyotype following removal of drug selection cassette using a Cre-LoxP system. Importantly, both F8 transcription and FVIII secretion were rescued in the candidate cell types for HA gene therapy including endothelial cells derived from the gene-corrected iPSCs. This is the first proof of an efficient in situ genetic correction of the large mutation/gene via targeted addition of a small sequence. Differentiation and transplantation of the corrected endothelial progenitor cells into the animal models is ongoing for in vivo validation.

Keywords: Hemophilia A, Intron 22 inversion of F8, In situ gene correction, iPSCs, TALENs

Presentation number: MG 27 Abstract number:

OPTIMIZATION OF AN EX VIVO GENE TRANSFER TO THE HAMSTRINGS TENDONS MUSCLE REMNANTS: POTENTIAL FOR GENETIC ENHANCEMENT OF BONE-TENDON HEALING

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The healing potential of a ruptured anterior cruciate ligament (ACL) is considered to be extremely poor. One of the strategies to enhance tendon graft-bone healing in ACL reconstruction is gene therapy. Recombinant bone morphogenetic proteins are the most potent osteoinductive agents available today and have demonstrated efficacy in promoting bone healing in tibial fractures and spinal fusions in prospective randomized controlled trials. Using “same day“ ex-vivo regional gene transfer it is possible to create human skeletal muscle cells that produce BMP-2 and subsequently return muscle graft at the place of healing with the surgery done within two hours. Using adenoviral vector carrying the luciferase reporter gene (AdLuc) we determined optimal virus concentration (10*8 plaque forming units) and period of contact (30 min) since time is the limiting parameter. The presence of $CaCl_2$ and $LnCl_3$ did not help bypass the inefficiency of receptor-dependent uptake of the vector. The human muscle grafts form 5 patients were transduced with adenoviral vector encoding bone morphogenetic protein-2 (AdBMP-2) according to the developed protocol. The amount of released BMP-2 protein from transduced muscle grafts was determined by ELISA through period of 21 days. Osteogenic potential was evaluated by RT-qPCR measuring relative gene expression of osteogenic margers Runx2, BSP and Dmp1 at days 14 and 21. Samples transduced with AdBMP-2 showed enhanced expression of osteogenic marker. Consistent with results given by RT-qPCR, immunohistochemical analysis revealed very strong staining of collagen type I fibrils in muscle tissue transduced with AdBMP-2. Taken together, our data introduce adenoviral vectors as a powerful tool in regenerative medicine and the clinical potential of a novel “same day” ex-vivo regional gene therapy approach to promote bone repair, which may make gene therapy cost-effective when adapted for human use.

Keywords: anterior cruciate ligament, adenoviral vector, BMP-2, muscle tissue, osteogenic induction

GENETIC BASIS OF DISEASE

Presentation number: MG 28 Abstract number:

SYSTEMATIC LITERATURE REVIEW OF THE Y MICRODELETION IN DIFFERENT WORLD POPULATIONS INCLUDING CASE SERIES FROM BOSNIA AND HERZEGOVINA

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Genetic factors related to male infertility are chromosomal abnormalities, gene mutations, and microdeletions on Y chromosome, which are detected in about 15% of all cases of male infertility. The aim of this study was to identify the site-specific microdeletions of Y chromosome in idiopathic azoospermic and oligozoospermic male patients from Bosnian population. During the 4 year period, 42 patient samples were received by the Human Genetics Laboratory, Clinical Center of the University of Sarajevo, referred for suspected male infertility for the detection of Y chromosomal microdeletion. PCR analysis for Y microdeletion was performed for each patient, while karyotype analysis was performed for 35 patients. In the analyzed period, Y microdeletion was detected in 11.9% of patients (n=5). Most patients had the complete deletion of AZFd region (n=4), followed by partial AZFc deletion (n=4), and complete AZFa or AZFb deletions (n=2 patients each). Additionally, 11.43%(4/35) had chromosomal abnormalities including translocations of autosomal chromosomes (n=2), an insertion of heterochromatin on chromosome 17q21 (n=1) and Klinefelter syndrome (n=1). Since the detection of Y microdeletion was established 4 years ago in Bosnia and Herzegovina, we evaluated the incidence and significance of chromosomal and Y microdeletion defects in order to create future guidelines for genetic diagnostic of male infertility.

Keywords: Y microdeletion, Bosnia and Herzegovina, male infertility, AZF, chromosomal abnormalities

Presentation number: MG 29 Abstract number:

YET UNREPORTED HETEROMORPHIC VARIANT IN CHROMOSOME 17

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Heteromorphic variants including Yq12 material, being inserted or added to autosomal chromosomes have been reported for chromosomes 1, 7, 11, 13, 14, 15, 21 and 22. Here we describe a novel insertion of Yq12 heterochromatin into a chromosome 17; to the best of our knowledge no similar cases have been reported previously. GTG-, C-banding, fluorescence in situ hybridization (FISH), and homemade human heterochromatin specific multicolor FISH probes set (HCM-mix) were used to define the abnormality in our male patient. A whole chromosome painting (wcp) probe for #17 together with a probe for Yq12 heterochromatin was hybridized to the patient sample. Additionally, Y microdeletion PCR was done to detect possible AZF subregional deletions. The male patient had normal sperm analysis and no AZF deletions on Y chromosome. GTG and C-banding showed an additional band on chromosome 17q21. FISH studies revealed that the insertion was derived from Yq12 heterochromatin. The heterochromatin insertion on 17q21 originating from Yq12 chromosome did not affect the spermatogenesis of aberration carrier and is probably not the cause of infertility in these partners. However, a new heteromorphic variant was identified in this case.

Keywords: Heteromorphic variants, chromosome 17, Yq12 chromosome, infertility, insertion

Presentation number: MG 30 Abstract number:

THE *MGAT3* GENE PROMOTER METHYLATION IN INFLAMMATORY BOWEL DISEASE AND CORRELATION WITH IGG GLYCOSYLATION

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Inflammatory bowel diseases (IBD) are intestinal disorders occurring due to inappropriate immune response to intestinal microbiome in susceptible individuals. Genome-wide association studies identified several genes to be important for both IgG glycosylation and IBD, including the *MGAT3*, a glyco-gene encoding for the enzyme (GnTIII) which adds bisecting GlcNAc to a glycan structure. We analyzed CpG methylation in the promoter of this gene from whole blood of IBD patients from two large independent cohorts using bisulfite pyrosequencing. Methylation level was generally high, with significant difference between healthy controls (HC) and Crohn's disease (CD) or ulcerative colitis (UC). In addition, the *MGAT3* promoter showed differential methylation between HC and CD at some CpG sites in B lymphocytes from the third independent cohort, as well as in CD3+ T-cells from inflamed bowel tissue of UC patients with active disease state. The *MGAT3* promoter methylation was correlated to glycan structures found on IgG molecules from the same blood samples of two large individual cohorts. Significant correlations were found between *MGAT3* promoter methylation and the FA2B/FA2 ratio, suggesting that activity of the GnT-III enzyme (the *MGAT3* gene product) might be altered in IBD. In line, FA2 was positively correlated with *MGAT3* promoter methylation, suggesting that the substrate abundance decreases when the enzyme is more actively transcribed due to decreased methylation. Significant correlation was found between the *MGAT3* promoter methylation and galactosylated and sialylated structures, as well, suggesting that these processes could be co-regulated with *MGAT3* expression. Taken together, the effects of *MGAT3* promoter methylation on IgG glycans in IBD suggest its potential as a biomarker or even a therapeutic target.

Keywords: IBD, MGAT3, DNA methylation, IgG glycans

Presentation number: MG 31 Abstract number:

DUCHENNE/BECKER MUSCULAR DYSTROPHIES IN THE CHILD POPULATION OF BOSNIA AND HERZEGOVINA

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Muscular dystrophies include more than 30 inherited disorders causing muscle to weaken and wither. Duchenne Muscular Dystrophy (DMD) represents the most severe form of this family of disorders. It is an X-linked disease, affecting 1 in 5000 male newborns and is caused by the absence of the protein dystrophin in skeletal, cardiac and smooth muscle. The purpose of our study was to determine the frequency, type and severity of muscular dystrophies among the children in Northeast Bosnia and Herzegovina. A retrospective study was performed among patients in the University Clinical Center Tuzla who were younger than 18 years, and they came from whole northeast Bosnia and Herzegovina. Patient's data has been collected from clinical records, biochemical findings, results of electroneuromyography, muscle biopsy, and molecular genetics tests, and then statistically analyzed. Our data showed that among 1600 patients, 69 patients (44 male, 25 female) had clinical records of muscular hypotonia or dystonia syndrome (4.31%), 16 patients (1%) had muscular dystrophy. Among male patients with hypotony of muscles, 36.36 % patients had muscular dystrophy. Genetic tests were done in 11 patients and they confirmed muscular dystrophy in all of them. Results of tests have shown the presence of deletions, duplications and some new mutations that were not previously described in the DMD gene. We present the first paper of frequency and severity of DMD among Bosnian children and presence of different mutations of DMD gene in Bosnian population. Anticipating future gene therapy for muscular dystrophies, this paper is a starting point for developing policies and strategies for these diseases in Bosnia and Herzegovina.

Keywords: Duchenne Muscular Dystrophy, BiH, DMD gene

Presentation number: MG 32 Abstract number:

LONG SURVIVAL IN TRISOMY 13 SYNDROME

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Genetic syndromes caused by chromosome aberration have usual pattern of multiple congenital developmental disorders including high mortality in early life. Patau syndrome – trisomy 13 is one of the most common chromosome anomalies clinically characterized by the presence of numerous malformations with a limited survival rate. 28 percent of newborns with Patau syndrome die within the first week of life. The median survival age is 2.5 days. We present a 10-year old girl with Patau syndrome, who we've been following since her birth up to now. She is the first of three children of young and healthy parents. Postnatal caryotypisation showed translocation trisomy 13 : 46,XX, der(4)t(4;13)(q33;q14.1). We did caryotypisation to her mother: 46, XX, t(4;13) (q33 ; q14.1), brother : 46, XY, t(4 ; 13) (q33;q14.1) and sister: 46,XX, healthy. Our patient has typical Patau phenotype with microcephaly, microophtalmia, skin defect on head, gothic palate, hexadactylism, congenital heart defect – double outlet right ventricle and generalized hypotonia. Congenital heart defect was operated when she was five months old. She stayed in hospital due to recurrent bronchpneumonia after the surgery. MRI shows signs of cortical atrophy, hypoplasia of corpus callosum and vermis. She developed epilepsy at the age of 8. Her psychomotor development is slower and she is being fed by both nasogastric tube and spoon. Based on our experience, every patient is a unique person and one should be very careful when deciding about patients' therapy. One should also focus on supporting the parents and also suggest them genetic counseling for next planned parenthood.

Keywords: Patau syndrome, trisomy 13, epilepsy, congenital heart defect, long survival

Presentation number: MG 33 Abstract number:

A SINGLE NUCLEOTIDE POLYMORPHISM OF DNA METHYLTRANSFERASE 3B GENE IS A POTENTIAL RISK FACTOR FOR RECURRENT SPONTANEOUS ABORTION

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Recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses before the 22nd week of gestation. It affects approximately 1% of couples and can be caused by several factors. However, the cause remains unidentified in about 50% of the cases, which are classified as idiopathic (IRSA). Among various possible etiological factors, aberrant DNA methylation has been suggested to be one of the possible causes of IRSA. Considering the growing evidence of the important roles of DNA methylation in gametogenesis and early pregnancy, as well as the results of multiple studies that indicate abnormal methylation patterns in the endometrium, spermatozoa and placenta of patients with IRSA, our aim was to investigate the potential association of DNA methyltransferase gene polymorphisms (DNMT1 rs2228611, DNMT3A rs1550117 and DNMT3B rs1569686) with IRSA in Slovenian reproductive couples. 146 couples with ≥3 idiopathic spontaneous abortions and 149 control women and men were included in this case-control study. Genotyping was performed using polymerase chain reaction and restriction fragment length polymorphism methods. We found a statistically significant higher frequency of the DNMT3B rs1569686 GG genotype (X2=7.37, P=0.025) and G allele (X2=6.33, P=0.012) in women with IRSA compared to controls. Additionally, the odds for IRSA in women were increased under the recessive genetic model (GGvsTG+TT: OR=1.92; 95% CI=1.18-3.09; P=0.008). There were no statistically significant differences in genotype and allele frequencies of any other tested polymorphism between IRSA patients and controls. Moreover, no significant associations occurred between the DNMT1 rs2228611 and DNMT3A rs1550117 polymorphisms and the risk of IRSA. Our results suggest that the GG genotype of the rs1569686 polymorphism in the DNMT3B gene in women might be a genetic marker for IRSA.

Keywords: DNA methyltransferases, pregnancy, recurrent spontaneous abortion, single nucleotide polymorphisms

Presentation number: MG 34 Abstract number:

GENE POLYMORPHISMS OF DNA METHYLTRANSFERASES IN WOMEN WITH SPONTANEOUS PRETERM BIRTH

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Preterm birth (PTB) is a birth that occurs before the 37th week of gestation and is the leading cause of neonatal mortality and morbidity. DNA methyltransferases (DNMTs) establish DNA methylation patterns at specific genome regions and contribute to gene regulation. Previous studies have reported on an association between spontaneous PTB (SPTB; birth with intact membranes) and epigenetic changes (i.e. methylation levels) in maternal blood, placenta and cord blood. Additionally, certain DNMT3B gene polymorphisms in women were found to be associated with an increased risk for SPTB. The aim of this study was to evaluate the potential association between SPTB and DNMT1, 3A, 3B and 3L gene polymorphisms in European Caucasian women, and their contribution to clinical characteristics of women with SPTB and their new-borns. A total of 113 women with SPTB and 119 women with term delivery were included in a case-control study. Genotyping of DNMT1 rs2228611 A/G, DNMT3A rs1550117 A/G, DNMT3B rs1569686 G/T and DNMT3L rs2070565 A/G single nucleotide polymorphisms was performed using polymerase chain reaction and restriction fragment length polymorphism methods. No statistically significant differences were found in the distribution of genotype or allele frequencies of tested polymorphisms between patients and controls. However, the DNMT3B rs1569686 minor allele (T) was more frequent in women with familial SPTB than women with non-familial SPTB (X2=7.65, P=0.006), contributing to a 4.02 increased odds for familial SPTB under the dominant genetic model (TG+TTvsGG) (95% CI=1.56-10.40, P=0.004). None of the other polymorphisms contributed to the clinical characteristics of women with SPTB and their new-borns (family history of SPTB, maternal and gestational age at delivery, fetal birth weight). The DNMT3B rs1569686 T allele in European Caucasian women might be associated with a positive family history of SPTB.

Keywords: DNA methyltransferases, spontaneous preterm birth, gene polymorphisms, DNA methylation, genetic association study

Presentation number: MG 35 Abstract number:

THE FOKI POLYMORPHISM IN VITAMIN D RECEPTOR GENE IN WOMEN WITH SPONTANEOUS PRETERM BIRTH INFLUENCES NEW-BORN BIRTH WEIGHT

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Preterm birth (PTB) is defined as birth of a new-born prior to the completed 37th week of gestation. Prior studies confirmed the association between low levels of vitamin D and increased risk of PTB. Considering that polymorphisms of vitamin D receptor (VDR) gene may modify the effects of vitamin D, several studies investigated the potential association between FokI and ApaI single nucleotide polymorphisms of the VDR gene and PTB in different races. The aim of our study was to evaluate the association between FokI and ApaI polymorphisms of the VDR gene and PTB in European Caucasian women, as well as their effect on clinical characteristics of women with PTB and their new-borns (smoking, family history of PTB, maternal and gestational age at delivery, new-born birth weight). A case-control study was conducted in 113 women with spontaneous PTB (SPTB; PTB with intact membranes) and 119 women with term delivery. Genotyping of FokI and ApaI polymorphisms of the VDR gene were performed using polymerase chain reaction and restriction fragment length polymorphism methods. No statistically significant differences were found in the distribution of genotype or allele frequencies of tested polymorphisms between patients and controls. We found a statistically significant effect of FokI polymorphism on new-born birth weight in women with SPTB using two-way ANOVA: Factorial design with Scheffe test ($F=4.53$, $P=0.013$), with the lowest birth weight identified in mothers carrying the FokI TT genotype ($P=0.021$). There was no statistically significant interaction between the FokI and ApaI polymorphisms on birth weight. Neither polymorphism was associated with any other clinical characteristic of women with SPTB and their new-borns. We determined that the TT genotype of the VDR FokI polymorphism is associated with new-born birth weight in European Caucasian women with SPTB.

Keywords: vitamin D receptor, spontaneous preterm birth, birth weight, gene polymorphisms, genetic association study

Presentation number: MG 36 Abstract number:

CLINICAL CHARACTERISTICS AND LONG SURVIVAL OF TRISOMY 18 IN TWO GIRLS

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Trisomy 18 (Edward's syndrome) is a condition with incidence of 1:3-8000 births. More that 85% of such newborns die before birth. Median survival of the others is 15 days to 1 month. Less than 4% may survive their first year, most of them are girls. Two girls with regular trisomy were reported, one is 2,5 and the other 4 years old. Both were born as prematures with intrauterine growth retardation by caesarian section. Both have clenched hands, rocker bottom feet and low set ears and short sternum. Cardiovascular malformations among them are: ventricular septal defect, ductus arteriosus persistence and persistent left venea cava superior. Both have cerebral malformations; one has agenesis of corpus callosum and the other ventricular dilatation and cerebellar hypoplasia. Both girls are hypotonic and have eating problems. Even though most patients with Edward's syndrome will die in the first few weeks, a few of them will survive for many years. The management of such babies remains a challenging medical and ethical issue. The majority of their problems are: hypotony, epilepsy, feeding difficulties and susceptibility for infections.

Keywords: Trisomy 18, long survival, feeding problems, cerebral malphormations, cardiovascullar malphormations

Presentation number: MG 37 Abstract number:

ASSOCIATION STUDY OF APOA5 GENE WITH METABOLIC SYNDROME IN TUNISIAN POPULATION

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Goal of the study Metabolic syndrome (MetS) is a complex disease characterised by multiple metabolic abnormalities including central obesity, hypertension, dyslipidemia and insulin resistance Apoliprotein A5 (APOA5) gene has been linked to MetS or its traits in several populations from Europe, Asia and Middle East. In North Africa, only the Moroccan population was investigated. Our aim is to assess the association between APOA5 gene variants with the susceptibility to MetS and its components in the Tunisian population. A total of 594 participants were genotyped for two polymorphisms rs3135506 and rs651821 located in APOA5 gene using KASPar technology. Statistical analyses were performed using R software. Our results showed that the SNP rs651821 increases the risk of MetS under the dominant model (OR=1.91(1.17-3.12) p=0.008) whereas the variant rs3135506 was not associated with MetS under any genotypic and allelic model. After stratification of the cohort according to the sex, only the variant rs651821 showed a significant association among the women group. The investigation of the impact of the geographic origin on the genotype distribution of APOA5 variants showed that the variant rs651821 was significantly associated with MetS only for the Northern population. This is the first study reporting the involvement of APOA5 gene in MetS development in Tunisia. Our study highlighted the significant association of the variant rs651821 with MetS and the association of the variant rs3135506 with TG levels. We reported also a gender-specific differences and an inter-regional variability. Further studies are needed to confirm the clinical relevance of these associations and to better understand the physiopathology of the MetS.

Keywords: metabolic sydrome, APOA5 gene, SNP, Tunisia, North Africa

Presentation number: MG 38 Abstract number:

RISK-ASSOCIATED SNPS IN OSTEOSARCOMA: A PILOT STUDY TO DISSECT ITS GENETIC HETEROGENEITY

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Osteosarcoma (OS) is a rare primary bone malignancy generally occurring during the adolescent growth spurt. Little is known about the aetiology of OS but several studies have provided consistent evidence of an inherited genetic risk and most of these researches deepen the knowledge about biologically plausible pathways for growth related genes. The aim of this pilot study is to contribute to dissect the genetic heterogeneity in OS risk through the genotyping of selected OS associated SNPs, that were selected with a minor allele frequency (MAF) greater than 1% in European populations. In order to speculate whether genetic variation in selected loci influences the risk of OS onset, the frequencies of 5 successfully genotyped polymorphisms (rs7386167 in CASC8; rs185852 in LOC101930033; rs2279744 in MDM2; rs231775 in CTLA4 and rs11866002 in CNOT1) were compared between 20 Italian OS cases and 100 matched controls. The variants are located in functionally different environments like non coding RNA (ncRNA), gene bodies, or intergenic regions. The obtained results point out a heterogeneous landscape that suggests how the role of these variants could be synergic, even though no staple haplotypes could be addressed in closely related markers. The remarkable finding in this study is that some SNPs associated with OS susceptibility are located in ncRNA-related complexes suggesting to broaden our knowledge in this topic in order to identify putative genetic target for early diagnosis and therapy.

Keywords: Osteosarcoma, Primary Bone Malignancy, Coding Variants, Non Coding RNA, Aetiology

Presentation number: MG 39 Abstract number:

GENETIC POLYMORPHISMS IN MICRORNA IS ASSOCIATED WITH DENTAL CARIES IN CHILDREN FROM AMAZONAS STATE, BRAZIL

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Polymorphisms in genes codifying microRNAs may alter the expression of the corresponding miRNA and influence a given disease. Therefore, the aim of this study was to evaluate the association between a polymorphism in microRNA202 with dental caries experience in children from Amazonas State, Brazil. A total of 102 children (10 to 12 years old) from Manaus public schools were examined. Manaus is the capital city of the state of Amazonas, Amazon region of Brazil. Dental caries experience was evaluated according to the DMFT and dmft index. Saliva samples were collected from each child for DNA extraction. The polymorphism rs12355840 in microRNA202 was evaluated by real-time PCR (TaqMan™). DMFT and dmft values were analyzed by means of analysis of variance (ANOVA) to test the association between dental caries and genotypes. A significant level of 5% was used. The CC genotype had a higher caries experience mean. A statistical association was observed for permanent dentition (p=0.01) and for combined mixed and permanent dentition (p=0.03). The polymorphism rs12355840 in microRNA202 could be a dental caries risk biomarker.

Keywords: microRNA, caries, children, polymorphism, oral

Presentation number: MG 40 Abstract number:

NON-CODING VARIANTS CONTRIBUTE TO THE CLINICAL HETEROGENEITY OF TTR-AMYLOIDOSIS

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Despite most of coding variants in TTR gene could determine amyloidogenic processes resulting in TTR amyloidosis (ATTR, OMIM ID: #105210), the non-coding ones represent key factors that contribute to the heterogeneous clinical display. In fact, some symptoms may be present in patients carrying different TTR disease-causing mutations but clinical phenotypes are not always consistent, and the same mutation may be associated with different clinical signs.Fifty-five recruited ATTR patients were re-sequenced for a 20 Kb region including TTR gene. Fifty-four identified variants have been annotated and their effect size and p-value were scored for 46 tissue in GTEx database. A LD clumping analysis was performed to obtain 46 tissues specific datasets accounting for LD structure and they have been used to estimate genetically determined TTR expression profiles across human tissues linked to potential pathogenic mechanisms.These patterns hint 3 patient clusters featured by specific profiles that could be linked to peculiar phenotypic display such as late onset, neurological involvement and gastrointestinal symptoms. This study advances the knowledge of ATTR in terms of novel data regarding the role of non-coding variation and the expression profile of TTR gene in patients introducing an innovative approach to investigate the mechanism at the basis of the genotype-phenotype correlation of TTR-amyloidosis.

Keywords: Transthyretin, Genotype-Phenotype Correlation, Gene-Expression, Symptoms, Rare Disease

Presentation number: MG 41 Abstract number:

ALTERED SLEEP STRUCTURE AND BREATHING IN CHILDREN WITH DOWN SYNDROME A NEED FOR ROUTINE POLYSOMNOGRAPHIC STUDIES

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Down syndrome is the most common genetic cause of intellectual impairment. Children with intellectual disabilities have frequently more sleep issues than general population of children. Obstructive sleep apnea (OSA) has prevalence in general population 1-4%, but in children with DS it increases up to 63. Despite these facts, there are limited studies analyzing the sleep issues in children with DS and there is still no well recognizable sleep phenotype in DS, or listed as an important clinical feature of DS. There have been some animal model studies which investigated the genotype of the sleep in DS and so far few genes have been listed as gene candidates for the sleep phenotype not only in DS, but for obstructive sleep apnea syndrome in different disorders. Our study examines the sleep issues in a small cohort of Croatian children with DS, using overnight polysomnography. Our data showed that children with DS had short sleep latency, but long REM latency, increased total wake time, low number, but long duration of arousals, higher percentage of N1 NREM sleep and lower percentage of REM and N3 NREM sleep, compared to their age. All these issues led to the sleep fragmentation in our group of children with DS. The sleep disturbances proved to be the important part of clinical features in Down syndrome. Promoting the importance of having the routine sleep study in children with DS and addressing the awareness of sleep issues to the health care providers who care for DS and to the parents of children with DS, we can prevent majority of respiratory complications in children with DS.

Keywords: sleep, Down syndrome, sleep apnea, overnight polysomnography, trisomy 21

Presentation number: MG 42 Abstract number:

SLEEP DISORDERED BREATHING IN CHILDREN WITH PRADER WILLI SYNDROME

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Prader-Willi syndrome is a rare genetic disorder characterized by different physical, physiological and psychological abnormalities. The aim of study was to assess sleep-disordered breathing (SDB) in children with Prader-Willi syndrome through polysomnographic studies and to identify the role of obesity in the breathing difficulties. A total of 14 children previously diagnosed with Prader-Willi syndrome and 28 healthy controls, matched by age, gender and BMI, were enrolled in the study. Participants underwent standard polysomnographic study/studies (PSG) and additional data regarding sleeping difficulties were gathered from participants' parents. Mean apnea hypopnea index (AHI) among PWS group was 7.47± 9.48 where as in control group AHI was 1.19±1.9, (p=0.0015). Statistical analysis showed significant difference among groups for mixed apneas (p=0.0001), obstructive apneas (p=0.019), duration of longest apneic episodes (p=0.0005), average duration of apneic episodes (p=0.029), microarousal index (p=0.0499) and average and minimal saturation (p=0.0004 and p=0.0001). Although the difference in AHI and number of respiratory events correlates with underlying type of genetic disorder, it was not statistically relevant. No difference was noticed in sleep efficacy, sleep latency, total sleep time and total awakeness. Children with PWS were more like to have learning difficulties, exhibit aggression and have breathing difficulties while sleeping. PSG studies revealed that children with PWS have significantly higher incidence of sleep disordered breathing in comparison with BMI matched control group, suggesting that etiology of SDB is multifactorial and could not be associated solely with obesity.

Keywords: Prader-Willi syndrome, sleep disordered breathing, children, polysomnography, obesity

GENOME-BASED APPLICATIONS IN FORENSIC SCIENCE

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Presentation number: MG 43 Abstract number:

SEQUENCING WORKFLOW FOR THE WHOLE MITOCHONDRIAL GENOMES FROM CROATIAN REFERENCE SAMPLES

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Recognizing the huge potential of next generation sequencing (NGS) technology, Forensic Science Centre "Ivan Vucetic", located in Zagreb, established the first Croatian NGS forensic laboratory at the beginning of 2016. The primary goal of our laboratory is gradual introduction of NGS methods for DNA analysis into forensic casework, by means of development, validation and implementation of forensically relevant protocols.Analysis of mitochondrial DNA (mtDNA) in today's routine forensic practice is restricted to hypervariable sites residing in control region of the molecule. However, developing whole mtDNA sequencing is important because it considerably increases the power of discrimination when reporting the results of forensic analyses, and enables maximal recovery of mtDNA from highly degraded samples. Given that the emergence of NGS technology potentiated sequencing of the whole mitochondrial genome (16,569 bp) at acceptable time-cost ratio, we selected this particular analysis to be the first step in the process of introducing NGS into our work.Here we present the established NGS workflow that resulted in high-quality whole mtDNA sequences obtained from reference buccal swab samples. This method will be further employed for creating a national database of whole mtDNA sequences, and thus will make foundation for the use of mtDNA analysis in forensic casework.

Keywords: NGS, Whole mtDNA sequencing, Reference samples, Croatian population, Forensics

Presentation number: MG 44 Abstract number:

HIGH-QUALITY SEQUENCES OF WHOLE MITOCHONDRIAL GENOMES FROM ILLUMINA® MISEQ®

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Analysis of mitochondrial DNA (mtDNA) for forensic applications requires high-quality data. Our aim in this study was to obtain whole mtDNA sequences of sufficient quality in order to produce confident haplogroup determination for future database use. Reference samples of buccal swabs from 23 donors were extracted and purified on EZ1® Advanced XL (Qiagen®). Following PCR amplification of two overlapping fragments (9.1 and 11.2 kbp) for each sample, library preparation was performed with Nextera® XT Sample Prep Kit (Illumina®). Sequencing was run on MiSeq® (Illumina®) instrument, using MiSeq® Reagent kit v2. Analysis was performed with on-instrument software MiSeq® Reporter v2.5.1 and Sequencing Analysis Viewer v.1.8.46, and subsequently with applications from BaseSpace® Sequence Hub (Illumina®). Haplogroups were determined by HaploGrep2 tool. Results exhibited 92.9% of reads with Q-score above 30 and average coverage depth of 9,928 reads. Composition of haplogroups showed predominance of group H (44%), which is concordant with European haplogroup frequencies. Also, high-level heteroplasmies were detected in 5 samples. In conclusion, we obtained high-quality whole mtDNA sequences, which enabled confident variant calling and haplogroup determination. Further sequencing of larger Croatian population sample is under way, aimed at founding national database of whole mtDNA sequences.

Keywords: NGS, Whole mtDNA sequences, Illumina MiSeq, Data analysis, Mitochondrial haplogroups

Presentation number: MG 45 Abstract number:

EVALUATION OF FOUR MRNA MARKERS FOR WOUND AGE ESTIMATION

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Wound age, defined as the survival time of an individual after the infliction of a fatal injury, is an important theme in forensic pathology as sharp traumas account for many fatalities. To diagnose the cause and manner of death, as well as to help reconstruct a crime scene, it is essential to describe the findings of the wound as complete as possible including the survival time. A molecular approach was performed based on RT-qPCR, examining the relative expression of four target genes (CXCL9, DUSP1, FOS, IL-10) and two, previously validated, endogenous reference genes (GAPDH and PGK1), to investigate expression patterns linked to wound age estimation. Total RNAs from 18 pairs of human post-mortem wounded and normal (unwounded) skin tissue were extracted. Quantification, purity and integrity were assessed prior to qPCR. The obtained RINs (RNA Integrity Numbers) ranged from 2.0 to 7.8 representing extensive to moderate degradation of RNA. Final qPCR data were analyzed with the qBase+ software for gene expression analysis (www.qbaseplus.com). Preliminary results of gene expressions (normalized to reference genes GAPDH and PGK1) showed that IL-10 was the only marker with significant differential expression between wounded and reference skin samples ($p=0.0273$, $p \leq 0.05$; based on a non-parametric Wilcoxon test). The wounded samples displayed a 6.5-fold higher expression than the reference group (12 out of 18 pairs). CXCL9 also showed a 2.3-fold higher expression in the wounded samples pairs (13 out of 18 pairs), but non-significantly different than the reference group. On the contrary, DUSP1 and FOS exhibited higher gene expression in the reference samples group in 16 and 18 pairs, respectively. Based on these preliminary results, IL-10 could represent a potential marker for wound age estimation supporting other studies based on animal models that have shown IL-10 highly expressed in the early inflammatory phase. Classical wound age histology as well as immunochemistry must be performed in order to support the correlation of IL-10 with the survival time of each specific case. Larger-scale studies on human specimens are required to further establish the applicability of mRNA analysis for determination of wound age.

Keywords: forensic, wound age, mRNA, gene expression, skin wound

Presentation number: MG 46 Abstract number:

RNA PROFILING IN FORENSIC CASEWORK

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At the Netherlands Forensic Institute (NFI), cell type inference by means of messenger RNA (mRNA) profiling was first applied in casework in 2010. Since then, mRNA profiling has been applied in over 200 cases. In approximately 80% of the RNA cases, identification of body fluids (e.g. blood, vaginal mucosa, menstrual secretion, semen, saliva) is requested; in 20% of the cases identification of organ tissues (brain, lung, liver, skeletal muscle, heart, kidney and skin) is asked. Body fluid inferring cases mainly involve sexual assault cases in which most often the presence of vaginal mucosa cells on penile swabs or in finger nail dirt is disputed. Inference of organ tissues is mainly requested on objects involved in violent crimes, such as bullets and knives. Here, we present how these two mRNA profiling systems have evolved throughout the years. Among others, we included a nasal mucosa mRNA marker, even though this bodily secretion itself has limited forensic relevance. We extended both multiplexes with sex-specific RNA markers that positively identify male or female cell material, providing for the first time a true female marker and overlapping information in DNA and RNA profiles. We present the remarkable stability of RNA in severely degraded samples of excavated human remains buried up to 42 years. Additionally, we discuss current difficulties in relating DNA and RNA results obtained from the same sample, and describe possible future solutions that will allow for the association of donor and cell type.

Keywords: mRNA profiling, cell type inference, organ tissue identification, body fluid identification

MOLECULAR DIAGNOSTICS: CURRENT TECHNOLOGY AND APPLICATIONS

10th ISABS Conference on Forensic and Anthropologic Genetics
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Presentation number: MG 47 Abstract number:

IMMUNOLOGICAL STABILITY OF CLOSTRIDIUM DIFFICILE TOXINS A AND B IN CLINICAL SPECIMENS

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Clostridium difficile is an intestinal pathogen with a range of clinical outcomes from asymptomatic diarrhea to megacolon. C. difficile infection (CDI) is recognized as the most frequent etiologic agent of infectious healthcare-associated diarrhea. The main C. difficile virulence factors are toxins A and B primarily targeting colonic epithelial cells. Detection of these toxins in fecal specimens is a common approach for CDI diagnostics. The impact of storage on stability and detection of Clostridium difficile toxins in feces is poorly understood but it may have clinical importance for diagnostic test results. The study objective was to investigate immunological stability of C. difficile toxins in clinical stool specimens under different storage conditions and evaluate stability using toxin detection by enzyme immunoassay (EIA). Prospectively collected clinical stool specimens positive for CDI by qPCR (Xpert® C. difficile/Epi, Cepheid, CA), were used for EIA testing by C. difficile Tox A/B IITM kit (TechLab, VA). The initial EIA testing was performed within 36 hours of the specimen collection. Aliquots of the positives were stored aerobically at refrigerated (4 / 10oC) and freezer (-30oC / -80oC) conditions. The same storage temperatures were applied for identical samples diluted in storage buffer. EIA testing was measurement of both toxins (A and B) quantity by optical density (450/620 nm abs) in each sample aliquot on days of storage 0 (the initial testing), 14, 30, 60, 90, and 120. C. difficile toxin testing revealed a broad range of quantities in specimens and good detection in undiluted stools up to 120 days of storage. Good detection were observed also in diluted samples at refrigerated and -80oC temperatures. However, dilution detrimentally affects toxin detection at -30oC. Undiluted stool stored at both freezing conditions demonstrated steady toxins detection comparable to detection at refrigerated temperature. Storage of undiluted clinical stool specimens at refrigerated, -30oC and -80oC temperatures for up to 120 days has no discernible effect on immunological stability of C. difficile cytotoxins. However, storage at -30oC has a detrimental effect on C. difficile toxin stability in diluted specimens.

Keywords: Clostridium difficile , toxins, EIA, storage, stability

Presentation number: MG 48 Abstract number:

SENSING OF LUNG CANCER MUTATIONS USING QUARTZ CRYSTAL MICROBALANCE

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Lung cancer is one of the most common severe disease driving to the death of human. Lung cancer can be divided into two cases of small-cell lung cancer (SCLC) and non-SCLC (NSCLC), and about 80% of lung cancers belong to the case of NSCLC. From several studies, correlation between epidermal growth factor receptor (EGFR) and NSCLCs has been investigated.[1] Therefore, EGFR inhibitor drugs such as gefitinib and erlotinib have been used as lung cancer treatments. However, the treatments result showed low response (10~20%) in clinical trials due to EGFR mutations that cause the drug resistance. [1] Patients with resistance to EGFR inhibitor drugs usually are positive to KRAS mutation. Therefore, assessment of EGFR and KRAS mutation is essential for target therapies of NSCLC patient. In order to overcome the limitation of conventional therapies, overall EGFR and KRAS mutations have to be monitored. In this work, only detection of EGFR will be presented. A variety of techniques have been presented for the detection of EGFR mutations. The standard detection method of EGFR mutation in ctDNA relies on real-time polymerase chain reaction (PCR).[2] Real-time PCR method provides high sensitive detection performance. However, as the amplification step increases, cost effect and complexity increase as well. Other types of technology such as BEAMing, next generation sequencing (NGS), electrochemical sensor and silicon nanowire field-effect transistor have been presented. However, those technologies have limitations of low sensitivity, high cost and complexity of data analyzation. In this report, we propose a label-free and high-sensitive detection method of lung cancer using quartz crystal microbalance based platform. The proposed platform is able to sense lung cancer mutant DNA with a limit of detection of 1nM.

Keywords: lung cancer DNA, quartz crystal microbalance, resonance frequency, mutation

Presentation number: MG 49 Abstract number:

DETECTION OF GERM-LINE MUTATIONS IN CFTR AND BRCA1/BRCA2 GENES BY ARRAY-BASED DNA-IMAGING METHODS

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We present an array-based method for detection of mutations in human genes. The method for detection of CFTR mutations includes the multiplex PCR amplification of CFTR exons for typing of 113 mutations: GLU7TER, TRP57TER, GLY85GLU, GLY91ARG, GLU92LYS, GLU92TER, TYR109CYS, ASP110HIS, ARG117HIS, LEU206TRP, GLU217GLY, PHE311LEU, ARG334TRP, THR338ILE, ARG347PRO, ARG347LEU, ARG347HIS, ALA349VAL, ARG352GLN, GLN359LYS, ALA455GLU, GLY458VAL, MET470VAL, GLY480CYS, SER492PHE, GLN493TER, ILE506VAL, ILE507DEL, PHE508DEL, PHE508CYS, VAL520PHE, CYS524TER, ALA534GLU, GLY542TER, SER549ASN, SER549ILE, SER549ARG, GLY551ASP, GLY551SER, GLN552TER, ARG553TER, ARG553GLN, ILE556VAL, ALA559THR, ARG560THR, ARG560LYS, ALA561GLU, TYR563ASN, PRO574HIS, GLY576ALA in 4 PCR reactions. The method for detection of BRCA1/BRCA2 mutations includes the multiplex PCR amplification of BRCA1/2 exons for typing of mutations in BRCA1: c.1016_1016dupA, c.1292_1292dupT, c.1380_1380dupA, c.1556delA, c.1687C>T, c.181T>G, c.211A>G, c.2197_2201delGAGAA, c.2338C>T, c.2475delC, c.2685_2686delAA, c.2722G>T, c.3052_3056dupAACAT, c.3228_3229delAG, c.3485delA, c.3626delT, c.3700_3704delGTAAA, c.4035delA, c.4097-2A>G, c.4327C>T, c.470_471delCT, c.4964_4982del19, c.5123C>A, c.5251C>T, c.5266dupC, c.5277+1G>A, c.5419delA, c.66_66dupA, c.68_69delAG, c.697_698delGT, c.70_73dupTGTC, BRCA2: c.2808_2811delACAA, c.3847_3848delGT, c.5351dupA, c.5946delT, c.6275_6276delTT, c.6468_6469delTC, c.7480C>T, c.771_775delTCAAA, c.8327T>G, c.8537_8538delAG, c.9026_9030delATCAT, c.9117+G>T, c.9118-2A>G, c.9403delC in 8 PCR reactions. The amplified fragments are pulled, purified and then subjected to cyclic matrix mini-sequencing with the use of dideoxynucleotides labelled with different fluorescent dyes and oligonucleotides immobilized on array and analysed using a GenePix 4300A (Molecular Devices) scanner at 4 different ranges of excitation and cut-off lengths for specific dideoxynucleotides. The use of an array divided into sub-arrays dedicated to the analysis of positive and negative control as well as two analyses of target sample allowed to conduct the typing of a high amount of mutations in a single analysis with ensured control of highest test quality.

Keywords: CFTR, BRCA1, BRCA2, mutations, array

Presentation number: MG 50 Abstract number:

PATHOGEN IDENTIFICATION BY ON-ARRAY DNA IMAGING METHODS

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Rapid, simple and credible identification of pathogens plays a crucial role in pregnant woman care and perinatal care of the new-born infants. We present two tests based on the DNA IMAGING technology for identification of pathogens which are hazardous during the course of pregnancy and for new-born infants. The DNA IMAGINING NEONATAL test allows for semi-quantitative identification of 23 pathogens and 3 groups of pathogens, which are considered as particularly dangerous for new-born infants, including premature babies. The DNA IMAGING PREGNANCY test allows for semi-quantitative identification of 19 pathogens and 3 groups of pathogens, which considered as particularly dangerous for women during pregnancy. The method includes the isolation of genetic material, multiplex PCR amplification and on array single-base extension with dideoxy nucleotides (ddATP, ddCTP, ddGTP, ddUTP) labelled with Cy3, by DynaSeq DNA Polymerase or ThermoSequenase. Four sub-arrays were distinguished on the array, which includes immobilized starters for minisequencing dedicated to: negative PCR control, positive control and two subarrays for the tested sample. The minisequencing reaction is conducted in 30 cycles in the thermocycler with a “flat” type block. The arrays are analysed with a array scanner. In order to control the test and easily evaluate the result, each of the spots includes a spot control labelled with the Cy5 fluorochrome in addition to the minisequencing starters, which is analysed in a different colour channel. The evaluation of the test result is based on the identification of a positive signal for a given location at the sub-matrix. The use of cyclic minisequencing notably increases the reliability and sensitivity of the reaction, and eliminates the need to precisely control the hybridization which was necessary in previously methods. The validation analyses confirmed the specificity of the method towards the analysed pathogens. The traceability of the method was established at a level of 10 copies of pathogens / reaction. Analysis of controls and two repetitions of the tested sample in dedicated sub-matrices of an individual test allows for a simultaneous, rapid, sensitive and credible identification of numerous pathogens during a single study, which is necessary for routine diagnostic of infectious diseases.

Keywords: pathogen, identification, on-array, diagnostic, DNA-IMAGING

Presentation number: MG 51 Abstract number:

DETECTION OF JAK2 AND CALR MUTATIONS IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS FROM BOSNIA AND HERZEGOVINA

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To detect frequency of mutations in JAK2 (Janus kinase 2) and CALR (Calreticulin) genes in samples of patients with polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) from Bosnia and Herzegovina. DNA was extracted from peripheral blood samples from 280 patients during 7 years. Quantitative real time PCR (qPCR, MutaQuant Kit - Qiagen) was performed for detection of JAK2 V617F mutation. PCR and agarose gel electrophoresis were used for detection of CALR mutation in JAK2 negative patients. Primers were designed to detect wild-type CALR (product: 357 bp), type I (product: 305 bp) and type II (product: 272 bp) mutations.Results: 113/280 patients were JAK2 positive (40.36%), and were not tested for CALR mutation. 14/167 JAK2 negative patients had CALR mutations (8.38%); 8 patients had type I (deletion of 52 bp) and 6 patients had type II (insertion of 5bp) mutation. Low frequency of CALR mutations is due to the diagnosis issues; most of the JAK2 negative patients are suspected or confirmed PV cases in which CALR mutations are not observed, or do not have clear diagnosis.

Keywords: JAK2, CALR, mutations, myeloproliferative neoplasms, PCR

Presentation number: MG 52 Abstract number:

DIVERSITY OF ACE AND ACTN3 POLYMORPHISMS IN BOSNIAN-HERZEGOVINIAN POPULATION

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Human ACE gene encodes angiotensin I-converting enzime with a key role in renin-angiotensin (RAS) and kinin-kallikrein (KKS) systems in the regulation of hemodynamics. ACTN3 gene encodes the muscle α-actinin-3 isoform that stabilises the muscle contractile apparatus. ACE I/D and ACTN3 R577X polymorphisms are both extensively studied for possible association with the human physical performance. Aim was to estimate diversity of ACE and ACTN3 polymorphisms in population of Bosnia and Herzegovina and to evaluate possible association of these gene variants, gender and sports activity. Genomic DNA was extracted from blood and buccal epithelial cells using salting-out method. Total of 242 unrealted healthy individuals were tested, with regard to their gender (N=132 women, N=110 men) and sports activity (N=90 active, N=152 non-active). Genotyping was done by PCR with locus-specific primers. Statistical analyses revealed no significant differences in genotype and allele frequencies of both ACE/ACTN3 genes in Bosnian-Herzegovinians, as well as between compared subgroups of men and women, athletes and non-athletes (P=0.05). Allele frequency distribution showed no deviation from Hardy-Weinberg equilibrium. We also compared our data with available data of other populations. Since no extensive research studies of ACE and ACTN3 genes were conducted in Bosnian-Herzegovinian population, except a few clinical studies, this study can provide further information about genetic diversity of human populations in western Balkan region. It can also serve as an incentive for future studies with larger number of variables, larger cohorts, as well as more applicative context in terms of developing more individualized approach in sports training.

Keywords: ACE, ACTN3 gene variants, genetic diversity, Bosnian-Herzegovinians

Presentation number: MG 53 Abstract number:

IMPACT OF CD117 EXPRESSION ON THE SURVIVAL OF GIST PATIENTS

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Gastrointestinal stromal tumors (GIST) are common mesenchymal neoplasms arising within the gastrointestinal tract from precursors of the connective-tissue cells. The objective of the study is to analyze immunohistochemical and histopathological features of GIST with a focus on CD117 expression as a relevant prognostic factor for ten-year overall survival of patients in the Federation of Bosnia and Herzegovina. Retrospectively studied, all GIST patients (n=145) in the Federation of Bosnia and Herzegovina was diagnosed in the period from 06/2005–12/2015. Diagnosis of GIST was based on both morphology and immunohistochemical profile that included cKit (CD117) (polyclonal rabbit; anti-human CD117; Dako), CD34 (Clone Qbend 10, Dako), SMA (CloneHHF35, Dako), Desmin (Clone D33, Dako), S-100 (Polyclonal Rabbit Anti-s- 100, Dako), and DOG1 (NCL-L- DOG1 Clone K-9, Novocastra). Among 145 patients, 82 (57%) patients were males and 63 (43%) were females, with a median age of diagnosis of 62 years. Regarding macroscopic tumor parameters, 62% of tumors displayed a diameter >5 cm. Using AFIP classifications, 33% of tumors were classified as low grade, while 58% were classified as high grade. The most common locations for GIST in this research were the stomach (54%), followed by the small intestine (35%), and the large intestine (6%). Moreover, a lack of significant immunohistochemical differences was found in the frequency of CD34 and SMA expression, depending on the site of occurrence. CD117 was positive in 86% of cases which did not have a significant impact on overall survival. Patients with advanced disease who were not treated with imatinib, or were treated with imatinib, showed no statistically different survival between CD117+ and CD117- tumors. However, there was a trend towards better longer survival of patients with CD117 negative tumors in patients who were not treated with imatinib.

Keywords: Gastrointestinal stromal tumor (GIST),CD117 (cKit) expression, treatment, outcome, developing country

Presentation number: MG 54 Abstract number:

FORENSIC IDENTIFICATION OF SOIL USING METAPOPULATIONAL ANALYSIS OF THE BACTERIAL IV 16SRNA REGION

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Forensic analysis of soil uses elemental analysis, macro- and microscopic analysis of soil and botany-based analysis. We present the results of initial analyses of 70 soil samples in terms of the composition of soil bacteria population for use in forensic analysis of soil.The studies were employed due to the discovery of two young female bodies in an aquatic tank. The soil samples from shoe soles of men suspected of murder were secured and the results of their macroscopic analysis matched those obtained for samples originating from the crime scene. Soil samples from similar areas and the residences of suspects and victims were also subjected to studies.Total DNA was extracted from 500 mg of each soil sample using Genomic Mini AX Soil kit (A&A Biotechnology). Region IV of bacterial 16S RNA gene was amplified using universal primers 515F and 806R: containing reverse complement of 3' Illumina adapter, golay barcode, reverse primer pad, reverse primer linker and reverse primer. The libraries were constructed from amplicons using NEBNext® DNA Library Prep Master Mix Set for Illumina (New England Biolabs UK). Sequencing was conducted on an Illumina MiSeq (Illumina, USA) using paired-end (2x250) MiSeq Reagent Kits v2 (Illumina, USA). The sequencing data was processed using CLC Genomic Workbench 8.5 and CLC Microbial Genomics Module 1.2. (Qiagen, USA). Metapopulational comparisons allow for an unequivocal identification of soil samples. Based on the obtained results it should be concluded that the described method may be useful for forensic identification of soil. The potential issues for the proposed study methods are mixed soils, stacked from different areas and in different time, which may notably limit the applicability of the method, which does not search for indicator species and the identification is based on a qualitative-quantitative criterion.

Keywords: soil, identification, bacterial, metapopulation, 16S RNA

MOLECULAR THERAPY

Presentation number: MG 55 Abstract number:

TOXICOLOGY AND ANTITUMOR EVALUATION OF TWO NOVEL MONOMETHINE CYANINE DERIVATIVES *IN VIVO*

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The area of small, biological active molecules that interact with specific cell compartment are in focus of today's science. Small molecules with low toxicity and specific mode of interaction are interesting in the area of molecular therapy especially for cancer treatment and future of personalised medicine. One, very interesting biologically active group of small molecules are monomethine cyanine dyes (MCDs) with preferable binding mechanism in to a minor groove of DNA. In this study we evaluate toxicological and antitumor effect of two, new monomethine cyanine derivatives designated as monomethine cyanine derivatives no. 4 and no.8. Highly inbreeded hybrid mouse WT strain created as combination of 129/Sv and C_{57}BL/6j were used for acute and chronic toxicology study and BalbC female mice were used for antitumor study. Four mice for each sex were used in experimental groups. Haematological (E,L, Hg, HE, MCV, MCV, MCHC, Plt) and biochemical (AST, ALT, AP, LDH, glucose, urea, creatinine, Na, K) parameters, histomorfological analysis of body organs (brain, hart, spleen, bone, stomach, intestine, colon, kidney, liver, lungs) and size of grown tumour are measured as tool for *in vivo* effect. Obtained results are analysed by multifactorial variance analysis (MANOVA) and Fisher LSD test in STATISTICA 8.0 program. One time application (7mg/kg) with MCD 4 in evaluation of acute toxicity resulted with increased levels of AST, ALT and LDH and decreased glucose. Chronic toxicity (3x7mg/kg) because of the prolonged exposure and multiple MCD 4 application did not show significant change on the male mice. MCD 4 applied to the female mice raised levels of AST, ALT and LDH and decreases ALP. Results points to enhanced sensibility of female mice compared to males in prolonged time of exposure to MCD 4. Chronic toxicity caused by the MCD 8 did not point to any significantly altered biochemical parameter no matter the sex of the mouse. Results pointed to stronger MCD 4 toxic effect in chronic exposure to female in comparison to male mice. There were no significant suppression of implanted tumour regardless to applied concentration of tested derivatives.

Keywords: monomethine cyanine derivatives, antitumor evaluation, MCD 4

Presentation number: MG 56 Abstract number:

CHANGE IN GENE EXPRESSION UNDER EPIGENETIC AGENTS IN MOUSE TERATOMA *IN VITRO*

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Testicular Germ Cell Tumours (TGCT), although rare, are the most frequent malignancies in young male population and believed to be initiated by epimutations, i.e. aberrant epigenetics, already *in utero*. Among various, teratoma is the most differentiated TGCT type encompassing all three germ layer derived tissues. Mouse teratoma is a well-established *in vitro* model which may be obtained by cultivating 7,5–days-old C3H mouse embryos and represent an ideal system to investigate the effect of the most prominent epigenetic drugs and agents. After embryo isolation, they were treated for two hours with 5-azacytidine, Trichostatin A, Valproat, esiNanog, esiOct3/4 and esiTrrap, respectively. Embryos/teratomas treated with esiGFP served as a methodological control. The embryos/teratomas were measured at day 0 and for the consequent 7 days of culturing, after which teratomas were scrapped and stored at -80°C until further analysis. RNA was isolated with TRIzol and was analysed by qPCR and ddPCR. Epigenetic drugs and agents reduced significantly teratoma growth, all except esiNanog and esiTrrap. Most prominent decrease in growth was determined in 5-azaC and esiOct3/4 treated embryos/teratomas. qPCR analysis has shown decrease in stem cell, germ layer and differentiation marker expression after treatment by esiOct3/4, 5azaC and Valproate. ddPCR was done to verify the results and has shown similar gene expression. This data notifies that epigenetic drugs and agents seem to have a significant effect on embryo/teratoma growth through change in the differentiation and stemness gene expression.

Keywords: Testicular Germ Cell Tumours (TGCT), teratoma, epigenetic agents

Presentation number: MG 57 Abstract number:

„STEP UP“ AND „TOP DOWN“ THERAPEUTIC APPROACH IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Ulcerative colitis and Crohn's disease are autoimmune idiopathic diseases whose incidence is constantly increasing, consequently representing a significant health problem of a developed Western society. They are characterized by chronic inflammation of the gastrointestinal wall and the high occurrence of relapse. Drug groups used in the treatment of inflammatory bowel disease are aminosalicylates, corticosteroids, immunomodulators and biological agents. By developing new drugs, therapeutic approach has changed from the simpler “step up” to the “top down” approach, where more complex and more effective drugs are applied from the start. The basic goal of the treatment is to develop a deep remission and its longer duration, along with the smaller number of relapses. The aim of this study was to determine frequency of disease relapses in various types of the therapy. The research was based on medical documentation of patients affected by inflammatory bowel disease, treated at the Department of gastroenterology and hepatology, at Clinical Hospital Osijek. In total, 79 patients were involved, of which 40 of them were diagnosed with ulcerative colitis and 39 with Crohn's disease. Patients with Crohn's disease achieved the longest remission, up to 2450 days, when recieving biological therapy. Much shorter remission is seen in patients taking aminosalicylates. However, there was no significant difference in remission duration between different treatment strategies (Mann-Whitney U test, P = 0,2836). For patients with ulcerative colitis, the best results, with the remission up to 2500 days, gave the combination of aminosalicylates and immunosupresives. Still, there was no significant difference in remission duration between different treatment strategies (Mann-Whitney U test, P = P = 0,4762). Almost all patients in this study started treatment with a "step up" approach, and only at later stages of the disease were treated with biological therapy. Because of the above-mentioned reason, there was no statistically significant difference between the "step up" and "top down" therapeutic approaches, and further assessment of these investigated groups is required.

Keywords: Ulcerative colitis, Crohn's disease, „step up“ therapeutic approach, „top down“ therapeutic approach, remission

PERSONALIZED GENOMICS

Presentation number: MG 58 Abstract number:

DRUG SENSITIVITY PREDICTION USING A COMBINED GENE EXPRESSION PROFILE IN BREAST CANCER

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Chemotherapy targets all rapidly growing cells, not only cancer cells, and thus is often associated with unpleasant side effects. Therefore, examination of the chemosensitivity based on genotypes is needed in order to reduce the side effects. Various computational approaches have been proposed for predicting chemosensitivity based on gene expression profiles. A linear regression model can be used to predict the response of cancer cells to chemotherapeutic drugs, based on genomic features of the cells, and appropriate sample size for this method depends on the number of predictors. We used principal component analysis (PCA) and identified a combined gene expression profile to reduce the number of predictors. The coefficients of determination (R2) of prediction models with combined gene expression and several independent gene expressions were similar. Corresponding F values, which represent model significances were improved by use of a combined gene expression profile, indicating that the use of a combined gene expression profile is helpful in predicting drug sensitivity. Even better, a prediction model can be used even with small samples because of the reduced number of predictors. Combined gene expression analysis is expected to contribute to more personalized management of breast cancer cases by enabling more effective targeting of existing therapies. This procedure for identifying a cell-type-specific gene expression profile can be extended to other chemotherapeutic treatments and many other heterogeneous cancer types.

Keywords: Gene expression, Drug sensitivity, Combined predictor, breast cancer

Presentation number: MG 59 Abstract number:

A CASE STUDY OF PRIMARY IMMUNODEFICIENCY IN A PERSONALIZED MEDICINE DIAGNOSTIC PERSPECTIVE: NOVEL MUTATIONS IN AN ATAXIA TELANGIECTASIA PATIENT IDENTIFIED BY NEXT GENERATION SEQUENCING

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In a translational medicine concept, we aim to combine clinical, immunological and genetic aspects of the primary immunodeficiency diagnostics in order to improve the treatment decisions and prevention strategies of these disorders. In this approach, the assessment of the immune imbalance by flow cytometry combined with the next generation sequencing (NGS) technology represents an unprecedented tool in immunogenetic diagnostics of rare and monogenic diseases in the new era of precision medicine. A 16 year-old male patient was admitted to our hospital due to recurrent respiratory infections and hypogammaglobulinemia, and was diagnosed with ataxia telangiectasia (A-T) - a rare genetic disorder with neurologic impairment and immune deficiency. We performed the flow cytometry immunophenotyping of peripheral blood and the targeted NGS analysis using the TrueSeq Custom Amplicon panel followed by sequencing on the MiniSeq system (Illumina, USA) to screen for the variants of the immunodeficiency genes involved in the predominantly antibody deficiency and common immunodeficiencies with associated or syndromic features. Immunophenotyping revealed a decrease of the CD4+ T lymphocytes, B lymphocytes, transitional B lymphocytes, class-switched memory B lymphocytes and plasmablasts; an increase of the CD3+HLA-DR+ activated T lymphocytes; and an appearance of a peculiar CD45dimCD38+CD27+CD56+ population. The targeted NGS analysis identified 3 novel missense variants in i) meiotic recombination 11 (MRE11) gene, ii) ataxia telangiectasia and Rad3-related protein (ATR) gene, and iii) B-cell linker protein (BLNK) gene, with the in silico predictions as possibly deleterious. We here report novel variants detected by NGS in the A-T patient previously not reported in clinical practice. Given their potentially pathogenic nature, i.e. the phenotypic association with leukemia (BLNK gene), the appearance of the CD45dimCD38+CD27+CD56+ population, and an increased alpha-fetoprotein level, a further treatment of the patient requires a close monitoring for the potential development of malignancy - the main cause of premature death of A-T patients, by means of a tight integration of immunogenetic diagnostic tools in a personalise medicine perspective.

Keywords: primary immunodeficiency, next generation sequencing, personalized medicine

Presentation number: MG 60 Abstract number:

PHARMACOGENETICALLY INTERESTING NAT2 GENE IN CROATIAN ROMA POPULATION

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Arylamine N-acetyltransferase 2 (NAT2) plays an important role in metabolism of therapeutic drugs and exogenous chemicals present in the diet and the environment. In addition, numerous association studies confirmed a link between NAT2 acetylator phenotypes and cancer risk. However, these studies were predominantly conducted in populations of European origin. Roma population is a transnational minority population of Indian origin for which data on variability of NAT2 gene is very limited, presenting a problem in regard to information on drug efficacy and safety. As many Roma people live from collecting secondary raw material, they are exposed to exogenous possibly cancerogenic chemicals, which can increase cancer risk in genetically susceptible individuals. The aim of this study was to determine genetic variability of NAT2 in Croatian Roma population. NAT2 gene polymorphisms (rs1041983, rs1801280, rs1799929, rs1799930, rs1208, rs1799931, rs1801279 and rs1805158) were genotyped in a sample of 440 individuals from three Roma groups as a part of genotyping project aimed at determining variability of ADME and several other selected genes involved in drug metabolism. Genotyping was performed using KASP method. Loci rs1801279 and rs1805158 were monomorphic in all three Roma groups. Allele distribution of 3 polymorphic loci, rs1801280, rs1799929 and rs1208, differed significantly among the investigated groups. In addition, MAFs in Croatian Roma are notably different from the average global frequency. LD between investigated loci is very high in all three Roma groups. Results of this study confirm expected specific genetic profile of NAT2 in Croatian Roma population which results from their high level of isolation. This genetic specificities should be taken into account in pharmacotherapy. In addition, very high LD level suggest a possibility of using tag SNPs in genotyping analysis of these loci in Roma populations.

Keywords: NAT2, Roma population, pharmacogenetics, ADME, isolates

Presentation number: MG 61 Abstract number:

DPYD AND UGT1A1 POLYMORPHISMS AND TOXICITY OF 5- FLUOROURACIL AND IRINOTECAN IN CROATIAN PATIENTS WITH METASTATIC COLORECTAL CANCER

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Treatment with 5- fluorouracil (5-FU), in combination with irinotecan is still one of the pillars of oncologic approach to patients with metastatic colorectal cancer. Dihydropyrimidine- dehydrogenase (DPD) and UDP-glucuronyl transferase (UGT1A1) are responsible for the metabolism of 5-FU and irinotecan respectively. Aim of the study was to test if DPD and UGT1A1 enzyme deficiency due to gene variants can result in increased risk of 5-FU and irinotecan toxicity. FOLFIRI-treated patients (N=114) were genotyped for UGT1A1*28 as well as for five DPYD polymorphisms (*2A, *13, c.2846A>T, c.1236G>A and c.496A>G). Adverse events were monitored for three months from the beginning of the treatment. Observed group included grade III and IV toxicity (N=52), whereas control group comprised grade I and II (N=62). DNA was isolated from whole blood (3ml) and genotyped according to manufacturer's propositions using real-time PCR (LightCycler® for UGT1A1 and TaqMan® for DPYD). Frequencies of the polymorphisms were tested by non-parametric statistical tests and binary logistic regression. A total of 206 adverse events were recorded during observation period (75 of high and 131 of low grade). Subjects in observed group (toxicity grade III and IV) developed adverse effects more rapidly and accumulated greater total number of events. UGT1A1*28 variant was detected in 58% of tested subjects. Both homozygotes and heterozygotes were at significantly increased risk for toxicity (OR=20.58 and 5.47 respectively). Aggregated DPYD polymorphisms (N=33; 28.9%) distributed unevenly with higher frequency of carriers in observed group (38.46% vs. 20.97%), thus creating a statistically significant increase of risk for severe toxicity among carriers of DPYD variants (OR=2.36). UGT1A1*28 variant had stronger influence on toxicity-risk than DPYD polymorphisms considering patients given irinotecan in combination with 5-FU. Significant association and predictive value of UGT1A1*28 polymorphism among patient given FOLFIRI-protocol is shown, while mutation status of DPYD has shown weaker but still significant influence. Frequencies of detected polymorphisms emphasize the importance of genetic background of the patients.

Keywords: DPYD, UGT1A1, polymorphism, 5-fluorouracil, irinotecan

Presentation number: MG 62 Abstract number:

STEADY-STATE PHARMACOKINETICS OF MYCOPHENOLIC ACID IN RENAL TRANSPLANT PATIENTS: EXPLORATORY ANALYSIS OF THE EFFECTS OF CYCLOSPORINE, RECIPIENT SAND DONORS ABCC2 GENE VARIANTS AND THEIR INTERACTIONS

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To evaluate the impact of recipients' and donors' polymorphisms in multidrug resistance-associated protein 2 (MRP2) gene ABCC2 -24C>T and 1249G>A on disposition of mycophenolic acid (MPA) and their interaction with cyclosporine (CsA) (compared to tacrolimus, TAC) in stable de novo adult renal transplant patients of Croatian origin. A total of 68 recipient-donor pairs were genotyped. Steady-state pharmacokinetics of MPA was assessed by the model-independent method. Adjusted for MPA formulation, renal function, type of calcineurin inhibitor and recipients' and donors' genotypes at the two loci, donors' A-allele at 1249G>A was associated with a reduced peak (29%) and early (AUC0-2, 33%) exposure and increased MPA clearance (26%). Donors' A-allele combined with CsA was associated with 78% higher MPA clearance, 49% lower early and 48% lower total exposure as compared to wild type homozygosity + TAC. Recipients' SNPs per se did not reflect on MPA disposition. However, A-allele at 1249G>A + CsA (compared to wild type + TAC) was associated with a numerically greater increase in MPA clearance (59% vs. 41%), reduction in total exposure (36% vs. 27%) and increase in absorption rate (Cmax/AUC) (56% vs. 37%) than observed for the main effect of CsA. Less pronounced effects were observed for the combination of variant allele at -24C>T and CsA. Conclusion. Considering MPA disposition, data indicate: donors' ABCC2 1249G>A polymorphism increases clearance and reduces exposure; CsA increases clearance and reduces exposure by inhibiting MRP2 in the gut, the liver and the kidney; donors' ABCC2 1249G>A polymorphism enhances the renal CsA effect, while recipients' polymorphism seems to enhance the liver and the gut CsA effects.

Keywords: renal transplantation, mycophenolic acid, cyclosporine, tacrolimus, ABCC2 protein

PRENATAL DIAGNOSTICS

Presentation number: MG 63 Abstract number:

CLINICAL STUDY OF CONGENITAL HYDRONEPHROSIS IN A 6 YEAR PERIOD

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Hydronephrosis refers to distension and dilation of the renal pelvis and calyces. Structural abnormalities of the junctions between the kidney, ureter, and bladder that lead to hydronephrosis can occur during embriogenesis. To diagnose congenital % hydronephrosis in hospitalized newborns. Prospective clinical study of newborns diagnosed with hydronephrosis congenita in the Neonatal Department, for Term Neonates coming from home, of the Institute for Child and Youth Health Care of Vojvodina during a 6 year period. From 2010 to 2016 in total 1071 (62% male and 38% female) newborns were hospitalized due to high fever, sleepiness and rejecting breastfeeding. 439 were diagnosed with urinal infection. Ultrasound showed that 29 of them (23 female and 6 male) suffer of prenatally undiagnosed congenital hydronephrosis (67%) or diagnosed congenital hydronephrosis that was considered mild (33%) and without need for urological examination before one month of age. In 28% of cases prenatal ultrasound was performed in 13th and 23th week of gestational age but not in the 32th week of pregnancy when hydronephrosis has a better diagnose rate. Majority of these cases were admitted during September-December (57%) which means they were conceived January-March when there is a high incidence of infections which are the leading teratogenic factor during organogenesis (in the first 8 weeks of pregnancy). All newborns with prenatally diagnosed hydronephrosis should be examined upon birth by a team of an urologist, nephrologist and neonatologist. Our recommendation: ultrasound after 32th week of gestation for all.

Keywords: prenatal, congenital, hydronephroses, newborn, clinical

Presentation number: MG 64 Abstract number:

ANTENATAL DETECTION OF CHROMOSOMAL ABNORMALITIES COMBINING QF-PCR AND CYTOGENETIC ANALYSIS

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To compare the diagnostic values and limitations of both, quantitative fluorescent polymerase chain reaction (QF-PCR) and conventional cytogenetic analysis in prenatal diagnosis of chromosomal abnormalities. A prospective study included simultaneous QF-PCR and cytogenetic analysis of 133 prenatal samples routinely obtained by amniocentesis or chorionic villus sampling (CVS). Additionally, QF-PCR analysis was performed on 14 tissue samples collected after termination of pregnancy (TOP) for which karyotyping could not be performed due to culture growth failure. Among 133 prenatal samples, cytogenetic analysis revealed chromosomal abnormalities in 12 cases (9%), including four cases of trisomy 21, one triploidy, three cases of Turner syndrome, one 47,XXX karyotype, one trisomy 9, a case with supernumerary marker chromosome, and one unbalanced structural rearrangement. Nine out of 12 aberrations (75%) were also detected with QF-PCR. However, all cases of the most common aneuploidies were successfully disclosed with QF-PCR, resulting in 100% detection rate for chromosomes 21, 18, 13, X and Y. Using a set of markers specific for chromosomes 21, 18 and 13, QF-PCR analysis of tissue samples collected after TOP revealed chromosomopathy in 21.4% of cases (two trisomy 18 cases and one triploidy). Comparison of STR markers confirmed monozygosity in two monochorionic/diamniotic twin pregnancies. QF-PCR has been shown as a rapid and reliable method for prenatal diagnosis of the most common chromosomal aneuploidies, and as an adequate alternative to conventional karyotyping in cases where cytogenetic analysis is disabled due to failure of culturing process. However, conventional cytogenetics still presents a gold standard for detection of structural aberrations and rare aneuploidies. Combining approach using both QF-PCR and cytogenetics provides information important not only for the current pregnancy management, but also for genetic counseling of parents and their families.

Keywords: chromosomal abnormalities, cytogenetic analysis, miscarriage, prenatal diagnosis, QF-PCR

REGENERATIVE MEDICINE

Presentation number: MG 65 Abstract number:

ACTIVATION OF NOTCH SIGNALING BY CONTROLLED RELEASE JAGGED 1 ENHANCES ANGIOGENESIS IN VITRO

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Vascular penetration is essential to deliver cells and growth factors that promote bone callus formation surrounding bone allografts in large bone defect repair surgery. Although multiple signaling pathways are involved in regulation of angiogenesis, increasing evidence shows that Jagged1-mediated Notch signaling also plays a crucial role in angiogenesis during development. Given the key function of JAG1/Notch in angiogenesis and the importance of early vascularization in tissue repair, we hypothesized that controlled release Jagged1 may promote angiogenesis by potentially releasing angiogenic factors over an extended period of time at the bone defective site. Human microvascular endothelial cells (HMECs) were exposed to either Jagged1 or DAPT (a Notch signaling inhibitor) for a period of three days and then plated on a dish coated with growth factor reduced matrigel. Photographs were taken periodically to monitor the tube formation. Total RNA was isolated and then reverse transcribed to cDNA before final amplification by RT-PCR. Tetramethylbenzidine (TMB) assay was performed using peroxidase/PBS solution. HMECs exposed to Jagged1 resulted in a measurable increase in capillary tube formation with thicker capillary tubes and more connections. Jagged1 also showed an increased expression of angiogenic promoting genes. HMECs treated with DAPT showed fewer, thinner tube formation with lower expression of the angiogenic genes. The results from TMB peroxidase protein release assay showed that the Jagged1 ligands cross-linked on the surface of bone allografts could hold on to and slowly release Jagged1 protein over days and possibly even weeks.

Keywords: Jagged1, Notch, Angiogenesis, allograft, endothelial cells

STEM CELLS

Presentation number: MG 66 Abstract number:

QUALITATIVE AND QUANTITATIVE MEASUREMENTS OF HUMAN ADIPOSE-DERIVED STEM CELLS TO USE AS CELL-ASSISTED LIPOTRANSFER FOR BREAST RECONSTRUCTION IN BREAST CANCER PATIENTS

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To establish a standardized protocol to identify and quantify human adipose-derived stem cells (ADSCs) for cell-assisted lipotransfer. Stromal vascular fraction (SVF) was isolated from lipoaspirate obtained from ten breast cancer patients aged 32 – 62 years. Freshly isolated SVF was analyzed for ADSCs by staining with fluorochrome-conjugated monoclonal antibodies against CD13, CD31, CD34, CD45, CD73, CD90, CD105, and CD146 prior to polychromatic flow cytometry analysis. SVF was cultured to obtain ADSCs. Expanded ADSCs were re-confirmed for their phenotypes and were detected for their differentiation ability towards adipogenic, osteogenic, and chondrogenic lineages by quantitative polymerase chain reaction (qPCR). SVF consisted of blood leukocytes, pericytes, endothelial progenitor cells and ADSCs. The average amount of ADSCs was 47,000 cells per milliliter lipoaspirate. There is no significant difference in quantity of ADSCs between patients at middle adulthood (32 – 44 years) and older adulthood (45 – 62 years). ADSC population is homogeneous with expression of CD13+CD31-CD34+CD45-CD73+CD90+CD105-CD146-, however, the characteristic of ADSCs was changed over serial passages with downregulation of CD34 and upregulation of CD105. Multilineage differentiation of ADSCs was confirmed for both early and late passages. Our developed protocols for isolation and characterization of human ADSCs from breast cancer patients are robust and reproducible with a homogeneous phenotype, suggesting a promising tool for autologous cell-assisted lipotransfer to use in breast reconstruction.

Keywords: ADSC, stromal vascular fraction, cell-assisted lipotransfer, reconstruction, breast cancer

Presentation number: MG 67 Abstract number:

PERIPHERAL BLOOD PREDICTORS FOR OBTAINING A DESIRED COLLECTION OF CD34+ CELLS ‰ 2,5X106/KG OF BODY WEIGHT, IN ONE DAY

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In lymphoma treatments, there is a high probability of a successful engraftment with transfusions of autologous hematopoietic stem cell where CD34+ cell counts are higher or equal to 2,5x106/kg. To determine differential blood counts of CD34+ and other cells affecting a successful collection of at least 2,5x106/kg bm of CD34+ cells in the process of apheresis in one day. The study was retrospective-prospective. We have analyzed 44 patients, with 69 apheresis (MM 23(33,3%), HL 29(42,0%) and NHL 17 (24,6%))completed in the process of autologous transplantation. Apheresis results were divided into two groups: collections with CD34+ counts less than 2,5x106/kg and those with values higher than 2,5x106/kg. We have examined relationships between collections of CD34+ cells by absolute counts of CD34+ cells in peripheral blood and differential blood counts by apheresis in one day. Patients' average age was 45+/-16.03 years, with the youngest being 18 and the oldest 67 years old. Men were represented with 52.3% and female with 47.7%. 44 patients (63,8%) received a transfusion with CD34+ cells count ≥2,5x106/kg. In that group, the absolute blood count of CD34+ cells was greater than 20.01x106/L in peripheral blood in 97,7% of cases. That difference is statistically significant with p=0.0001. A significant impact was found for absolute count of white blood cells in a range from 3440 to 12600 (p=0.001), Neutrophils (2245-10900, p=0.0001) and Monocytes (342-1345, p=0.002). Chi-square test was used for comparison analysis, $p < 0.05$ was considered significant. Absolute blood counts of CD34+, WBC, Neutrophils and Monocytes in peripheral blood on the day of apheresis significantly impact a successful collection of CD34+ cells higher than 2,5x106/kg bm.Key words: CD34+ cells, peripheral blood, apheresis

Keywords: CD34+ cells, peripheral blood, apheresis

TRANSLATIONAL MEDICINE

Presentation number: MG 68 Abstract number:

AP-1 OVEREXPRESSION MEDIATES TAMOXIFEN RESISTANCE IN ER-POSITIVE BREAST CANCER

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Clinically, upregulated transcription factor AP-1 activity has been associated with tamoxifen resistance in estrogen receptor (ER) alpha-positive breast tumors. However, our understanding of how AP-1 governs the ER transcriptome and the molecular determinants conferring tamoxifen resistance remains largely unknown. Here, we use an integrative analysis combining the genomic landscapes of Jun, an AP-1 family member, and ER with gain-of-function transcriptome analysis to comprehensively decipher the role of AP-1 in ER-positive breast cancer cells. Induced Jun triggers oncogenic gene signatures highly associated with cell proliferation. Moreover, overexpression of Jun mediates tamoxifen resistance in ER-positive cells. Integrated omics data reveal 25 AP-1 direct target genes as the most perturbed genes in Jun-overexpressed cells. Functional studies on one of the 25 genes, TGFBI, show that TGFBI knockdown inhibits tamoxifen-resistant cell survival and renders Jun-overexpressed cells sensitive to tamoxifen treatment. We also show that TGFBI is associated with poor clinical outcome in breast cancer patients. Overall, this work illuminates the pathways through which ER-positive breast cancer cells acquire tamoxifen resistant properties.

Keywords: Estrogen receptor, breast cancer, resistance, ChIP-seq, Jun

ANTHROPOLOGY AND HEALTH

Presentation number: AH 1 Abstract number:

INDIVIDUAL IDENTIFICATION BY ISOMETRIC SCALING OF EXTERNAL EAR: PRELIMINARY TEST

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Individual identification using biometric information is used various parts of human body. Law enforcement currently used to verify the identification by surveillance camera in public areas and arrest criminals using facial recognition technology. This study is a preliminary test to develop measurements of the external ear that are not affected by image angles for individual identification.Taken 452 images from among photographs of the face in Koreans, those were selected with all the photographs both of 45-degree and lateral views in isometric scales. Fifteen variables around the external ear of the face measured by computer program Mimics, respectively. The differences according to sex, age, and directions of photos were analyzed by SPSS. Eleven among 15 measurements, except 4 measurements indicating the external ear position, were compared between images in lateral and 45-degree views. Lobule width was not statistically different between lateral and 45-degree views in all four groups. Four groups were as follows: males and females, young and old groups. Lobule length and ear length below tragus were not different in females, young and old groups. Based on results of this study, it was found that the earlobes were less affected by the image angles than the other parts of the external ear. The earlobes would be helpful for individual identification from a morphometrical perspective. Henceforth, data would be added for statistical verification of these results.

Keywords: external ear, sex determination, isometrics, morphometrics, angular difference

ABSTRACTS OF THE FIFTH DAYS OF HUMAN GENETICS – PROF. LJILJANA ZERGOLLERN-ČUPAK

10th ISABS Conference on Forensic and Anthropologic Genetics
and Mayo Clinic Lectures in Individualized Medicine
June 19-24, 2017, Dubrovnik, Croatia

PHYLIPS WORKSHOP

ASSESSMENT OF INFORMATION FLOW AND BIOLOGICAL PATHWAY DYSREGULATION IN CANCER USING MULTI-OMICS DATA

Nevenka Dimitrova

Philips Research North America, Cambridge, Massachusetts, USA

Here we present a method that estimates patient-specific biological pathway interaction activities using genome-wide, multi-modality molecular measurements. The estimates of the pathway interaction activities are modeled as units of information flow within the biological network that capture functional changes in tumors associated with prognosis and therapy response. Curated pathway models were obtained from NCI-PID, Pathway Commons and KEGG. Our pathway modeling framework focused on modeling regulatory interactions where two or more gene products interact to effect either a transcriptional or post-translational change in another gene/protein. Discrete modalities of gene expression and copy number data along with the curated pathway models were used to estimate the activities of all interactions in the pathways per tumor sample. Full joint-distribution of all interaction activities in a given pathway and tumor sample were estimated by a rejection sampling strategy that identified differential expression and copy-number estimates compared to normal samples. We used copy number variation and gene expression data as well as and platinum-free survival data from 380 ovarian cancer patients the Cancer Genome Atlas. Our goal was to identify pathways associated with poor prognosis. In a two-loop cross-validation setting, InFlo showed disregulations in interleukin and FOXM1 pathways were consistently associated with ER positivity in the breast cancer dataset, thus recapitulating the downstream modulation of FOXM1 pathway by estrogen receptor signaling. InFlo showed higher outer-loop specificity in identifying pathways associated with ER-positivity when compared with PathOlogist, a previously published pathway modeling framework. In ovarian cancer, InFlo found PI3K pathway interactions were associated with platinum free survival ($p \leq 0.002$) with down-regulation specific interactions in the PI3K pathway being associated with poor response to platinum-based chemotherapy. This association was not evident from direct gene expression or copy-number analysis.

DIGITAL TRANSFORMATION IN HEALTH

Jeoren Tas

Royal Philips N.V.

Not before were healthcare systems challenged the way they are today. The world population is aging and many people today are living with one or more chronic conditions. As demand for care is increasing, there is growing shortage of qualified caregivers and budgets are increasingly under pressure. At the same time new digital, connected and cloud technologies are emerging rapidly offering major opportunities to caregivers and governments to fundamentally transform the way healthcare is delivered. As our world has become digital the amount and granularity of the medical information we have available has increased exponentially. With all this data we are able to gain deeper, denser and more longitudinal insights of patients than ever before. Yet, the true value does not lie in the amounts of data we have at hand. It lies in combining this information, creating comprehensive contextual insights that support care professionals to deliver first-time-right precision medicine and bridge high quality continuous care from hospitals to homes. Jeroen Tas, Chief Innovation & Strategy Officer at Philips, will share his views on how the latest industry trends are fundamentally disrupting the health technology landscape and offer unprecedented opportunities to spur science, research and innovation in genomics, precision diagnostics and population health management. Over the past decade Philips transformed itself into a focused health technology company and is working in close collaboration with leading clinical and technology partners to enable new models of healthcare delivery.

SHORT BIOGRAPHIES OF INVITED LECTURES

Amélie Bonnefond (PhD) is currently a senior research scientist in the lab headed by Philippe Froguel in Lille (France). The (short) scientific career of Amélie Bonnefond has been focused so far on the dissection of the genetic etiologies of type 2 diabetes (T2D) and obesity, through integrative studies combining large-scale or focused human genetic studies and functional investigations (cell/animal models), towards a better stratification of patients, and subsequent precision medicine. To date, she has published more than 90 Pubmed indexed papers (including articles published in Nature, Nature Genetics, Cell Metabolism and JCI). Her current H-index is 32. In 2012, she received the Rising Star award from the European Association for the Study of Diabetes (EASD). Recently, she has been recipient of the Starting Grant Award from the European Research Council (ERC).

Wojciech Branicki is a professor of biological sciences and forensic DNA expert. Currently Wojciech Branicki is leading a research group at Małopolska Centre of Biotechnology of the Jagiellonian University in Krakow and has a position of forensic DNA expert at the Central Forensic Laboratory of the Police in Warsaw. His major area of interest is exploring the significance of DNA variation for phenotypic diversity observed in the human population. He works on the practical application of DNA markers in forensic genetics and anthropology. His research work led to the development of prediction models for human appearance traits and age. He is also involved in education at the Jagiellonian University lecturing in genetics and forensic genetics. Wojciech Branicki is a member of the International Society for Forensic Genetics, Polish Society of Human Genetics and the Forensic Genetics Commission of the Polish Society of Legal Medicine and Criminology.

Dr. Bruce Budowle is the Director of the Center for Human Identification. His current efforts focus on the areas of human forensic identification, microbial forensics, molecular biology, massively parallel sequencing, and emerging infectious disease. Some of Dr. Budowle's efforts also are in counter terrorism, including identification of victims from mass disasters and in efforts involving microbial forensics and bioterrorism. Dr. Budowle was an advisor to New York State in the effort to identify the victims from the WTC attack. In the area of microbial forensics, Dr. Budowle has been the chair of the Scientific Working Group on Microbial Genetics and Forensics, served on the Steering Committee for the Colloquium on Microbial Forensics, an organizer of four Microbial Forensics Meetings held at The Banbury Center in the Cold Spring Harbor Laboratory, and on steering committees for NAS sponsored meetings. He has published approximately 595 articles, made more than 720 presentations (many of which were as an invited speaker at national and international meetings), and testified in well over 250 criminal cases in the areas of molecular biology, population genetics, statistics, quality assurance, and forensic biology.

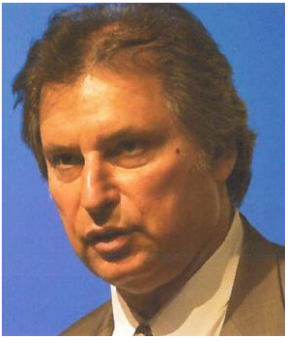
Dr. **Cassandra Calloway** is an Assistant Scientist at Children's Hospital Oakland Research Institute and Assistant Professor in the Department of Pediatrics in the School of Medicine at the University of California, San Francisco. She is also the DNA Program Coordinator for the Forensic Science Graduate Group and Assistant Adjunct Professor at the University of California, Davis. Dr. Calloway is a leader in the field of Forensic Genetics and over the last 18 years her research has primarily focused on the development of new genotyping assays for analysis of mtDNA and nuclear DNA markers for forensic human identification and clinical genetic applications. Currently, Dr. Calloway is leading an effort to develop sensitive methods for analysis of the entire mitochondrial genome and select nuclear SNP markers in degraded and mixed DNA samples using next-generation sequencing (NGS) with funding through the National Institute of Justice (NIJ). Dr. Calloway led the effort to develop commercial kits for analysis of mtDNA, including the first commercial forensic mtDNA kit in 2001. She is also leading an effort to develop a probe capture NGS assay for noninvasive prenatal testing of beta-hemoglobinopathies.

Jiri Damborsky is the Josef Loschmidt Chair Professor of Chemistry and Professor of Biochemistry at the Faculty of Science at Masaryk University in Brno, Czech Republic and a group leader at the International Centre for Clinical Research. Research of his group focuses on protein and metabolic engineering. His group develops new concepts and software tools for protein engineering (CAVER, CAVERDOCK, HOTSPOT WIZARD, PREDICTSNP, FIREPROT, CALFITTER, CAVETTA), and uses them for the rational design of enzymes. He has published >180 original articles, 15 book chapters and filed 6 international patents. He is a co-founder of the first biotechnology spin-off from Masaryk University Enantis Ltd. Among the awards and distinctions he has received is the award EMBO/HHMI Scientist of the European Molecular Biology Organisation and the Howard Hughes Medical Institute.

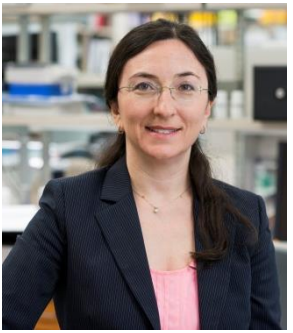
Peter de Knijff is full professor in population genetics and evolutionary genetics at the Leiden University Medical Center (LUMC). His main research lines are the development and use of polymorphic markers on the human Y chromosome and fundamental population genetic and evolutionary genetics. Between 2008 and 2014 he was the scientific director of the Forensic Genomics Consortium Netherlands (FGCN); the strategic alliance of the Netherlands Forensic Institute (NFI), the Forensic Laboratory for DNA Research of Leiden University Medical Center (FLDO - LUMC), and the Department for Forensic Molecular Biology of the Erasmus Medical Center, (FMB-EMC) with the aim to substantially improve routine forensic genetic research. In December 2015 his lab was the first forensic laboratory worldwide to receive formal ISO 17025 accreditation to use MPS for forensic case-work. In addition he is also actively involved explaining (forensic) science to the general public, students, and criminal justice professionals. He does this by means of (popular) lectures, courses, contributions to books and journals written in Dutch, news-paper articles, and interviews for radio. He also regularly appears in court as a forensic DNA expert.

Dr. **Jan van Deursen** received his Ph.D. in Cell Biology at the University of Nijmegen, the Netherlands in 1993. He started his own lab at St Jude's Children's Research Hospital in 1996. He joined Mayo Clinic in 1999, where he is currently a Professor of Biochemistry/Molecular Biology and Pediatrics at Mayo Clinic, Rochester. He is the Vita Valley Named Professor of Cellular Senescence and directs the Senescence program of the Robert and Arlene Kogod Center on Aging, the Cell Biology program of the Mayo Clinic Comprehensive Cancer Center, and the Mayo Clinic Gene Knockout and Transgenic Core Facility. Beginning in early 2012, he is also the Chair of the Biochemistry/Molecular Biology Department.

Nevenka Dimitrova, Ph.D., has been at Philips since 1995. She is the Chief Technology Officer of Genome Informatics at Philips. With her technology passion and intrapreneurial mind, she has been in diverse areas of signal processing, cognition, genomics, healthcare informatics. She believes that innovation happens at the confluence of diverse disciplines and cultures, and has led collaborations with with research institutes and companies. Her bibliography contains over 130 scientific articles and 50 issued patents. Recently she has been given the Gilles Holst award – the highest science and technology peer recognition prize at Philips.



Dr. **Henry Erlich** is currently a Senior Scientist at Children's Hospital Oakland Research Institute. His research has focused on the study of genetic variation in human populations and on the development and application of new genotyping technologies and analytic methods to the genetics of common complex disease, forensics, evolution, and population genetics. Dr Erlich co-developed the polymerase chain reaction (PCR) in the mid '80s and applied it to analyze the host genetics of a variety of autoimmune diseases. In addition, Dr Erlich has contributed to the understanding of the genetics of infectious disease and cervical cancer and pioneered the development of DNA-based HLA typing for clinical applications in transplantation, pharmacogenetics and disease susceptibility. In the past decade, Dr Erlich's lab has applied next generation DNA sequencing (NGS) to the analysis of the highly polymorphic genes (HLA and KIR) that play a key role in adaptive and innate immunity, as well as to forensics, focusing on mitochondrial DNA for analysis of mixed samples and to developing a non-invasive prenatal test for sickle cell anemia and beta-thalassemia.



Nilüfer Ertekin-Taner, MD, PhD is a Professor of Neurology and Professor of Neuroscience at the Mayo Clinic, Jacksonville, FL, USA. She is a neurogeneticist and board-certified behavioural neurologist. Dr. Ertekin-Taner's laboratory aims to discover and characterize genetic factors underlying the complex genetics of Alzheimer's disease (AD) and related neurodegenerative conditions. Dr. Ertekin-Taner is the Principal Investigator (PI) of numerous NIH and foundation grants. She leads multiple collaborative projects aimed at gene and pathway discoveries in AD and other neurodegenerative diseases, as part of the NIH initiatives Accelerating Medicines Partnership Alzheimer's Disease (AMP-AD) and Molecular Mechanisms of the Vascular Etiology of AD (M2OVE-AD) consortia. Dr. Ertekin-Taner is the PI of the Florida Consortium for African-American Alzheimer's Disease Studies (FCA3DS). As a neurologist in the Memory Disorders Clinic at Mayo Clinic in Jacksonville, Florida, Dr. Ertekin-Taner continues to evaluate and manage patients with AD and other dementias and aspires to improve the quality of care that we provide to our patients and their families.

Magnus Essand has published seventy original peer-reviewed scientific articles and eleven peer-reviewed overview articles. He has tutored ten PhDs to completion of their PhD theses and is currently the main supervisor of three PhD students and four postdocs. Essand is a scientific reviewer for many international journals and funding agencies. The research in Essand's group mainly concerns development and advancements of translational cancer immunotherapies, with the focus on oncolytic viruses, chimeric antigen receptor (CAR) T-cells and dendritic cell-based vaccines. Most of the work is preclinical and based on mouse models but two oncolytic viruses developed in Essand's laboratory are now being evaluated in clinical phase I trials for neuroendocrine cancer and prostate cancer. Essand is also involved in a CAR T-cell trial for lymphoma and leukemia patients and a new CAR T-cell expansion protocol developed in his laboratory is about to be evaluated in an upcoming clinical trial.

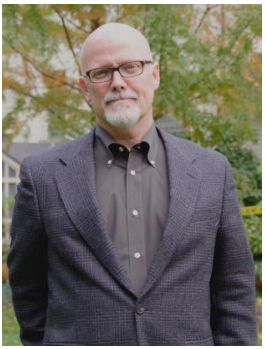
Dr. Gianrico Farrugia is CEO of Mayo Clinic in Florida and Vice President of Mayo Clinic. He is also a consultant in the Division of Gastroenterology and Hepatology and the Department of Physiology and Biomedical Engineering, as well as a professor of Medicine and Physiology in the Mayo Clinic College of Medicine. He recently served as the director of the Mayo Clinic Center for Individualized Medicine with a focus on integrating genomics into routine clinical practice. Dr. Farrugia's research interests include genomics and the treatment of disorders of gastrointestinal motility. He has published more than 250 articles on these topics. His research is funded by the National Institutes of Health through the National Institute of Diabetes and Digestive and Kidney Diseases, among other organizations. Dr. Farrugia has received many honors throughout his career, including top teacher awards, Mayo Clinic Research Career Achievement Award and the Janssen Award for Outstanding Science in Gastroenterology.

William A. Faubion, M.D. is currently Professor of Medicine, Immunology, and Pediatrics at Mayo Clinic Rochester. He is the only board certified pediatric and adult gastroenterologist with advanced training in both clinical Inflammatory Bowel Disease (Bill Sandborn, Mayo Clinic 1999-2000) and basic immunology (Cox Terhorst, Harvard MS, 2001-2003). At Mayo Clinic, Dr. Faubion is Chair of Research within the GI division, director of the Pediatric IBD Program, the IBD Translational Research Program, and the GI T32 training grant. He has chaired the steering committee for a Helmsley foundation funded consortium of translational IBD centers representing Mayo Clinic, Massachusetts General, UNC, University of Chicago, Washington University, Mt. Sinai, and Cedars Sinai. The quality, originality, and importance of his team's research is supported by his publication record (100 publications, H index 32, i10 index 57) in high quality journals (i.e. Gastroenterology, CGH, JBC, CMGH, and JI) and research portfolio (federal grants [R01 PI, R01 Co-I], foundation grants and industry grants). As recognition of this stable research portfolio, he is currently a standing member of the GMPB NIDDK study section and the CCFA grant review committee.

After his PhD on 'Ancient DNA from Europe's first farmers' in 2006, **Wolfgang Haak** 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 April 2015 he is leading the 'Molecular Anthropology' group at the newly founded Max-Planck Institute for the Science of Human History in Jena, Germany. His works is placed at the interface of human populations genetics, archaeology, anthropology, linguistics, and occasionally covers medical and forensic aspects. The main aim of his group is to investigate and evaluate ancient human genome-wide data in the light of data from neighboring disciplines to generate a detailed and comprehensive portrait of human prehistory over the last 20,000 years. The portfolio ranges from global outlooks on population affinities, migrations and past demography to intra-group relationships, and also encompasses the interaction with and response to changing environmental factors, such as climate, diet and disease.

Diana Hall received her PhD in Forensic Genetics from the Catholic University of Rome for her research on background linkage disequilibrium across human populations, carried out at the Department of Human Genetics of the University of Chicago. She continued her postdoctoral studies on disease mapping of complex traits at the Rockefeller University of New York. Since 2010, Diana Hall is responsible for research and development at the Forensic Genetics Unit of the University Hospital of Lausanne-Geneva. Diana's current research projects focus on novel molecular approaches for DNA mixture resolutions. The applications of her methods include forensic casework, non-invasive prenatal paternity testing, solid organ transplant monitoring and individual biogeographical ancestry inference

Zdenko Herceg is a molecular biologist with specialization in epigenetics and cancer research. He is currently Section and Group Head at International Agency for Research on Cancer - World Health Organisation (IARC-WHO, Lyon, France). Prior to joining IARC (1997), he was a post-doctoral scientist at the Research Institute of Molecular Pathology (IMP) in Vienna, Austria. He earned his Ph.D. (in 1995) from the University of St Andrews (UK) in the field of oncogenic transformation of human epithelial cells. He has authored more than 100 original peer-reviewed research publications in the field of epigenetics, mechanisms of carcinogenesis, and cancer research. He is principal investigator of several major international projects funded by European Union, NIH and Gates Foundation. Recent research projects aims at better understanding of epigenetic mechanisms in human cancer and providing critical information for the development of novel epigenetics-based strategies for cancer prevention and treatment.



Dr. **Mitch Holland** 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.

Robert Huber has been a member of the Max-Planck-Gesellschaft and Director at the Max-Planck-Institut für Biochemie until his retirement in 2005. Since 1976, he also serves at the TUM as a Professor. He holds appointments as Guest Professor at the Universität Duisburg-Essen (Germany), the Cardiff University (Great Britain), the Universidad Autonoma de Barcelona (Spain), and the Seoul National University (Korea). He serves as a member of the Board and/or Scientific Advisory Board of a number of pharmaceutical and crop science companies, and he is co-founder of two companies, Proteros and Suppremol, located in Martinsried and offering services for drug discovery and development and for the development of novel therapies for autoimmune diseases, respectively. Huber has made major contributions to the understanding of the structure and function of biological macromolecules. He has studied proteases and their natural and synthetic inhibitors, metalloenzymes (iron,

nickel, molybdenum, copper), proteins of the immune system (antibodies and antibody receptors), protein hormones and their receptors, protein kinases, enzymes of amino acid biosynthesis, enzymes of cofactor and vitamin biosynthesis and proteins of energy and electron transfer. He has been honoured by numerous honorary doctorates, professorships, memberships in learned societies and awards, including the Otto-Warburg Medal, the Emil von Behring Medal, the Sir Hans Krebs Medal, the The Linus Pauling Medal, Max Tishler Prize and, in 1988, the Nobel Prize for Chemistry together with H. Michel and J. Deisenhofer.

Dr. **Mattias Jakobsson** 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. Dr. 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. Dr. Jakobsson is a Wallenberg Academy Fellow and supported by the European Research Council.

Manfred Kayser currently is Professor of Forensic Molecular Biology and Head of the Department of Genetic Identification (formerly known as Dept. of Forensic Molecular Biology) at Erasmus University Medical Center Rotterdam. He received his diploma in biology from University of Leipzig in 1994, his Ph.D. in biology/genetics with summa cum laude from Humboldt University Berlin in 1998, and his habilitation in genetics from University of Leipzig in 2004. After postdoctoral research at the Department of Anthropology, Pennsylvania State University, he was staff scientist and later Heisenberg Fellow of the German Research Council, at the Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology Leipzig, before his full professorship appointment at Erasmus University in 2004. His research interest is in various aspects of forensic and anthropological genetics. In the forensic genetics world, he is well known for the introduction and further development of forensic Y-chromosome analysis, and his pioneering work on establishing Forensic DNA Phenotyping as new subfield. He (co)authored >225 articles in peer-reviewed scientific journals, books, and encyclopedias (H factor 55), serves as academic editor, editorial board member, and ad hoc reviewer for various scientific journal, and regularly accepts invitations to present at international conferences and institutes worldwide.

Manolis Kellis 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.

Turi King started her career reading Archaeology and Anthropology at the University of Cambridge before moving into the field of genetics at the University of Leicester. Her research combines genetics with history, archaeology, forensics and geography. Projects include the examining the link between the Y chromosome and inherited surnames in Britain and elsewhere, the genetic legacy of the Vikings in the north of England and norther France and the Romany in Britain. Given her background in archaeology, her work has expanded to include work on ancient remains such as the Norton Priory remains and those from Stirling Castle. She is perhaps best known for her work leading the genetic analysis in the King Richard III case.

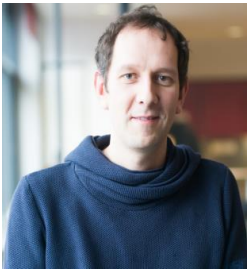
Eric W. Klee, Ph.D., is a senior associate consultant II-research in the Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, with joint appointments in the Department of Medical Genetics and in the Division of Laboratory Genetics, Department of Laboratory Medicine and Pathology. He serves as director of the Bioinformatics Core, Department of Laboratory Medicine and Pathology, and associate director of Bioinformatics, Mayo Clinic Center for Individualized Medicine. Dr. Klee holds the academic rank of assistant professor of medical informatics, Mayo Clinic College of Medicine. Dr. Klee's research focuses primarily on two major areas. First, with the Center for Individualized Medicine and the Department of Laboratory Medicine and Pathology, he leads the bioinformatics initiative centered on discovering how clinicians can apply information gathered from molecular-level data to diagnose and treat individual medical conditions. Second, Dr. Klee is a member of the Mayo Addiction Research Center, where he leads a laboratory team using zebrafish as models to identify novel therapeutic strategies for treating alcohol abuse and tobacco dependence. He consistently publishes in high-impact scientific journals and is frequently invited to give presentations on his research. He has authored numerous journal articles, abstracts and other written publications

Dr. **Michael S. Kobor** is a Professor in the Department of Medical Genetics at the University of British Columbia (UBC) in Canada, and a Senior Scientist at the Centre for Molecular Medicine and Therapeutics in the BC Children's Hospital. He was recently appointed as the Lead for the "Healthy Starts" Theme at BC Children's Hospital. Dr. Kobor also serves as the Director of the Program on Social Epigenetics at the Human Early Learning Partnership (HELP) at UBC's School of Population and Public Health. Dr. Kobor's own research program is focused on illuminating the developmental origins of health and disease. Building upon deep expertise in gene regulation and epigenetics developed over the course of his career, Dr. Kobor's translational research in human populations is taking a life course approach to understand human health. Dr. Kobor holds the Sunny Hill BC Leadership Chair in Child Development, the Canada Research Chair in Social Epigenetics and is a Senior Fellow of the Canadian Institute for Advanced Research (CIFAR) Child and Brain Development Program.

Gordan Lauc is a Professor of Biochemistry and Molecular Biology at the University of Zagreb, Honorary Professor at the University of Edinburgh and Kings College London, and member of the Johns Hopkins Society of Scholars. He graduated molecular biology at the University of Zagreb in 1992, and obtained PhD in Biochemistry and the University of Zagreb in 1995. He got his postdoctoral training at the Institute for Medical Physics and Biophysics in Münster and Johns Hopkins University in Baltimore. Dr. Lauc is author of over 150 research papers and eight international patents, which were cited over 1,800 times. His laboratory performed the first large scale studies of the human plasma glycome (in 2009) and human IgG glycome (in 2011), which were the basis for the subsequent first GWAS of the human plasma and IgG glycomes, as well as numerous large clinical and epidemiological studies. He is a currently president of the International Glycoscience Organisation, Board Member of the Genos Glycoscience Research Laboratory and Director of the National Centre of Excellence in Personalized Healthcare.

Jessica Metcalf brings together the fields of evolutionary biology, microbial ecology, and microbiome science to study the interactions between microbes and animals during life and after death. She leads innovative microbiome research projects that span the fields of medicine and forensics by combining experimental ecology, large genomic datasets, and bioinformatics tools.

Dr. **Henry Lee** 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 for New Haven Police department, Expert Advisor for China National Chief Prosecutor's Office.



Matthias Meyer is a biochemist and head of the "advanced DNA sequencing techniques" group at the Max-Planck-Institute for Evolutionary Anthropology in Leipzig, Germany. He has developed many methods that improve the scope of DNA sequencing in evolutionary studies, a work that has led to the generation of the first high-quality genome sequences from archaic humans as well as the recovery of the oldest DNA sequences known to date from fossils discovered outside the permafrost.

Rasmus Nielsen is currently a Professor of Computational Biology in the Department of Integrative Biology and the Department of Statistics at UC Berkeley. He works on statistical and population genetic analyses of genomic data, in particular methods for detecting natural selection, describing population genetic variation, inferring demography, and methods for association mapping. Much of his current research concerns statistical analyses of Next-Generation Sequencing (NGS) data, both in the context of medical genetics and population genetics. Many of the methods he has developed are heavily used by other researchers, including the phylogeny based methods for detecting positive selection implemented in PAML, the methods for inferring demographic histories implemented in the IM and IMa programs, the method for detecting selective sweeps implemented in SweepFinder, and the methods for analysing NGS data implemented in ANGSD. He has published >200 peer reviewed papers, invited book chapters and review papers (including 32 in Science or Nature) with a total H-index of 101, and many of these papers focus on methods development and theory. However, much of his recent research has also focused on the application of evolutionary genetics for understanding molecular function, for example for understanding the genetic basis of the regulation of haemoglobin concentration in high-altitude adapted populations or diet and cold adaptation in the Inuit of Greenland.

Dr. **Tamas Ordog** is Consultant and Associate Professor in the Department of Physiology and Biomedical Engineering and the Division of Gastroenterology and Hepatology at the Mayo Clinic, Rochester, Minnesota. He is also founding Director of the Epigenomics Program of the Mayo Clinic Center for Individualized Medicine. Dr. Ordog has published 85 peer-reviewed papers and book chapters and mentored 55 postdoctoral fellows, undergraduate and graduate students, medical students, residents, junior faculty and research staff. His research has been continuously funded by the National Institute of Health since 2002. Dr. Ordog received the Masters Award in Gastroenterology from the American Gastroenterological Association Institute, PriCara and Esai in 2008 and Honorary Membership and Géza Hetényi Medal from the Hungarian Gastroenterological Association in 2010. He has served on numerous National Institutes of Health study sections. His current research interests focus on epigenetic control of cellular phenotypes in the lineage of gastrointestinal pacemaker and neuromodulator cells in diabetes, aging, caloric restriction and oncogenesis and in response to targeted oncolytic therapy; transcriptional and epigenetic regulation of nitric oxide biosynthesis in enteric neurons; as well as on the development of high-throughput/ultralow-input epigenetic technology. As Director of the Mayo Clinic CIM Epigenomics Program, he is leading the effort to implement epigenomic testing of cancer patients in the Individualized Medicine Clinic.

Professor **Timothy Palmbach** joined the faculty of the University of New Haven in 2004 and is Chair of the Forensic Science Department. Professor Palmbach is founder of Center for Forensic Investigation of Trafficking in Persons at UNH. This center is committed to providing research, training, and investigative assistance to government agencies in the US and abroad. Since 2013 Professor Palmbach has been engaged with the implementation of advanced forensic investigative methods in the war against trafficking in persons (TIP) and related issues such as counter terrorism. He worked in conjunction with non-governmental organizations and government officials to actually employ the collection of DNA based evidence. His international work in this area includes the countries of Nepal, Costa Rica, Poland, Italy, Croatia, Bosnia, Djibouti, and Jordan. Throughout his 34-year career Timothy Palmbach has used his expertise in forensic science and investigations for matters of international interest, human rights, and counter terrorism.

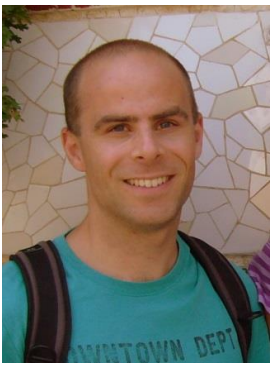
Walther Parson holds an associate professorship at the Institute of Legal Medicine, Innsbruck, Austria and an adjunct professorship at PennState, PA, USA. He set up the Austrian National DNA Database Lab in the late Nineties and is currently overseeing the High Through-put DNA Database Laboratory and Forensic Molecular Biology Research in Innsbruck. He received international scientific prizes and serves on various international boards (EDNAP, ENFSI, EAFS, ICMP). He is the current president of the International Society for Forensic Genetics (ISFG). WP leads an active group of researchers and has authored more than 300 peer-reviewed original articles. He is curating the forensically relevant databases EMPOP (www.empop.online) and STRidER

(www.strider.online). He was repeatedly consigned to handle international requests on DNA fingerprinting of historic persons (Russian Tsar family, Mozart, Schiller) and mass fatalities (Chile, Mexico). His current research is focussing on Massively Parallel Sequencing techniques.

Professor **Dieter Saur** is a Consultant and Senior Group Leader at Technische Universität München (TUM), School of Medicine. His lab investigates fundamental aspects of cancer biology using next-generation small and large animal models and imaging strategies and applies this knowledge towards the development of innovative therapeutic and diagnostic strategies in the clinic. His research focuses on how oncogenic signaling networks, tissue damage, the tumor microenvironment and inflammatory pathways contribute to tumor initiation, progression, metastasis and treatment resistance using novel advanced animal models that faithfully recapitulate human cancer. His lab translates this knowledge into novel and effective methods for early tumor detection, cancer subtype specific therapies as well as response prediction strategies. Prof. Saur completed a medical residency and a medical gastroenterology (GI) and GI oncology fellowship at TUM in 2006. Since 2007 he is a Consultant in Gastroenterology, Hepatology, GI Oncology and Endoscopy and a Senior Clinical Lecturer in Internal Medicine. His clinical interests include translational oncology and imaging studies to improve early detection and treatment response of GI cancer. His clinical practice is at the University Hospital Klinikum rechts der Isar in Munich.

Jeroen Tas is the Chief Innovation & Strategy Officer since 2017, driving innovations in smart systems, software and services to improve people's health, embedding artificial intelligence and the Internet of Things. Jeroen has long been a highly respected thought leader. He was responsible for turning around our healthcare IT business and has been instrumental in establishing HealthSuite as the new open industry standard for the 'Healthcare Internet of Things' Cloud platform. He co-founded and served as President, COO and vice-chairman of the board for MphasiS and prior to that he was the head of Transaction Technology, Inc., Citigroup's tech lab, responsible for the innovation and development of the bank's customer-facing systems, including Internet banking and self-service devices. Jeroen is the 2004 winner of the E&Y Entrepreneur of the Year Award in the Information technology category for the New York region. He also won the Dutch CIO of the year 2013 Award, NASSCOM Global CIO Award 2014, the World Innovation Congress 2014 CIO Leadership Award, CIO Net European CIO of 2014 Award, the IT Executive 2014 Award and the Accenture 2015 Innovator of the Year award. He holds a Master's Degree in computer science and business administration from the VU University, Amsterdam.

Chris Tyler-Smith is a team leader at The Wellcome Trust Sanger Institute in the UK. His team, Human Evolution, generates data on worldwide genetic variation in humans and closely-related species such as gorillas and chimpanzees, and uses this information to investigate the human past. They are interested in the way humans have spread around the world, diverged, mixed, and also adapted to their environments throughout prehistoric and historic times. They have contributed to large international projects such as the 1000 Genomes Project and African Genome Variation Project. Their current focus is on understanding genetic variation in Africa more thoroughly, as well as variation in other parts of the world including the Middle East, the Himalayas, the Pacific and the Americas. They also want to understand the functional consequences of genetic variants, including knockouts of human genes in healthy people, and advantageous human variants, which we model in mice.



Dr. **Elmar Tobi** his main focus has been the mediating role of epigenetic marks in the well-described relationship between the prenatal environment and adult health, working in the epigenetic epidemiology group of Dr. Bas Heijmans (Leiden University Medical Center). In 2008 he was joint first author on the paper that was first to described an association between a transient prenatal exposure and adult DNA methylation levels in humans (Heijmans &Tobi et al. PNAS 2008). With the help of the Dutch Hunger Winter Families study he showed that, analogues to animal experiments, the epigenome of humans is especially sensitive to the environment during the first stages of human development. Beside his work on human cohort studies, he has a strong interest in developing methodology, both in and outside the laboratory. He was part of the beta testing team of the epiTYPER technology to measure DNA methylation of candidate regions (Suchiman et al. Frontiers in Genetics, 2015) and helped to characterize statistical methodology and build tools to process and analyze DNA methylation data (van Itersson et al. Bioinformatics 2014, Tobi et al. Nature Comm. 2014). In close collaboration with evolutionary biologists at Wageningen University (Prof. Zwaan, Dr. van den Heuvel) and Lund University (Prof. Uller) he is working on a novel hypothesis to explain the DNA methylation patterns associated with environmental exposures during early gestation. In 2016 he received a grant within the talent scheme program of the Dutch government to set up a novel model to work on gestational diabetes and its profound influence on child metabolic health.

George Vazmastis

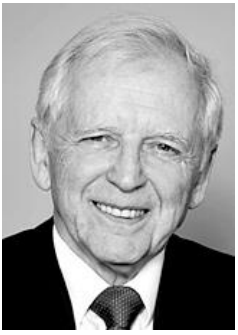
Eric D. Wieben, Ph.D. is the Director of the Medical Genome Facility and co-Director of the Clinomics Program within the Center for Individualized Medicine at the Mayo Clinic. Dr. Wieben earned a B.S. degree in Biological Sciences with highest distinction at Indiana University and a Ph.D. in Biology from Yale University. He has academic appointments in the Department of Biochemistry and Molecular Biology (a department he chaired for 7 years before assuming his present roles) and the Department of Clinical Genomics. He is the Mayo Project Leader for the Minnesota Partnership in Biotechnology and Medical Genomics, a unique state-funded research collaboration between the Mayo Clinic and the University of Minnesota supporting research innovation in the medical sciences. Dr. Wieben also directs the Office of External Research collaborations at Mayo, managing collaborations with major research centers around the world. His research interests are centered on the application of genomic technologies to improve the prediction, prevention, diagnosis and treatment of human disease. Much of his current work focuses on the diagnosis, pathogenesis, and treatment of inherited rare disease, with a particular focus on trinucleotide repeat expansions in hereditary eye disease.

Eske Willerslev is the Prince Philip Chair in Ecology and Evolution at Department of Zoology, University of Cambridge. He is also Lundbeck Foundation Professorship at University of Copenhagen and is the director for Centre of Excellence in GeoGenetics. Willerslev is an evolutionary geneticist recognized for his studies on human evolution and dispersal, microbial long-term survival and evolution, megafaunal extinctions, DNA degradation, and environmental DNA. He is particularly known for sequencing the first ancient human genome and establishing the field of environmental DNA, where modern and ancient DNA from organisms such as higher plants and animals are obtained directly from environmental samples such as sediments, ice and water. At the age of 33, Willerslev became Full Professor at University of Copenhagen - the youngest in Denmark at the time. Willerslev has been visiting researcher at the MD Anderson Cancer Research Centre in Austin, Texas and independent Welcome Trust Fellow at Oxford. He is foreign associate of the National Academy of Sciences, member of the Royal Danish Academy of Sciences and Letters, horary doctor at University of Oslo and have been Visiting Professor at Oxford University and a Miller Visiting Professor at UC Berkeley. Willerslev is interested in processes forming contemporary human genetic diversity and distribution, the evolution of human diseases, and the processes underlying culture development, and in the impact of humans and climates on the environment through time including extinctions. He has more than 200 peer-reviewed papers, including more than 40 papers published in the journals Nature and Science.

Ada Yonath obtained her Ph.D. degree in X-ray crystallography at the Weizmann Institute of Science, Rehovot, Israel. As a postdoctoral student at Carnegie Mellon University and Massachusetts Institute of Technology she became interested in the structure of macromolecular assemblies, an interest she pursued following her return to the Weizmann where she has stayed ever since. At the Weizmann she established the first protein crystallography laboratory in the country, in which she later focused on the structural basis of protein biosynthesis, particularly ribosomes. Dr. Yonath pioneered the currently broadly used techniques for ribosome crystallization and biological crystallography at cryogenic temperatures that allowed the unprecedented insight into the structure of complex biological macromolecules. An important focus of her studies has been the mode of action of antibiotics that bind ribosomes that revealed the mechanisms of resistance to antibiotics and facilitated the structure based drug improvement and design. Currently Dr. Yonath holds the Martin S. Kimmel Professorial Chair at the Weizmann Institute where she directs the Mazer Center for Structural Biology and the Kimmelman Center for Biomolecular Structure and Assembly. For her contributions she received numerous prizes including the European Crystallography Prize, the Israel Prize, the Wolf Prize in Chemistry, the Paul Ehrlich and Ludwig Darmstaedter Prize, and the 2009 Nobel Prize in Chemistry (with Thomas Steitz and Venkatraman Ramakrishnan). Dr. Yonath is a member of the United States National Academy of Sciences, the American Academy of Arts and Sciences, the Israel Academy of Sciences and Humanities, the European Academy of Sciences and Art and the European Molecular Biology Organization. Dr. Yonath has been a frequent lecturer at the International School of Biophysics, a series of triennial events held in Croatia.

Zhang

Vlatka Zoldoš, PhD, graduated Biology at University of Zagreb, Faculty of Science, in 1992. She obtained PhD in Molecular Biology at the University of Paris-Sud XI and University of Zagreb in 2000 within the program of dual mentorships (thèse en cotutelle). Currently she is a professor at University of Zagreb and Head of the Division of Molecular Biology. In 2008, she established the Laboratory for Epigenetics at the Division of Molecular Biology, at the Biology Department, where she is pioneering the field of epigenetic regulation of protein glycosylation. Her team of 11 researchers participated in several FP7 and Horizon 2020 projects and is a part of the National Centre of Research Excellence in Personalised Healthcare and the National Centre of Competences in Personalized Medicine.



Prof. Dr. **Harald Zur Hausen** is a German virologist and was awarded half of the 2008 Nobel Prize for his discovery of the role of human papilloma viruses (HPV) in cervical cancer. Ultimately his work led to the introduction in 2006 of a vaccine to combat HPV. He became an assistant professor at the University of Pennsylvania but in 1969 returned to Germany to head a research team at the University of Würzburg, where he soon succeeded in proving that EBV DNA persists in every tumour cell in Burkitt's lymphoma. In 1972 he moved to the University of Erlangen-Nuremberg as chairman of the newly established Institute of Clinical Virology and began to examine the established theory that cervical cancer may be caused by a virus. In 1977 zur Hausen was appointed chairman of the Institute of Virology of the University of Freiburg. He took most of his team with him, including Lutz Gissmann and Ethel-Michele de Villiers, who would later become his wife. In 1979 they isolated the first DNA from genital warts, HPV-6. By 1983 they had reached HPV-16, which they discovered was present in around half of cervical cancer cases. The following year they isolated HPV-18, accounting for a further fifth of cases. Incredibly, pharmaceutical companies at first dismissed zur Hausen's suggestion of developing a cancer vaccine, one firm saying there was 'no market' for it. Fortunately this view later changed and the HPV vaccine became available in 2006. From 1983-2003 zur Hausen served as the Scientific Director of the German Cancer Research Centre. He acted as Editor-in-Chief of the International Journal of Cancer from 2000 to the end of 2009.