International Society for Applied Biological Sciences

PROGRAM AND ABSTRACTS

8th

ISABS Conference on Forensic, Anthropologic and Medical Genetics and Mayo Clinic Lectures in Translational Medicine

Split, Croatia, June 24 – 28, 2013

www.isabs.hr

CONFERENCE ORGANIZED BY:

International Society for Applied Biological Sciences (ISABS)

ORGANIZED UNDER THE AUSPICES OF:

Croatian Academy of Sciences and Arts

GENERAL SPONSOR:

OFFICIAL JOURNAL:

Croatian Medical Journal

CO-ORGANIZED BY:

PROGRAM AND ABSTRACTS

The Eighth ISABS Conference
in Forensic, Anthropologic and Medical Genetics
and Mayo Clinic Lectures in Translational Medicine

June 24—28, 2013, Split, Croatia

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PROGRAM AND ABSTRACTS

THE EIGHTH ISABS CONFERENCE IN FORENSIC, ANTHROPOLOGIC AND MEDICAL GENETICS AND MAYO CLINIC LECTURES IN TRANSLATIONAL MEDICINE

JUNE 24—28, 2013
Le Meridien Lav Hotel, Split,
CROATIA

www.isabs.hr info@isabs.hr

Le MERIDIEN

Grljevačka 2A, Podstrana
21312 Split

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WELCOME TO THE EIGHTH ISABS CONFERENCE IN FORENSIC, ANTHROPOLOGIC AND MEDICAL GENETICS AND MAYO CLINIC LECTURES IN TRANSLATIONAL MEDICINE!

The conference is next in the series of biennial events organized by the International Society for Applied Biological Sciences, a society dedicated to promotion of applied molecular biology (www.isabs.hr). This conference is organized with participation of Mayo Clinic, Penn State University, University of New Haven, George Washington University, Universities of Split, Osijek and Rijeka, Ruđer Bošković Institute, Institute of Anthropology, Genos Ltd, Croatian Society of Human Genetics, Croatian Forensic Association, Children's Hospital Srebrnjak, St. Catherine Hospital, Croatian Society of Human Genetics, Croatian Academy of Technical Sciences, Croatian Academy of Legal Sciences and the City of Split. 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 ninth 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.

This year we are pleased to introduce the up-to-date advances in genetics applied to the crossing of forensic science, anthropology and translational medicine. More than 500 participants and 80 lecturers including Nobel Laureates Aaron Ciechanover, Robert Huber and Ada Yonath will participate in the conference. Principal sponsors of the conference are Pliva, Inc., Belupo, Inc. and the Seventh Framework Program of the European Union.

As before, the conference is structured to allow close interaction of the international faculty and attendees. Together with formal presentations, there will be meet-the-professor sessions and other social occasions that are meant to enhance opportunities for scientific intercourse, but also to introduce the participants to the city of Split, the ancient maritime capital of Croatia and a vibrant modern Mediterranean city.

Enjoy!

Moses Schanfield, Dragan Primorac, Stanimir Vuk-Pavlović
Program/Conference Directors

ISABS CONFERENCES JOIN FORCES WITH THE FP7-INTEGRA-LIFE PROJECT

From the very beginning in 1997, ISABS Conferences have been the flagship of Croatian science, demonstrating that events of global importance can have their home in Croatia. Since the time of Ancient Rome, for nearly 2000 years, Croatia was on the forefront of science and education in Europe. However, after the end of World War I, the Treaty of Versailles exiled Croatia to the Balkans, with devastating consequences to its science and culture. After 95 years of exile, on July 1st, 2013, only three days after the conclusion of the 8th ISABS Conference, Croatia will "return home" as the 28th member state of the European Union.

Research and innovation are strategic objectives of the European Union, and the Framework Programme (Horizon 2020 in the future) is its main tool for their promotion. Among the programmes of the FP7 are the highly competitive Research Potential (REGPOT) projects aimed at supporting centres of excellence in the EU Convergence and Outermost regions. Integra-Life is one such Research Potential project whereby the European Commission will invest over 3,2 million Euros in the network of five research groups at the University of Zagreb.

The main objective of Integra-Life project is to functionally integrate and reinforce most promising research groups in molecular life sciences at the Faculty of Science and Faculty of Pharmacy and Biochemistry, University of Zagreb. All participating research groups have already demonstrated excellence in research and success in raising international research funds. Integra-Life will support these groups through coherent acquiring and upgrading of equipment, recruiting experienced researchers, and fostering exchange with 17 eminent European partnering organisations.

By teaming up with ISABS Conferences, Integra-Life hopes to improve the visibility of its researchers and enable them to initiate novel collaborations with leading researchers from Europe and the US.

Gordan Lauc
Coordinator, Integra-Life project

8TH ISABS CONFERENCE, SPLIT, CROATIA, JUNE 24-28, 2013

• *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.

• *Program/Conference Directors*

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Timothy Palmbach (University of New Haven, West Haven, CT, USA)
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Molecular and Cellular Medicine

Malcolm Brenner (Baylor College of Medicine, Houston, Texas, USA)
Aaron Ciechanover (Technion, Haifa, Israel)
Henry Erlich (Roche Molecular Systems, Inc., Alameda, CA, USA)
Robert Huber (Max Planck Institute for Biochemistry, Martinsried, Germany)
Doron Lancet (Weizmann Institute of Science, Rehovot, Israel)
Ada Yonath (Weizmann Institute of Science, Rehovot, Israel)
Andre Terzic (Mayo Clinic, Rochester, MN, USA)

Molecular Anthropology

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Richard Villems (University of Tartu and Estonian Biocentre, Tartu, Estonia)
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• *Conference Logistics*

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INTERNATIONAL SOCIETY FOR APPLIED BIOLOGICAL SCIENCES

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

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Anamarija Slović (Zagreb, Croatia)
Ante Vulić (Zagreb, Croatia)

RECIPIENTS OF THE YOUNG INVESTIGATOR AWARD

2013
- Matko Čančer, Sweden (Gene therapy)
- Dora Markulin and Branka Gršković, Croatia (Genome-based applications in forensic science)
- Slavé Petrovski, USA (Personalized genomics)
- Antoinette Westen, The Netherlands (Genome-based applications in forensic science)

2011
- Mark Barash, Australia (Forensic DNA phenotyping)
- Rebecca Just, USA (Genome-based applications in forensic science)
- Renato Polimanti, Italy (Molecular Anthropology)
- Martina Smolić, Croatia (Molecular therapy)

2009
- Chiara Barbieri, Germany (Molecular anthropology)
- Fernanda Toledo Gonçalves, Brasil (Individualized medicine)
- Pavlo Feliksovich Tatarskyy, Ukraine (Individualized medicine)
- Antoinette Westen, The Netherlands (Forensic genetics)

2007
- Kaye Ballantyne, Australia (Molecular anthropology)
- Grzegorz Kaczmarczyk, Poland (Forensic genetics)
- Tomislav Domazet-Lošo, Croatia (Molecular anthropology)
- Coralie Frassati, Switzerland (Molecular anthropology)
- Taeko Kashima, Japan (Molecular anthropology)
- Agnieszka Krzyzańska, Poland (Forensic genetics)

2005
- Tracy Johnson, USA (Forensic genetics)
- Mirela Baus Loncar, Germany (Molecular and cellular medicine)
- Vedrana Montana, USA (Molecular and cellular medicine)
- Caroline Round, United Kingdom (Forensic genetics)

2003
- Chiara Magri, Italy (Molecular and cellular medicine)
- Robert J. Shelton, CO, USA (Forensic genetics)

2001
- Rima Dada, India
- Katja Drobnič, Slovenia
- Anna Gareeva, Russia
- Nguyen Hoai Giang, Vietnam
- Tomasz Kupiec, Poland
- Lucia Cifuentes Ovalle, Chile

SCIENTIFIC PROGRAM INFORMATION

Certificate of Attendance will be issued at the registration desk.

Young Investigator Awards
The 2013 ISABS Young Investigator Award Committee reviewed all abstracts submitted for YIA and selected four 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 26 in the evening.

Credits
Croatian Medical Chamber has approved to award 20 points to participants or 30 points to lecturers of the 8th ISABS Conference in Forensic, Anthropologic and Medical 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 medical doctors in compliance with their national policy.

Sponsor Exhibition
Setup: June 24, 2013
Dismantling: June 28, 2013 by noon

Poster Setup
Monday, June 24, 2013
Tuesday, June 25, 2013 until noon
Poster board numbers can be found in the author index at the back of this brochure. Staff at the registration & info desk will help you locate the board.

Poster Discussion
Tuesday, June 25, 2013, 19:00 - 20:00
Thursday, June 27, 2013, 18:30 - 19:30
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 28, 2013, 08:00 - 12:00
Posters left on boards after noon on Friday will be discarded.

Meet the Professor
Tuesday, June 25, 2013, 19:00 - 21:00
Thursday, June 27, 2013, 18:30 - 20:30
This program is intended particularly for the students and the youngest members of the audience, but is available to all participants. To make speakers available to all, each speaker will be assigned a table so that participants can come freely and mingle with the speakers. Take advantage of the chance to meet some of the greatest scientists of our time. Remember, they are here for you!

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

Registration & Info Desk Hours

Sunday, June 23, 2013	18:00 - 20:00
Monday, June 24, 2013	08:00 - 20:00
Tuesday, June 25, 2013	08:00 - 16:00
Wednesday, June 26, 2013	08:00 - 16:00
Thursday, June 27, 2013	08:00 - 16:00
Friday, June 28, 2013	08:00 - 16:00

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 8th ISABS Conference in Forensic, Anthropologic and Medical Genetics and Mayo Clinic Lectures in Translational Medicine is declared as a non-smoking event.

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

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

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

GENERAL INFORMATION

Badges will be provided at registration to participants, accompanying persons, exhibitors and press. Badges are required for admission to all conference facilities, scientific and social events during the duration of 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). Approximate exchange rates are 1 EUR = 7,48 HRK and 1 USD = 5,60 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 bankcards and credit cards are located at numerous sites in Split.

Electricity Supply: 220-240 V, 50 Hz

Travel and Health Insurance: Participants are advised to make their own arrangements pertinent to health and travel. By registering for the 8th ISABS Conference in Forensic, Anthropologic and Medical Genetics and Mayo Clinic Lectures in Translational Medicine, participants agree that neither the organizers and their agents nor the sponsors and exhibitors nor the Le Meridien Lav hotel, Split assume any liability whatsoever.

Restaurants: Most restaurants in Split 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 Split 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 Split city centre and in front of hotels. All hotel staff will be glad to help you.

Hotel Information: Le Meridien Lav, amongst the largest of Split hotels, is a five star facility that features 365 modern rooms and 17 sea-view suite, perfect to accommodate a family or a group. Embark on a journey of coastal discovery, easily explore the imposing Dalmatian coast, admire scenic towns of Trogir, Omiš, Solin and Makarska as well as the nature parks of Biokovo and Krka - an hour drive from the hotel. Le Meridien's unique beachfront location invites guests to avail themselves of the inspirational panoramic views of the Adriatic sea.

INVITED SPEAKERS

• *Keynote Addresses*

Aaron Ciechanover (Nobel Prize in Chemistry 2004; Technion, Haifa, Israel)
Robert Huber (Nobel Prize in Chemistry 1988; Max-Planck-Institute, Martinsried, Germany)
Ada Yonath (Nobel Prize in Chemistry 2009; Weizmann Institute of Science, Rehovot, Israel)

• *Inaugural Plenary Session: Science and Public Security*

Zvia Agur (Institute for Medical BioMathematics, Bene Ataroth, Israel)
Christopher Asplen (Global Alliance for Rapid DNA Testing, Chalfont, Pennsylvania, USA): Future impact of rapid DNA technology on law enforcement
Isaac Ben-Israel (Tel Aviv University, Tel Aviv, Israel)
Henry Lee (University of New Haven, West Haven and Connecticut State Police, Meriden, Connecticut, USA)

• *Forensic Program*

Šimun Anđelinović (University of Split, Split, Croatia)
Kenneth Aschheim (New York University, New York, New York, USA)
Kaye Ballantyne (Victoria Police Forensic Services Department, Macleod, Victoria, Australia)
Corina Benschop (Netherlands Forensic Institute, The Hague, The Netherlands)
Frederick Bieber (Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts, USA)
Veronique Bourdon (Azur Génétique, Nice, France)
Zoran Budimlija (Office of Chief Medical Examiner, New York City, New York, USA)
Cassandra Calloway (Children's Hospital & Research Center Oakland, Oakland, California, USA)
Katja Drobnič (National Forensic Laboratory, Ljubljana, Slovenia)
Santo Davide Ferrara (University of Padua, Padua, Italy)
Benjamin Figura (Office of Chief Medical Examiner, New York, New York, USA)
Dan Frumkin (Nucleix, Tel Aviv, Israel)
Cordula Haas (Institute of Legal Medicine, University of Zürich, Zürich, Switzerland)
Mitchell Holland (Penn State University, University Park, Pennsylvania, USA)
Edwin Huffine (Bode Technology, Lorton, Virginia, USA)
Manfred Kayser (University of Rotterdam, Rotterdam, The Netherlands)
Peter de Knijff (University of Leiden, Leiden, The Netherlands)
Timothy Palmbach (University of New Haven, West Haven, Connecticut, USA)

Walther Parson (Institute of Legal Medicine, Innsbruck Medical University, Insbruck, Austria)
Dragan Primorac (University of Split and University of Osijek, Croatia; Penn State University and University of New Haven, USA)
Antti Sajantila (University of Helsinki, Helsinki, Finland)
Moses Schanfield (George Washington University, Washington, District of Columbia, USA)
Peter Vallone (National Institute of Standards and Technology, Gaithersburg, Maryland, USA)
Elisa Wurmbach (Office of Chief Medical Examiner, New York City, New York, USA)

• *Translational Medicine Program*

Chiara Bonini (San Raffaele Scientific Institute, Milan, Italy)
Malcolm Brenner (Baylor College of Medicine, Houston, Texas, USA)
Renier Brentjens (Memorial Sloan-Kettering Cancer Center, New York, New York, USA)
Robert Deans (Athersys Inc, Cleveland, Ohio, USA)
Henry Erlich (Roche Molecular Systems, Pleasanton, California, USA)
Arezou Ghazani (Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts, USA)
David Goldstein (Duke University, Durham, North Carolina, USA)
Eric Halioua (Promethera Biosciences, Mont-Saint-Guibert, Belgium)
Michael Jensen (Seattle Children's Hospital, Seattle, Washington, USA)
Moien Nihad Kanaan (Bethlehem University, Bethlehem, Palestine)
Doron Lancet (Weizmann Institute of Science, Rehovot, Israel)
Steven Moran (Mayo Clinic, Rochester, Minnesota, USA)
Carmen Perez-Terzic (Mayo Clinic, Rochester, Minnesota, USA)
Jef Pinxteren (ReGenesys, Heverlee, Belgium)
Franklyn Prendergast (Mayo Clinic, Rochester, Minnesota, USA)
Yoel Rak (Tel-Aviv University, Tel Aviv, Israel)
Dražen Raucher (University of Mississippi, Jackson, Mississippi, USA)
Isobel Scarisbrick (Mayo Clinic, Rochester, Minnesota, USA)
John Sinden (ReNeuron, Guildford, Surrey, United Kingdom)
Rafael Sierra (Mayo Clinic, Rochester, Minnesota, USA)
David I. Smith (Mayo Clinic, Rochester, Minnesota, USA)
Andre Terzic (Mayo Clinic, Rochester, Minnesota, USA)
Peter Underhill (Stanford University, Stanford, California, USA)
Richard Villems (University of Tartu and Estonian Biocentre, Tartu, Estonia)
Stanimir Vuk-Pavlović (Mayo Clinic, Rochester, Minnesota, USA)
Peter de Waele (Cardio3 Biosciences, Mont-Saint-Guibert, Belgium)
Scott Waldman (Thomas Jefferson University, Philadelphia, Pennsylvania, USA)
Raphael Zidovetzki (University of California Riverside, Riverside, California, USA)

• *Workshop by the Croatian Academy of Legal Sciences: Medicine and the Law-Medical Expertise and Admissibility of Evidence Obtained Through Analysis in Court Proceedings*

Davor Derenčinović (Faculty of Law, University of Zagreb, Zagreb, Croatia)
Zlata Đurđević (Faculty of Law, University of Zagreb, Zagreb, Croatia)
Peter Henning (Wayne State University Law School, Detroit, Michigan, USA)
Ivana Milas Klarić (Faculty of Law, University of Zagreb, Zagreb, Croatia)
Maja Munivrana Vajda (Faculty of Law, University of Zagreb, Zagreb, Croatia)
Sunčana Roksandić Vidlička (Faculty of Law, University of Zagreb, Zagreb, Croatia)

• *Workshop: Communication Skills in Science and Medicine*

Lovorka Brajković (School of Medicine, University of Zagreb, Zagreb, Croatia)
Marijana Braš (School of Medicine, University of Zagreb, Zagreb, Croatia)
Veljko Đorđević (School of Medicine, University of Zagreb, Zagreb, Croatia)

• *Workshop: Adapting Clinical Trials to Modern Cancer Therapy*

Zwi Berneman (University of Antwerp, Antwerp, Belgium)
Moran Elishmereni (Institute for Medical BioMathematics, Tel Aviv, Israel)
Mauro Gasparini (Politecnico di Torino, Torino, Italy)
Yuri Kogan (Institute for Medical BioMathematics, Bene Ataroth, Israel)
Manish Kohli (Mayo Clinic, Rochester, Minnesota, USA)

• *Workshop: Glycoscience in Personalized Medicine*

Harry Campbell (University of Edinburgh, Edinburgh, Scotland, United Kingdom)
Gordan Lauc (University of Zagreb, Zagreb, Croatia)
Falk Nimmerjahn (Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany)
Shin-Ichiro Nishimura (Hokkaido University, Sapporo, Japan)
Igor Rudan (University of Edinburgh, Edinburgh, Scotland, United Kingdom)
Tim Spector (Kings College London, London, England, United Kingdom)
Vlatka Zoldoš (University of Zagreb, Zagreb, Croatia)

SCIENTIFIC PROGRAM

• *Monday, June 24, 2013*

08:00 - 18:00 **Registration**
Poster setup

16:00 **First ISABS 2.5 km run and walk around Mt. Marjan**

20:00 **Welcome drink**

• *Tuesday, June 25, 2013*

08:00 - 18:00 **Registration**
08:00 - 11:00 **Poster setup**

08:30 **Directors' Inaugural Plenary Session**
Public Security: Forensic, Medical and Policy Aspects
08:30 ***Introduction from the chair***
08:35 **Zvia Agur** (Bene Ataroth, Israel): Can one theory guide both personalized medicine and national security policies?
09:10 **Christopher Asplen** (Chalfont, PA, USA): Future impact of rapid DNA technology on law enforcement
09:45 **Break**
10:00 **Opening Ceremony**
10:30 **Isaac Ben-Israel** (Tel Aviv, Israel): Forensics of cyber crime-The problem of attribution
11:05 **Henry Lee** (West Haven, CT, USA): Application of DNA evidence in public safety
11:40 **Discussion**
12:00 **Adjourn**

12:30 **Opening of Commercial Exhibits**

14:00 *Keynote Address*
Robert Huber (Martinsried, Germany): Protein structures at the interface of physics, chemistry and biology (Introduced by S. Vuk-Pavlović)

Parallel Scientific Sessions

T1: Genetically Engineered T Cells

15:00 ***Introduction from the chair:*** **Malcolm Brenner**
15:05 **Renier Brentjens** (New York, NY, USA): Moving CAR modified T cell therapy of cancer forward in the clinic

15:45 **Oral Presentation: Yuri Kogan** (Bene Ataroth, Israel): Reconsidering the paradigm of cancer immunotherapy by computationally aided real-time personalization
16:00 **Michael Jensen** (Seattle, WA, USA): Enhancing IQ of chimeric antigen receptor-redirected T cells
16:40 **Chiara Bonini** (Milan, Italy): TCR gene editing for treatment of hematological malignancies
17:20 **Oral Presentation: Diana Dudziak** (Erlangen, Germany): Antigen targeting to dendritic cells as therapeutic application in the fight of cancer
17:35 **Malcolm Brenner** (Houston, TX, USA): How to make T cells a standard of care for cancer?
18:15 **Discussion**

F1: Forensic Sciences in Disaster Management

15:00 ***Introduction from the chair:*** **Frederick Bieber**
15:05 **Zoran Budimlija** (New York, NY, USA): Mass disaster management: The science and the New York City experience
15:50 **Benjamin Figura** (New York, NY, USA): Anthropology and fingerprinting: New techniques for identification
16:25 **Oral Presentation: Frank Wendt** (University Park, PA, USA): Rapid human identification; evaluation of Rapidhit200
16:40 **Kenneth Aschheim** (New York, NY, USA): Forensic odontology in disaster victim identification: New technologies
17:15 **Elisa Wurmbach** (New York, NY, USA): Improved eye and skin color prediction based on eight SNPs
17:50 **Frederick Bieber** (Boston, MA, USA): Role of statistical genetics in mass disaster management
18:25 **Discussion**

19:00 ***Poster Session I***
Meet the Professor I

• *Wednesday, June 26, 2013*

Parallel Scientific Sessions

T2: Advances in Regenerative Medicine:
Experience at Mayo Clinic in Rochester, Minnesota, USA

08:00 ***Introduction from the chair:*** **Carmen Terzic**
08:05 **Stanimir Vuk-Pavlović**: Stemness arisen
08:30 **Andre Terzic**: Regenerative medicine: a new paradigm
09:05 **Carmen Terzic**: CXCR4 and FLK-1 identify circulating cells associated with improved cardiac function in patients following myocardial infarction

09:40 **Steven Moran**: Hand and face transplantation at Mayo Clinic
10:15 **Rafael Sierra**: Hip decompression by bone marrow concentrate in early osteonecrosis of femoral head
10:50 **Isobel Scarisbrick**: Pharmacological approach to spinal cord injury regeneration
11:25 **Discussion**

F2: NextGen Sequencing for Human Identification

08:00 ***Introduction from the chair:*** **Mitchell Holland**
08:05 **Mitchell Holland** (University Park, PA, USA): Forensic applications of NGS on454 LifeSciences GS Junior and Illumina MiSeq instruments
08:40 **Walther Parson** (Innsbruck, Austria): Forensic applications of NGS on LifeTechnologies Ion PGM instrument
09:15 **Peter Vallone** (Gaithersburg, MD, USA): Characterization of reference standards with NGS platforms
09:50 **Oral Presentation: Hallie Altshuler** (University park, PA, USA): Evaluation of direct PCR amplification using swabs and washing reagents
10:05 **Peter de Knijff** (Leiden, The Netherlands): Deep sequencing of 750 complete Dutch mtDNA genomes using NGS
10:40 **Oral Presentation: Nicola Oldroyd** (Essex, UK): Next Generation Sequencing methods for forensic analysis
10:55 **Corina Benschop** (The Hague, The Netherlands): Low template DNA analysis and interpretation
11:30 **Discussion**

W1: Academy of Croatian Legal Sciences Workshop

12:00 **Medicine and the Law: Medical Expertise and Admissibility of Evidence Obtained through DNA Analysis in Court Proceedings**

- ***Introduction from the chair:*** **Željko Horvatić** and **Davor Derenčinović**
- **Davor Derenčinović** (Zagreb, Croatia): International legal standards on the use of DNA analysis in the criminal justice system
- **Ivana Milas Klarić** (Zagreb, Croatia): Medical expertise in family law proceedings: From relativity to absolute values
- **Peter J. Henning** (Detroit, MI, USA): Legal issues for researchers and medical practitioners: it is not just the science anymore
- **Zlata Đurđević** ((Zagreb, Croatia): Use of information obtained by DNA analysis in criminal proceedings
- **Maja Munivrana Vajda** (Zagreb, Croatia) Criminal responsibility for disclosure of confidential medical information
- **Sunčana Roksandić Vidlička**, **Aleksandar Maršavelski** (Zagreb, Croatia): DNA and privacy protection through criminal law

14:00 *Keynote Address*
Aaron Ciechanover (Haifa, Israel): Revolution of personalized medicine: Will we cure all diseases and at what cost? (Introduced by M. Schanfield)

Parallel Scientific Sessions

T3: Captured Next-Gen Sequencing for Deciphering Rare Monogenic Diseases

15:00 ***Introduction from the chair:*** **Doron Lancet**
15:05 **David Goldstein** (Durham, NC, USA): Genome-wide identification of pathogenic mutations in patients with neurological and developmental disease
15:40 **Moien Kanaan** (Bethlehem, Palestine): Next Generation Sequencing studies of hereditary disorders in highly inbred populations
16:15 **Oral presentation: Vesna Boraska** (Cambridge, UK and Split, Croatia): WTCCC3 and GCAN: a genome-wide association study of anorexia nervosa
16:30 **Rafael Zidovetzki** (Riverside, CA, USA): Genetic scrutiny of systemic lupuserythematosus, a prototypic autoimmune disease
17:05 **David I. Smith** (Rochester, MN, USA): Next generation transcriptome analysis of human cancer
17:40 **Doron Lancet** (Rehovot, Israel): Biological tales of human disease mutations
18:15 **Discussion**

F3: Recent Advances in Forensic Molecular Biology

15:00 ***Introduction from the chair:*** **Manfred Kayser**
15:05 **Kaye Ballantyne** (Macleod, Vic., Australia): Improved forensic Y chromosome analysis
15:40 **Manfred Kayser** (Rotterdam, The Netherlands): Forensic DNA phenotyping
16:15 **Cordula Haas** (Zurich, Switzerland): Gene expression in forensic sciences
16:50 **Dan Frumkin** (Tel Aviv, Israel): Forensic epigenetics
17:25 **Dragan Primorac** (Osijek and Split, Croatia; University Park, PA and New Haven, CT, USA): Epigenetic predictors of age
18:00 **Antti Sajantilla** (Helsinki, Finland): Progress in forensic pharmacogenomics
18:35 **Discussion**

19:00 ***Sharing the Wealth of Personal Experience***
Robert Huber (Martinsried, Germany): How I became a scientist

20:30 ***Conference Reception and Dinner***
Young Investigator Awards Presentation

- **Matko Čančer** (Uppsala, Sweden): Oncolytic vaccinia virus expression disialoganglioside mimotope mediates strong cytotoxic effect on murine neuroblastoma, glioma and melanoma cancer cell lines in vitro
- **Dora Markulin** and **Branka Gršković** (Zagreb, Croatia): Relation of touch DNA from different surfaces with donor age and gender
- **Slavé Petrovski** (Durham, NC, USA): A genome-wide ranking of genic intolerance to functional variation facilitates predicting disease causing genes and interpreting personal genomes
- **Antoinette Westen** (The Hague, The Netherlands): Improved analysis of long STR amplicons from degraded single source and mixed DNA

• *Thursday, June 27, 2013*

Parallel Scientific Sessions

T4: Advances in Regenerative Medicine: The Industry Perspective

08:00 *Introduction from the chair:* **Robert Deans**
08:05 **Robert Deans** (Cleveland, OH, USA): Stem cell treatment in allogeneic bone marrow transplantation
08:40 **Eric Halioua** (Mont-Saint-Guibert, Belgium): Liver stem cells for treatment of liverbased inborn errors of metabolism: A Promethera Biosciences practical case
09:15 **John Sinden** (Guildford, Surrey, UK): Developing a neural stem cell therapy for stroke disability: the clinical pathway
09:50 **Peter de Waele** (Mont-Saint-Guibert, Belgium): Treating congestive heart failure by autologous adherent progenitors
10:25 **Jef Pinxteren** (Heverlee, Belgium): Quality control validation of cell expansion systems for isolation and culture of cell therapy products
11:00 **Discussion**

F4: Advanced Methods in Forensic Practice

08:00 *Introduction from the chair:* **Henry Lee**
08:35 **Santo Davide Ferrara** (Padua, Italy): Perspectives of biomedicolegal sciences
09:05 **Katja Drobnič** (Ljubljana, Slovenia): Advanced methods in forensic practice
09:35 **Cassandra Calloway** (Oakland, CA, USA): Analysis of DNA mixtures and degraded DNA by clonal sequencing
10:05 **Frederick Bieber** (Cambridge, MA, USA): Utility of offender hits and the promise of familial searching
10:35 **Oral Presentation: Charissa van Kooten** (Hague, The Netherlands): Familial searching combining autosomal and Y chromosomal STRs and surnames
10:50 **Timothy Palmbach** (New Haven, CT, USA): Leveraging the power of DNA analysis in the war on human trafficking
11:20 **Veronique Bourdon** (Nice, France): Optimization of human mtDNA control region sequencing for forensic applications
11:50 **Discussion**

W2: Communication Skills in Science and Medicine

12:00 **Lovorka Brajković** (Zagreb, Croatia)
12:30 **Marijana Braš** (Zagreb, Croatia)
13:00 **Veljko Đorđević** (Zagreb, Croatia)
13:30 **Discussion**

14:00 *Keynote Address*
Ada Yonath (Rehovot, Israel): From basic science to better antibiotics
(Introduced by D. Primorac)

Parallel Scientific Sessions

T5: Pharmacogenomics and Personalized Medicine

15:00 *Introduction from the chair: Franklyn Prendergast*
15:05 **Scott Waldman** (Philadelphia, PA, USA): A common molecular mechanism at the intersection of obesity and colorectal cancer
15:35 **Henry Erlich** (Alameda, CA, USA): Next Generation Sequencing in analysis of HLA polymorphism: clinical and research applications
16:05 **Oral Presentation: Manuela de Gregori** (Pavia, Italy): How pharmacogenetics might help to predict postoperative pain and response to patient controlled analgesia by morphine
16:20 **Oral Presentation: Mohamed Gad** (Cairo, Egypt): SNP analysis of DDAH2 gene in cardiovascular disease
16:35 **Arezou Ghazani** (Boston, MA, USA): Advances in nanotechnology and diagnostics
17:05 **Dražen Raucher** (Jackson, MS, USA): Thermoresponsive biopolymers for tumor — specific drug delivery
17:35 **Franklyn Prendergast** (Rochester, MN, USA): Heterogeneity and accuracy of diagnostics for personalized medicine
18:05 **Discussion**

F5: Anthropological Genetics

15:00 **Introduction from the chair: Richard Villems**
15:05 **Yoel Rak** (Tel Aviv, Israel): Neanderthal man's place in nature
15:35 **Oral Presentation: Vladimir Paar** (Zagreb, Croatia): Large tandem, higher order repeats (HOR) and regularly dispersed repeat units contribute substantially to divergence between human and chimpanzee Y chromosomes
15:50 **Peter Underhill** (Stanford, CA, USA): Patterns of Y-chromosome diversification
16:20 **Moses Schanfield** (Washington, DC, USA): Population variation in X chromosome STR markers: Forensic and anthropologic considerations
16:50 **Richard Willems** (Tartu, Estonia): Genetics of Slavic-speaking peoples — Patrilineal, matrilineal and autosomal portraits
17:20 **Edwin Huffine** (Lorton, VA, USA): From forensic investigations to medical applications — the growing impact of DNA testing
17:50 **Šimun Anđelinović** (Split, Croatia): Virtual autopsy and forensic anthropology of mummified Catholic saints
18:20 **Discussion**

18:30 ***Poster Session II***
Meet the Professor II

• *Friday, June 28, 2013*

08:00 *Scientific Workshops with Audience Participation*

W3: Adapting Clinical Trials to Modern Cancer Therapy
Zvia Agur, moderator

- **Zwi Berneman** (Antwerp, Belgium): Dendritic cell vaccination in acute myeloid leukemia
- **Mauro Gasparini** (Torino, Italy): New statistical tools for novel approaches to clinical trials in personalized medicine
- **Manish Kohli** (Rochester, MN, USA): Use of biomarkers in personalized therapy of prostate cancer
- **Moran Elishmereni** (Bene Ataroth, Israel): Personalized modeling for efficient planning of combinatorial hormone therapy for prostate cancer patients
- **Yuri Kogan** (Bene Ataroth, Israel): Improving efficacy of immunotherapy by dynamic personalization—the prostate cancer case study
- **Discussion**

W4: Glycoscience in Personalized Medicine
Gordan Lauc, moderator

- **Gordan Lauc** (Zagreb, Croatia): Protein glycosylation in personalized medicine
- **Falk Nimmerjahn** (Erlangen, Germany): How does intravenous immunoglobulin therapy modulate immunity?
- **Harry Campbell** (Edinburgh, UK): Protein glycosylation in colorectal cancer
- **Tim Spector** (London, UK): IgG glycosylation in TwinsUK
- **Igor Rudan** (Edinburgh, UK): GWAS analysis of the human plasma glycome
- **Vlatka Zoldoš** (Zagreb, Croatia): Epigenetic regulation of protein glycosylation
- **Shin-Ichiro Nishimura** (Sapporo, Japan): Towards personalized medicine by glycan biomarkers discovered by glycoblotting-assisted high throughput serum glycomics
- **Discussion**

12:30 *Conclusion of the Meeting and Announcement of the Next ISABS Conference*

KEYNOTE ADDRESSES

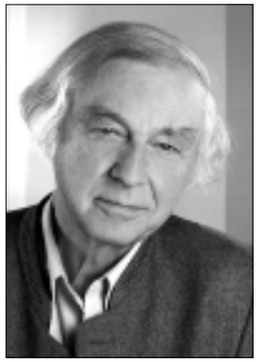
Aaron Ciechanover

Nobel Prize in Chemistry 2004;
Technion, Haifa, Israel

REVOLUTION OF PERSONALIZED MEDICINE: WILL WE CURE ALL DISEASES AND AT WHAT COST?

Aaron Ciechanover was born in Haifa, Israel in 1947. He is a Distinguished Research Professor in the Technion - Israel Institute of Technology in Haifa. He received his M.Sc. (1971) and M.D. (1973) from the Hebrew University in Jerusalem. He then completed his national service (1973-1976) as military physician, and continued his studies to obtain a doctorate in biological sciences in the Faculty of Medicine in the Technion (D.Sc.; 1982). There, as a graduate student with Dr. Avram Hershko and in collaboration with Dr. Irwin A. Rose from the Fox Chase Cancer Center in Philadelphia, USA, he discovered that covalent attachment of ubiquitin to a target protein signals it for degradation. They deciphered the mechanism of conjugation, described the general proteolytic functions of the system, and proposed a model according to which this modification serves as a recognition signal for a specific downstream protease. As a postdoctoral fellow with Dr. Harvey Lodish at the M.I.T., he continued his studies on the ubiquitin system and made additional important discoveries. Along the years it has become clear that ubiquitin-mediated proteolysis plays major roles in numerous cellular processes, and that aberrations in the system underlie the pathogenetic mechanisms of many diseases, among them certain malignancies and neurodegenerative disorders. Consequently, the system has become an important platform for drug development. Among the numerous prizes Ciechanover received are the 2000 Albert Lasker Award, the 2003 Israel Prize, and the 2004 Nobel Prize (Chemistry; shared with Drs. Hershko and Rose). Among many academies, Ciechanover is member of the Israeli National Academy of Sciences and Humanities, the American Academy of Arts and Sciences (Foreign Fellow), the American Philosophical Society, the National Academy of Sciences of the USA and the Institute of Medicine of the National Academies of the USA (Foreign Associate), and the Russian Academy of Sciences (Foreign Member). Earlier this year, the University of Split awarded Dr. Ciechanover the honorary doctorate for his support of Croatian scientists and science.

(http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2004/ciechanover-autobio.html)



Robert Huber

Nobel Prize in Chemistry 1988;
Max-Planck Institute, Martinsried, Germany

PROTEIN STRUCTURES AT THE INTERFACE OF PHYSICS, CHEMISTRY AND BIOLOGY

Robert Huber was born in Munich in 1937. He studied chemistry at the Technische Universität München (TUM), where he also completed his Ph.D. and habilitation. Since 1972, he has been a member of the Max-Planck-Gesellschaft and Director at the Max-Planck-Institut für Biochemie until 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), and the Universidad Autonoma de Barcelona (Spain). Prof. Huber serves as a member of the Board and/or Scientific Advisory Board of a number of pharmaceutical and crop science companies. He co-founded two companies located in Martinsried: Proteros offering services for drug discovery and development and Suppremol developing novel therapies for autoimmune diseases. Prof. Huber has made major contributions to the understanding of the structure and function of biological macromolecules, particularly 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. In addition, he has contributed to the development of instruments for data collection and methods in protein crystallography, particularly Patterson methods, graphic methods, and refinement, to the use of electron rich metal clusters. Most recently, Prof. Huber contributed to the methods and instruments for crystal improvement. 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.

(http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1988/huber.html)

Ada Yonath

Nobel Prize in Chemistry 2009;
The Weizmann Institute of Science, Rehovot, Israel

FROM BASIC SCIENCE TO BETTER ANTIBIOTICS

Ada Yonath is a native of Jerusalem where, at the Hebrew University she earned her B.Sc. in chemistry and M.Sc. in biochemistry. She 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.

(http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2009/yonath-lecture.html)

ABSTRACTS
OF INVITED LECTURES

Inaugural Plenary Session:
Science and Public Security

Abstract number: ABS-191-ISABS-2013

CAN THE SAME THEORY GUIDE BOTH PERSONALIZED MEDICINE AND NATIONAL SECURITY POLICIES?

Agur Z

Institute for Medical BioMathematics (IMBM), Bene Ataroth, Israel

Theory suggests the existence of a universal Resonance Phenomenon whereby population growth is maximized when the periodicity of environmental disturbances is a multiple (integer or fractional) of the characteristic periodicity of the population (i.e., generation time). This implies that efficient control of phenomena, as diverse as smallpox transmission and cancer proliferation, which display clear periodicity, should involve a periodic strategy with a period different from that of the phenomenon being counteracted. Earlier we proposed the application of periodic vaccination (termed pulse vaccination) in response to a terrorist attack by the smallpox virus. Our simulation studies indicate that post-attack vaccination with precisely calculated inter-vaccination intervals results in much more effective long-term population protection against smallpox, compared to one-time mass vaccination, either prophylactic or post-attack. Universality of the resonance phenomenon offers an attractive possibility for the control of cancer as well. An example is combination drug therapy, prospectively optimized for a particular mesenchymal chondrosarcoma (MCS) patient. Model simulations suggested that bevacizumab, in combination with once-weekly docetaxel, would be more efficacious than any other schedule, due to the internal MCS periodicity dependent on personal cytokinetic and angiogenic parameters. Weekly docetaxel in the patient stabilized his progressive metastatic disease and relieved pancytopenia.

Suggested Reading

1 Gorelik B., Ziv I., Shohat R., Wick M., Webb C., Hankins D., Sidransky D., Agur Z. Efficacy of once weekly docetaxel combined with bevacizumab for patients with intense angiogenesis: validation of a new theranostic method in mesenchymal chondrosarcoma xenografs. Cancer Research, 68: 9033-40, 2008.

2 Agur Z., Marron K., Shai H.,. Danon YL. Preparing for a smallpox bioterrorist attack: pulse vaccination as an optimal strategy. In "Risk Assessment and Risk Communication Strategies in Bioterrorism Preparedness", Proceedings of the NATO Advanced Research Workshop on Risk Assessment and Risk Communication in Bioterrorism, Ein-Gedi, Israel, June 2005. Series: NATO Security through Science Series. Green, M.S.Zenilman, J. Cohen, D. Wiser, I. Balicer, R.D. (Eds.) 2007, Springer.

3 Kheifetz Y., Kogan Y., Agur Z. Matrix and compact operator description of resonance and antiresonance in cell populations subjected to phase specific drugs. J. Med. Informatics Technol., 8, MM11-29, 2004.

4 Agur Z., Cojocaru L., Mazor G., Anderson R.M., Danon Y.L. Pulse mass measles vaccination across age cohorts Proc. Nat. Acad. Sci. USA, 90: 11698-11702, 1993.

5 Agur Z., Arnon R., Schechter B. Effect of the dosing interval on myelotoxicity and survival in mice treated by cytarabine. Eur. J. Cancer, 28A: 1085-1090, 1992.

Abstract number: ABS-322-ISABS-2013

FUTURE IMPACT OF RAPID DNA TECHNOLOGY ON LAW ENFORCEMENT

Asplen C

Global Alliance for Rapid DNA Testing, Chalfont, PA, USA

Rapid DNA Technology - the use of portable kits to quickly and accurately analyze human DNA for swift identification (60-90 minutes versus the industry standard of several hours) - will fundamentally change the way law enforcement utilizes forensic DNA technology. As various partnerships between the public and private sector establish the reliability, portability, operational simplicity and speed of DNA analysis, law enforcement agencies around the world are going to begin maximizing the intelligence gathering value of DNA technology. While traditional laboratory dynamics will continue to carry the weight of the most sensitive and complicated DNA testing, the ability of police to develop forensic DNA based leads and identifications in short periods of time, will significantly drive a greater utilization of DNA as an investigative tool rather than simply and evidentiary piece of evidence. This new technology will create a fundamental expansion of DNA use both in diverse application and in volume. This presentation will explain the current developmental dynamics of Rapid DNA testing and how, with Rapid DNA testing, DNA technology will begin to reach its crime solving potential.

Suggested Reading

1 www.RapidDNA.org

2 Rapid DNA Analysis is Coming - Rapidly (http://www.forensicmag.com/article/rapid-dna-analysis-coming-rapidly)

FORENSICS OF CYBER CRIME: THE PROBLEM OF ATTRIBUTION

Ben-Israel I

Tel Aviv University, Tel Aviv, Israel

Computers penetrate every field of our life. Their functions go well beyond information technology—storing, communicating and processing information. Computers serve also as controllers of essential critical infrastructure, e.g., power production, transportation, hospitals, etc. This situation creates a new weak point for the society: one can use cyber techniques to attack and destroy these computers resulting in real large-scale physical damage. Such attacks have certain immunity due the problem of attribution as it is highly difficult to locate the attacker. Forensic development in this area should involve new technologies and be based on international cooperation. The difference between information or data security and cyber security will be explained, and some of the lessons gained in Israel will be described.

Abstract number: ABS-322-ISABS-2013

DNA EVIDENCE IN NATIONAL AND INTERNATIONAL SECURITY

Lee H

Henry Lee Institute of Forensic Science, and University of New Haven, New Haven, Connecticut, USA

With the recent increase of crime across national boundaries, global crime investigation became among the most important aspects in law enforcement. In general, the term global crime refers to crime against international law (created by international treaties and conventions), against humanity and war crimes. In recent years, a new category—transnational crime has been added to the list. The increase in transnational crime has seriously affected the security of the world. Eight major areas of transnational crimes are drug trafficking, arms trafficking, human trafficking, money laundering, artifact smuggling, transnational gang activity, terrorism, and kidnapping and murder. How to use the newest crime fighting tools to reduce transnational crime and protect the integrity and security of the national and international community? Besides addressing the policy and international cooperation in law enforcement, equally important are the utilization of new forensic techniques, such as image recording and enhancement, artificial intelligence, data mining and timeline analysis, DNA and biometric databases, trace DNA analysis, as well as firearm and weapon tracing. The value of DNA evidence has been demonstrated in all aspects of criminal investigations. As science and technology continue to advance, the importance and value of DNA evidence in investigation of global crime will continue to grow linking international criminals to cases, tracking the movement of evidence and individual criminal, identifying international terrorist groups, linking bomb parts to bomb makers, identifying victims and human traffickers. I will discuss case examples to illustrate the importance of the application of DNA evidence in protecting national security and in investigation of global crime.

Suggested Reading

1 Lee HC, Ladd C, Miller Coyle H(1999) Forensic Biology. In: 9th Edition of McGraw-Hill Encyclopedia of Science and Technology. McGraw-Hill Publishers (available on-line at http://www.AccessScience.com)

2 Forensic Science Today, 2nd Ed (2008), Lee H, Taft MG, Taylor KA (2008) Lawyers & Judges Publishing

Forensic Program

Abstract number: ABS-331-ISABS-2013

VIRTUAL AUTOPSY AND FORENSIC ANTHROPOLOGY OF MUMMIFIED CATHOLIC SAINTS IN CROATIA

Anđelinović Š[1], Janković S[1], Vilović K[1], Mihanović F[1], Anterić I[2], Bašić Ž[2], Ferenček Z[3], Mršić G[3], Crnjac J[2]

[1]Department of Health Studies, and [2]Department of Forensic Science, university of Split, Split, Croatia; [3]Ivan Vučetić Forensic Science Centre, General Police Directorate, Ministry of Interior, Zagreb, Croatia

By noninvasive and nondestructive virtual autopsy techniques of forensic analysis we studied the anatomical and pathological changes of four mummified bodies from the Church of St. Blaž (St. Blaise) in Vodnjan (Istria, Croatia). We analyzed the images obtained by multi-slice computed tomography (MSCT) of bodies using Osirix software, which allows the three-dimensional examination of the interior of the body. As the bodies of St. Nicolosa Bursa and St. Ivan Olini were mummified, all soft tissue data were negative due to lack of water inside them. The remains of internal organs, muscles and ligaments were preserved. The body of St. Ivan Olini revealed the spina bifida occulta on the sacrum as well as a clubfoot. Images of the remains of St. Leon Bembo and St. Paul did not show any soft tissue. The remains of their bones were attached to metal and wooden handles, covered with fabric and filled with the material that is assumed to be cotton. Forensic teams from University Department for Forensic Sciences and Forensic Science Centre "Ivan Vučetić" performed anthropological analysis and photographically documented 670 relics (bones) for their catalogue and individualization. We will present the most important findings of MSCT imaging and anthropological analysis of the relics as well as the significance of their catalogue, preservation and presentation.

Abstract number: ABS-240-ISABS-2013

ROLE OF FORENSIC ODONTOLOGY IN MASS DISASTER IDENTIFICATION: FUNDAMENTALS AND NEW TECHNOLOGIES

Aschheim K

Office of Chief Medical Examiner, New York; New York University College of Dentistry, New York; The Mount Sinai Medical Center, New York, New York, USA, and American Dental Association, Chicago, Illinois USA

Despite advances in DNA and fingerprint technology, forensic odontology still plays an important role in disaster victim's identification (DVI). This comprehensive presentation will cover forensic odontology fundamentals relating to DVI and the dental response to some of New York City's DVI incidents, including the 9/11 World Trade Center attack and the aftermath of the crash of American Airlines Flight 587. Some of the lessons learned, and the current role dentistry plays, in New York City's Medical Examiner's Special Operations Response Team (MESORT), the interdisciplinary team that responds to complex fatality management scenarios will be discussed. Advances in both the hardware and software technology that have occurred in the last decade, including software developed by OCME, will be covered. We will explore the joint interoperability data initiative between the American Dental Association, the American National Standards Institute, the National Institute of Standards and Technology, and Interpol (Plass Data) and how this protocol will expedite the transfer of dental data. The presentation will conclude with an examination of some of the coding research performed at New York City's Office of the Chief Medical Examiner on coding granularity (simplicity), ambiguity and specificity.

Suggested Reading

1 Bruce-Chwatt RM (2010) A brief history of forensic odontology since 1775. J Forensic Leg Med 17: 127-130

2 Avon SL (2004) Forensic odontology: the roles and responsibilities of the dentist. J Can Dent Assoc 70: 453-458.

3 Pittayapat P et al. (2012) Forensic odontology in the disaster victim identification process. J Forensic Odontostomatol 30: 1-12.

4 ANSI/ADA Standard No. 1058-Forensic Dental Data Set: 2010 http://www.ada.org/805.aspx#1058 Accessed April 2013

5 ANSI/NIST-ITL 1-2011 Supplemental Dental and Oral Forensics, http://www.nist.gov/itl/iad/ig/ansi_standard_dental_forensics.cfm, Accessed April 2013

Abstract number: ABS-319-ISABS-2013

IMPROVED FORENSIC Y-CHROMOSOME ANALYSIS

Ballantyne K

Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Macleod, Victoria, Australia

The analysis of the Y-chromosome is now well established in forensic science, with applications as ranging from resolving mixtures in sexual assault cases, paternity testing for male offspring, narrowing suspect pools from familial searches and identifying biogeographical ancestry of male suspects. However, with the increased application of Y-STRs and the growing size of worldwide databases, there is a need to expand the core set of loci routinely tested to limit the occurrence of adventitious matches between unrelated male lineages. Furthermore, the main limitation of Y-STR testing, that male relatives can not be distinguished, can be alleviated by the judicious selection of rapidly mutating Y-STRs. The presentation will provide alternatives to the current panels of Y-STRs, and demonstrate how the application of additional markers can improve the forensic performance of Y-STR testing. Particular emphasis will be given to the Rapidly Mutating Y-STR panel, with examples of the increases obtainable in both male relative and male lineage differentiation given by this panel compared to current methods.

Suggested Reading

1 Ballantyne KN et al. (2012) A new future of forensic Y-chromosome analysis: rapidly mutating Y-STRs for differentiating male relatives and paternal lineages. Forensic Sci Intl Genetics 6: 208-218

2 Ballantyne KN, Kayser M. (2012) Additional Y-STRs in Forensics: Why, Which and When. Forensic Sci Rev 24: 63

Abstract number: ABS-169-ISABS-2013

LOW TEMPLATE DNA ANALYSIS AND INTERPRETATION

Benschop C

Netherlands Forensic Institute, The Hague, The Netherlands

DNA typing of minute amounts of DNA often results in partial DNA profiles. Several technical methods exist to improve DNA typing results and gain information from a crime stain sample. Nevertheless, low template (LT) DNA profiles can suffer from stochastic amplification effects like allelic dropout, locus dropout, heterozygote imbalance and/or (stutter) drop-in. The interpretation of LT DNA profiles is even more challenging when DNA of multiple contributors is present. With special focus on these challenging mixed LT DNA profiles, possibilities at the technical level, the development and use of consensus-based interpretation strategies, the efforts towards the application of statistical software tools and the setting of mixture interpretation guidelines will be discussed. The guidelines were developed using a set of purposeful LT DNA mixtures and comply with literature. The mixtures all have a major contributor and minor contributor(s); some mixtures have a high template major while in other mixtures the major presents alleles around the stochastic threshold. For DNA database purposes, it can be useful to deduce alleles from the major contributor. To this aim, a new and automated approach is introduced that functions upon categorisation of each locus: based on peak heights, heterozygote balance and ratio major to minor, three types of loci are distinguished. For each type of locus, different actions apply regarding deduction of the major. The locus categorisation is embedded in our guidelines, and aids both the deduction of the major contributor and the defining which loci are/are not suitable for DNA database purposes.

Abstract number: ABS-343-ISABS-2013

ROLE OF STATISTICAL GENETICS IN MASS DISASTER MANAGEMENT

Bieber F

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When statistical issues are involved in mass disaster DNA-based identifications both mathematical and policy are important in determination of thresholds for making identifications. Both direct and indirect DNA comparisons are typically needed, with kinship analysis typically necessary in a fair to large proportion of missing persons or victim identifications. This presentation will provide an overview of lessons learned and policies implemented in specific examples of DNA-identification of persons in mass casualties. Discussion will include determination of statistical thresholds, use of prior probabilities and likelihood ratios, and use of non-DNA data (i.e., "metadata") that can be collected about victims from autopsy and from family members. Administrative and technical review of data will be addressed, along with ethical guidelines and policies to consider so that harm to families is avoided when genetic and social pedigrees are not consistent.

Suggested Reading

1 National Institute of Justice Kinship and Data Analysis Panel (incl. Bieber FR), (2006) Lessons Learned From 9/11: DNA Identification in Mass Fatality Incidents, U.S. Dept. Justice,(NCJ 214781), Washington, DC,http://www.ncjrs.gov/pdffiles1/nij/214781.pdf

2 Budowle B, Bieber FR, Eisenberg A (2005) Forensic aspects of mass disasters: strategic considerations for DNA-based human identification. Legal Med 7: 230-243

3 Biesecker LG et al. (2005) DNA Identifications after the 9/11 World Trade Center attack. Science 310: 1122-1123

4 Ballantyne J (1997) Mass Disaster Genetics. Nature Genetics 15: 329-331

Abstract number: ABS-342-ISABS-2013

UTILITY OF OFFENDER HITS AND THE PROMISE OF FAMILIAL SEARCHING

Collins D[1,2], Lazer D[3], **Bieber FR**[1,4]

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Offender DNA databases are now in use in most developed countries, often leading to identification of suspects and resolution of forensic investigations. This presentation will provide a summary of the US experience to date and will discuss the ongoing debate concerning arrestee databases and familial searching. Organizations are now beginning to examine ways to improve their DNA databanks to increase efficiency and reduce costs. As part of this process we examined the extent to which use of kinship searches might help develop new investigative leads in an established DNA database of approximately 100,000 offender profiles. Offender profiles with a hit to one or more forensic profiles were searched against the existing offender index, using shared allele and kinship search configurations, optimized for identifying first degree relationships among 15 locus and 13 locus DNA profiles. Numerous target profiles, when searched against known offenders, identified at least one candidate with a matching Y-STR profile, documenting the known familial clustering of offenders. Next, to determine the current efficacy of the MSPFSG CODIS program, offender hits reported to one county over a 4-year period were examined for case disposition. The majority of cases were resolved with a plea bargain and sexual assaults were often closed with no prosecution. These results support the potential effectiveness of familial search strategies and demonstrate the need for better methods for downstream tracking of DNA hits to determine the resolution of the investigations they are designed to aid.

Suggested Reading

1 Bieber FR (2006) Turning base hits into earned runs: Improving the effectiveness of forensic DNA data bank programs. J Law Med Ethics 134: 222-233

2 Lazer D, Bieber FR (2010) Familial searching, its promise and perils, op-ed, Los Angeles Times, July 10, 2010

Abstract number: ABS-220-ISABS-2013

OPTIMIZATION OF HUMAN MTDNA CONTROL REGION SEQUENCING FOR FORENSIC APPLICATIONS

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Sequencing mitochondrial DNA (mtDNA) hypervariable regions I and II (HVI and HVII) is useful in forensic missing person, unidentified remains cases as well as some criminal cases. At the New York City Office of Chief Medical Examiner, mtDNA testing has been online since 2006. Based on the validations, typing is performed on an mtDNA genome equivalent to 100pg of nuclear DNA (nDNA), or 5ng of HVI/HVII amplicons per sequencing reaction. Testing normally takes two days. However, as casework volume increased since 2006, from testing of a few samples a year to a few hundreds, we are now limited by the cost and time of testing. Furthermore, samples with a low nDNA amount are not typed and no DNA profile is obtained for some criminal cases, missing persons or World Trade Center remains. Improvements in ease and sensitivity of testing will yield results from more samples in a timely fashion. Routinely, amplification of HVI and HVII is followed by Sanger sequencing using the BigDye® Terminator v3.1 Cycle Sequencing kit using 4µL of Ready Reaction Mix (RRM). Each sequencing reaction is then purified through column filtration before capillary electrophoresis. Using lower amounts of RRM and purification using BigDye® XTerminatorTM showed no loss of sequence length and increased the quality and the sensitivity of testing, allowing HVI and HVII typing from mitochondrial genome equivalent to 125 femtograms of nDNA, or 100pg of HVI/HVII amplicons. Furthermore, using this methodology, testing can be completed in one day, and the cost of testing is reduced.

Suggested Reading

1 Andrews RM et al. (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23: 147

2 Parsons TJet al. (1997) A high observed substitution rate in the human mitochondrial DNA control region. Nat Genet 15: 363-368

3 Butler JM (2011) Advanced topics in forensic DNA typing: methodology. London: Elsevier Academic Press

4 Fernandez C, Alonso A (2011) DNA electrophoresis protocols for forensic genetics Humana Press, pp. 367-380

5 Melton, T, Dimick, G, Higgins, B, Yon, M, Holland, C. (2012) Mitochondrial DNA analysis of 114 hairs measuring less than 1cm from a 19-year-old homicide. InvestGenet 3: 12

Abstract number: ABS-335-ISABS-2013

MASS DISASTER MANAGEMENT: 1. THE SCIENCE

Butcher BA, **Budimlija Z**

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Mass fatality management has changed radically with the advent of new identification technologies, most notably in DNA. However, the older methods such as fingerprinting, odontology, and anthropology have also advanced both in execution and in matching software. It is imperative that operational personnel doing recoveries of victims understand the science behind identification so that they can maximize the chance of obtaining good specimens and avoid the loss of valuable evidence. Following the 9/11 attacks on the World Trade Center, the New York City Medical Examiner launched an unprecedented effort to recover and identify remains of the 2753 victims. The effort continues to this day; to date, we have found more than 21,800 partial remains and identified 1632 victims, largely through DNA.

Abstract number: ABS-325-ISABS-2013

MASS DISASTER MANAGEMENT: 2. THE NEW YORK CITY EXPERIENCE

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During the World Trade Center victim identification project, samples of human provenance were collected over the course of 12 years. Due to environmental conditions and the length of recovery, DNA in the collected samples typically experienced high level of degradation. An additional problem was commingling of the remains. Due to the enormous forces during the evolution of the incident, many body/tissue fragments were compacted simultaneously, causing anthropological-DNA profiles conflicts. Among the project's greatest challenges is the high number of fragmented remains and the "open manifest" nature of the incident. As of October 2012, there were 1,633 identifications for the 2,753 people reported missing. Of these, 996 were identified by a single means; of which by DNA analysis only 879 of the victims. DNA analysis, combined with anthropologic expertise, has become the standard method for identification in these types of disasters where fragmentation is overwhelming. Throughout the course of the WTC human identification project to date, all of the recovered samples have been tested multiple times. After new technologies were developed, validated and approved for use in human identification, they too have been employed in this operation. In addition to classic STRs (short tandem repeats), mini-STRs, Single Nucleotide Polymorphism (SNPs), and mitochondrial DNA (mtDNA) analysis were used, adding more combined results and making possible additional fragment to fragment links or new body identifications. One of many legacies of this Project is design of more stringent policies and procedures specific to mass fatality incidents management. (Presented as part of the talk entitled.)

Suggested Reading

1 Biesecker LG et al. (2005) DNA identifications after the 9/11 World Trade Center attack. Science 310: 1122-1123.

2 Budimlija ZM et al. (2003) World Trade Center Human Identification Project: Experience with individual body identification cases. Croat Med J 44: 259-263.

3 Holland MM et al. (2003) Development of a quality, high throughput DNA analysis procedure for skeletal samples to assist with the identification of victims from the World Trade Center attacks. Croat Med J 44: 264-272

4 Huffine E et al. (2001) Mass identification of persons missing from the break-up of the former Yugoslavia: structure, function, and role of the iInternational Commission on Missing Persons. Croat Med J 42: 271-275

5 Marchi E, Chastain T (2002) The sequence of structural events that challenged the forensic effort of the World Trade Center disaster. American Laboratory 34: 13-17.

Abstract number: ABS-336-ISABS-2013

ANALYSIS OF DNA MIXTURES AND DEGRADED DNA BY CLONAL SEQUENCING

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Next-generation sequencing (NGS) technologies have revolutionized the field of genetics and these massively parallel clonal sequencing technologies have the potential to make a significant impact to the DNA forensics field. Recently, we have developed two NGS enrichment assays for sequencing mitochondrial DNA (mtDNA) from highly limited and degraded samples. We have developed a PCR assay targeting the mtDNA hypervariable regions using 8 sets of 454 MID tagged fusion primers in a combinatorial approach for deep sequencing 64 samples in parallel on a 454 GS Jr. This assay was shown to be highly sensitive for sequencing limited DNA amounts (~100 mtDNA copies) and detecting low level mixtures (~1%). We have also developed a solution phase sequence capture and NGS assay for targeted enrichment and deep sequencing of the entire mitochondrial genome for increased discrimination power. Using this SeqCap NGS assay, 100% sequence coverage of the mitochondrial genome with an ~80% on target rate was achieved. Additionally, a DNA fragmentation method using mechanical shearing was optimized for rapid library preparation. This method was shown to be DNA quantity and quality independent, essential for preparation of highly degraded or limited samples often encountered in forensic cases. This optimized fragmentation method coupled with the SeqCap NGS assay was successfully used for sequencing the entire mitochondrial genome of partially degraded, limited DNA samples as well as detection of minor sequences in a mixture. We present here results demonstrating the potential application of these two NGS mtDNA enrichment assays for sequence analysis of difficult forensic samples.

Abstract number: ABS-253-ISABS-2013

ADVANCED METHODS IN FORENSIC PRACTICE

Drobnič K

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Aim: The main focus in forensic genetics in the past 20 years was to increase the efficiency of extracting DNA from a wide variety of evidence as well as to improve DNA profiling technology by making it sensitive and robust enough to allow the individualization of a tiny stain if we have the DNA profile of the person who left it behind. In the past few years this narrowly specialized improvement approach of biological evidence examination has been replaced by a holistic approach that includes advances in screening tests and prediction of human externally visible characteristics. The goal of this lecture is to present the benefit of including different advanced molecular genetic methods such as mRNA profiling for the identification of biological evidence or cytochrome B analysis for the identification of human/nonhuman source in forensic examination on solving cases. **Methods:** The improved holistic approach was examined and compared with the traditional evidence examination from routine forensic casework. **Results:** In all analyzed cases, the holistic approach provided a better understanding of the contact between the source of the evidence and the target. **Conclusions:** The study confirmed that in the case of criminal offences against life and advanced methods such as mRNA profiling are necessary for a more specific identification of the source of the evidence.

Suggested Reading

1 Walsh S et al. (2013) Forensic Sci Intl Genetics 7: 98-115
2 Kayser M, de Knijff P (2011) Nature Rev Genetics 12: 179-192
3 Kastelic V, Drobnič K (2012) Croat Med J 53: 401-8.
4 Hadžić G, Lukan A, Drobnič K (2011) Forensic Sci Int Genetics Suppl Series 3: 222-23

FUTURE PERSPECTIVES OF BIOMEDICOLEGAL SCIENCES

Ferrara SD

University of Padova, Padova, Italy

The primary objective of the lecture is to raise awareness of the growing importance of translational forensic medicine for innovation of biomedicolegal sciences and of training the forensic experts for the future. The lecture will briefly review the state of the art of molecular biology contributions to biomedicine and focus on imaging and bioanalytical platforms currently used in clinical medicine; the goal is to envisage innovations in biomedicolegal sciences from pathology to genetics, criminology, psychopathology, clinical forensic medicine and toxicology. The lecture will conclude by exploring the present and future prospects for funding opportunities of translational forensic research projects, particularly in the European Framework Program "Horizon 2020". The necessity will be underlined for medicolegal expertise in the next ten years, achieved by creating strong collaborative transdisciplinary projects in the fight against crime and clinical forensic medicine involving small and medium enterprises.

ANTHROPOLOGY AND FINGERPRINTING: NEW TECHNIQUES FOR IDENTIFICATION

Figura B

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Forensic anthropology and fingerprinting have been components of disaster response for many years. However, there has been a significant shift in the utility of these disciplines over the past decade, based in part on lessons learned from the World Trade Center disaster. This presentation will discuss these shifts with a focus on more recent advances in both process and science. Forensic anthropology has traditionally been limited to the analysis of skeletonized remains for the establishment of a biological profile. Following 9/11, forensic anthropologists established clearly defined roles in initial morgue triage, field recovery operations, and disaster victim identification operations. Further, recent research (1, 2) has demonstrated the ability to quantify anthropologically derived aspects of the biological profile (e.g., age and gender) as well as recovery location information for direct incorporation into statistically based identification efforts. In the last five years it has become clear that fingerprinting for identification purposes, a process developed for use on the living, is greatly limited when dealing with the deceased. This is particularly true in the United States where decedent identification is a function of the medical examiner/coroner whereas fingerprinting is a generally a function of law enforcement. Database access, direct electronic submission, and alternative capture techniques have led to a significant increase in fingerprint utility for medical examiners/coroners. Future initiatives include the development of tools specifically designed for capturing fingerprints from deceased individuals and the establishment of a fingerprint specific database for missing and unidentified persons.

Suggested Reading

1 Figura BJ (2011) A Bayesian Approach to Disaster Victim Identification. State University of New York at Binghamton, Department of Anthropology

2 Steadman DW, Adams BJ, Konigsberg LW (2006) Statistical basis for positive identification in forensic anthropology. Am J Phys Anthropol 131: 15-26

Abstract number: ABS-218-ISABS-2013

FORENSIC EPIGENETICS

Frumkin D

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Human genome contains ~28 million CpG dinucleotides, of which 70-80% are methylated at any specific point in time. Because DNA methylation is a key regulator of gene expression, the precise methylation pattern varies widely between tissues and cells, may change with time, and reflects the cell's physiological and/or pathological situation. This fact, together with the possibility of obtaining an informative methylation profile quickly and inexpensively, opens the door to several promising forensic applications, such as differentiating between in vitro and in vivo generated DNA, monozygotic twin identification, age prediction, and tissue identification. Methylation-based identification of blood, saliva, semen, and skin has recently been demonstrated, and a forensic semen identification assay for sexual assault cases was developed and validated.

Suggested Reading

1 Frumkin et al. (2011) DNA methylation-based forensic tissue identification. Forensic Sci Int Genet 5: 517-524

2 Wasserstrom et al. (2013) Demonstration of DSI-semen—A novel DNA methylation-based forensic semen identification assay. Forensic Sci Int Genet 7: 136-142

3 LaRue et al. (2013) A validation study of the Nucleix DSI-Semen kit—a methylation-based assay for semen identification. Int J Legal Med 127: 299-308

4 An et al. (2012) Body fluid identification in forensics. BMP Rep 45: 545-553

5 Madi et al. (2012) The determination of tissue-specific DNA methylation patterns in forensic biofluids using bisulfite modification and pyrosequencing. Electrophoresis 33: 1736-1745

GENE EXPRESSION IN FORENSIC SCIENCES

Haas C

Institute of Legal Medicine, University of Zürich, Zürich, Switzerland

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are either proteins (mRNAs as intermediate products) or functional RNAs (rRNA, tRNA, miRNA, etc.). RNA was notorious for its rapid post-mortem and in vitro decay, but it showed an unexpectedly high stability in certain conditions and therefore found its way into forensic science. This presentation gives an overview on the applications of RNA in forensic science, which include identification of body fluids and tissues, pregnancy diagnostics and identification of newborns, estimation of the postmortem interval, determination of the age of stains, determination of the wound age and diagnosis of the cause and mechanism of death. To deal with these challenges, we take advantage of the differential expression of RNAs in different tissues, specific RNA degradation rates and changes of gene expression due to functional and morphological changes of cells.

Abstract number: ABS-326-ISABS-2013

ANALYSIS OF MITOCHONDRIAL DNA HETEROPLASMY USING A NEXT GENERATION DNA SEQUENCING APPROACH

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Next generation DNA sequencing (NGS) studies have resulted in the characterization and measurement of mitochondrial DNA (mtDNA) heteroplasmy at a molecular level, allowing for the routine reporting of heteroplasmy in forensic casework, and thus, significantly increasing the discrimination potential of the testing method. Of the three currently available benchtop NGS solutions (Roche 454 Junior, LifeTechnologies Ion Proton System PGM, and Illumina MiSeq), the MiSeq instrument and supporting reagent kits were chosen by our laboratory as a fast and accurate solution to data acquisition. Long range amplification of the mtDNA genome, followed by tagmentation using the NexteraXT™ kit and indexing of individual samples (library preparation), allowed for the analysis of up to 12 samples per run. In the current study, we performed whole mtDNA genome sequencing on more than 50 maternally related pairs of individuals at a coverage of typically more than 5,000 reads per nucleotide. The rate of mtDNA heteroplasmy across unrelated maternal lineages was assessed, including the identification of low-level heteroplasmic variants. An evaluation of the inheritance pattern of these low-level variants was performed for shared variants, and for those unique variants that would potentially distinguish maternal relatives. Thresholds for mtDNA heteroplasmy detection and parameters for mtDNA sequence analysis on the MiSeq instrument were developed, accounting for variation in features such as depth of coverage.

Suggested Reading

1 Holland et al. (2011) Croat Med J 52: 299-313

2 Li et al. (2010) Am J Hum Genet 87: 237-49

FROM FORENSIC INVESTIGATIONS TO MEDICAL APPLICATIONS: THE INCREASING IMPACT OF DNA TESTING

Huffine E

Bode Technology Group, Inc., Lorton, Virginia, USA

DNA technology has been among the most significant scientific developments of the past thirty years. Its use assists in solving decades old rape and missing person cases, and provides for individualized heath care based on patient's unique genetic characteristics. Forensic DNA testing has led to a reduction of crime in the areas where such testing is aggressively applied and helped in identifying missing persons from mass graves. DNA testing has also helped exonerate the innocent from crimes they did not commit. In all that forensic DNA testing has been a significant support to human rights. A relatively new and burgeoning area of DNA testing is personalized medical treatment based upon an individual's genetic sequence. Having the unique DNA sequence of an individual can help doctors and the patient understand which treatments may work best for the patient. Also, a genetic sequence can help determine which diseases an individual may be predisposed leading to preventative lifestyle adjustment. Individualization of medical treatment will have a huge impact on the quality of life and the effectiveness of medical treatment in the near future. Thus, the evolution of DNA testing and the understanding of its applications for both forensic and medical applications is having a tremendous impact on the individual as well as society. The full impact of these developments will transform society to even a larger degree than has already been witnessed.

Abstract number: ABS-161-ISABS-2013

FORENSIC DNA PHENOTYPING

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There will always be perpetrators whose STR profile is not already known to the investigating authorities and the remains of individuals for which it is impossible to locate ante mortem samples or biological relatives for providing reference samples. As current DNA profiling is a completely comparative approach, these people will consequently escape current means of DNA identification. In principle, information about a person's appearance, if obtainable from biological material, would be useful in a non-comparative way for tracing unknown persons. This rationale has initiated a new field within forensic genetics called Forensic DNA Phenotyping, where phenotypes usually refer to externally visible characteristics (EVCs). Recent applications of genome-wide association studies to a few EVCs, such as pigmentation traits and body height, have delivered various genes, but for many EVCs the genetic basis remains largely or completely unknown thus far. In the cases of eye and hair colour it has been demonstrated already that current genetic knowledge allows reliable and accurate phenotype prediction from DNA. Multiplex genotyping tools as well as prediction models have been developed and validated to allow DNA-based eye and hair colour prediction in forensic practise. The existing genetic knowledge of all other EVCs however, does not allow DNA prediction to be accurate enough for practical FDP as of yet. In this overview talk I will summarize current knowledge on the genetic determination and DNA prediction of human appearance traits with particular relevance for forensics.

Suggested Reading

1 Kayser and de Knijff (2011) Nat Rev Genet 12: 179-192

2 Liu et al. (2009) Curr Biol 19: R192-R193

3 Liu et al. (2012) PLoS Genet 8: e1002932

4 Walsh et al. (2011) Forensic Sci Int Genet 5: 170-180

6 Walsh et al. (2013) Ibid. 7: 98-115

Abstract number: ABS-312-ISABS-2013

MT-GONL: DEEP SEQUENCING OF 750+ COMPLETE DUTCH MTDNA GENOMES

de Knijff P[1], Vermaat M[1], Li M[2], van Oven M[3], den Dunnen J[1], Stoneking M[2], Kayser M[3], Laros J[1]

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The Genome of the Netherlands (GoNL) is a national collaboration aimed at establishing a map of Dutch genetic variation by whole genome sequencing of 250 Dutch trio families. This trio-based setup and the high mtDNA coverage (on average 1100 times) gives us the unique opportunity to study both population-wide and intra-human variation of the mitochondrial genome. We developed a number of techniques in which mtDNA can assist in quality control of whole genome sequencing experiments. The high coverage also enables us to easily detect sample specific heteroplasmy frequencies, heteroplasmy transmission patterns and contamination with a low percentage of foreign DNA. One such case is present in our data set and its contamination has been confirmed by autosome analysis. By looking for violations of the inheritance patterns we also identified sample swaps. One of the goals of our mtDNA study is to define the Dutch mtDNA phylogenetic tree. Preliminary results show that the data set contains more than 165 different mtDNA haplogroups, where H and its subclades are most abundant, representing about 40% of individuals. In some of our samples we noted a disagreement with respect to the defining polymorphisms for some haplogroups, indicating opportunities for refinement of the mtDNA phylogenetic tree. This abstract is submitted on behalf of the Genome of the GoNL Consortium (http://www.nlgenome.com).

Suggested Reading

1. Li M, Stoneking M (2012) A new approach for detecting low-level mutations in next-generation sequence data. Genome Biol 13: R34. doi: 10.1186/gb-2012-13-5-r34.

2. Behar DM et al. (2012) A Copernican reassessment of the human mitochondrial DNA tree from its root. Am J Hum Genet. 90: 675-684

Abstract number: ABS-165-ISABS-2013

LEVERAGING THE POWER OF DNA ANALYSIS IN THE WAR ON HUMAN TRAFFICKING

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The year 2012 marks the 150th anniversary of the Emancipation Proclamation in the United States. Currently, as many as 27 million men, women, and children are victims of modern slavery. The annual profit from these horrific crimes, approximately 32 billion is second only to the sale of illicit drugs. Investigative and prosecutorial initiatives rely heavily upon traditional law enforcement methods such as surveillance, use of informants, interview and interrogation of victim's and perpetrators. At some level these methods have proven to be valuable. However, the scope and magnitude of the problem will require far greater resources and methods to fully resolve this epic tragedy. Some of the resources should include tools forensic science and the use of modern technologies and biometric databases. Modern forensic science methods and equipment can provide a means to address many of the above listed issues associated with human trafficking and the sex trade. Advances in DNA analysis have provided forensic laboratories and the criminal justice community with a very powerful tool. Many of the procedures are specifically designed to address samples associated with sexual assault evidence, the type of evidence that can be obtained from sex trade victims and or the locations where these crimes occur. The ability to utilize forensic procedures such as DNA analysis and collection of fingerprint evidence can be accomplished in remote and dangerous environments. The US military, specifically United States Armed Criminal Investigation Laboratory (USACIL) has successfully deployed these techniques in battlefield conditions.

Suggested Reading

1 Trafficking in Persons Report 2012, Dept. of State, USA

2 Dept. of Defense Battlefield Forensic Material Collection Training Recommendations

Abstract number: ABS-172-ISABS-2013

FORENSIC APPLICATIONS OF NGS ON LIFETECHNOLOGIES ION PGM INSTRUMENT

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Our insights into the human mitochondrial phylogeny were mainly achieved by sequencing full mitochondrial genomes (mtGenomes). In forensic genetics, (partial) mtGenome information can be used to assign a haplotype to its phylogenetic background that may have a characteristic geographic distribution and thus may provide useful information in a forensic case. Even more relevant to forensics, the haplogroup-specific patterns of mutations form the basis for quality control of mtDNA sequences. The current method for establishing (partial) mtDNA haplotypes is chain termination sequencing, which is laborious, time-consuming, error-prone and expensive. With the emergence of Next Generation Sequencing technologies the body of mtDNA data may be extended in a much faster and cheaper way. Customized chemistries, protocols and analysis software packages could support the community and increase the validity of mtDNA analysis in forensics. We have evaluated the performance of mtGenome sequencing using the Personal Genome Machine (PGM) and compared the resulting haplotypes directly with conventional Sanger-type sequencing (STS). A total of 64 mtGenomes (> 1 million bases) were established that yielded high accordance with the corresponding STS haplotypes (< 0.02% differences). About two thirds of the differences were observed with or around homopolymeric sequence stretches. The sequence alignment algorithm turned out to play a significant role when analyzing mtDNA and further development of alignment software would be desirable to permit its application in forensic genetics.

Suggested Reading

1 Rothberg et al. (2011) An integrated semiconductor device enabling non-optical genome sequencing. Nature 475: 348-352

2 Holland et al (2011) Second generation sequencing allows for mtDNA mixture deconvolution and high resolution detection of heteroplasmy. Croat Med J 52: 299-313.

3 Irwin et al. (2011) mtGenome reference population databases and the future of forensic mtDNA analysis. Forensic Sci Int Genet 5: 222-225

4 Bandelt, Salas (2012) Current next generation sequencing technology may not meet forensic standards. Ibid. 6: 143-145

5 Fendt et al. (2009) Sequencing strategy for the whole mitochondrial genome resulting in high quality sequences. BMC Genomics 10: 139

Abstract number: ABS-231-ISABS-2013

PREDICTING AGE FROM BIOLOGICAL MARKERS IN FORENSIC TRACES

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The advance of genotyping technologies is making the goal of deciphering phenotype from genotype very realistic. Predicting human phenotypes from genotypes is a newly emerging field with relevance for personalized medicine and forensics. A new technique, Forensic DNA Phenotyping, allows investigators to predict suspects' hair and eye color by analyzing stand-alone DNA evidence. Several biochemical analyses, including DNA methylation patterns change, T-cell DNA rearrangements, have been developed in order to predict the age of individual. However, some important phenotypic aspects are not defined by genes, and thus cannot be deduced from the genome. The most important non-genetic information is the age of the culprit, since accurate prediction of age from biological traces at the crime scene could significantly help the investigation. Most human proteins are posttranslationally modified by the addition of complex oligosaccharide structures (glycans) and protein glycosylation could be a potential solution for this challenge since glycan structure and composition is defined by both genetic background and environment. Our recent study on over 5000 individuals from Europe and China revealed very strong association of some IgG glycans with age. Our glyco-age index composed of three IgG glycans explains some 60% of age variance and predicts age from measured glycans. However, IgG glycosylation analysis is not practical for forensic applications and we have developed a method, which uses total blood glycans to predict age. The method has been validated on 600 dried blood spots.

Suggested Reading

1 Kayser M, Schneider PM (2009) DNA-based prediction of human externally visible characteristics in forensics: motivations, scientific challenges, and ethical considerations. Forensic Sci Int 3: 154-161

2 Thanabalasingham G et al. (2012) Glycan profiling of plasma proteins enables diagnostic discrimination between diabetes subtypes. Diabetes

3 Ruiz Y et al. (2012) Further development of forensic eye color predictive tests. Forensic Sci Int 7: 28-40.

4 Zubakov D et al. (2010) Estimating human age from T-cell DNA rearrangements. Current Biol 22: R970-R971

5 Kayser M, de Knijff P (2011) Improving human forensics through advances in genetics, genomics and molecular biology. Nat Rev Genet 12: 179-192.

POSTMORTEM PHARMACOGENETICS AND CAUSE OF DEATH INVESTIGATION

Sajantila A

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Establishing the cause of death in medico-legal terms requires expertise of forensic pathology, imaging, toxicology, biochemistry and genetics. Medico-legal autopsies produce reliable data in investigating unexpected deaths due to natural causes, injury or toxic agents and are fundamental for the legal protection of citizenry. The new genomic technology provides the high throughput procedures for disease and pharmacogenetic testing; these procedures can also be applied to investigation of the medico-legal cause of death. The cost-effective use of pharmacogenetics in medico-legal investigation requires integrating research into forensic pathology, toxicology and genetics. To fully utilize the new technology and concepts, it is crucial that pharmacogenetic methods be integrated into medico-legal autopsy premises and that academic research in this field be activated. This talk will focus on the basic concepts of pharmacogenetics in the context of postmortem investigation and will show illustrative cases and possibilities of using pharmacogenetics to foster understanding of mechanisms underlying different types of completed suicides.

Abstract number: ABS-293-ISABS-2013

PRELIMINARY REPORT ON ANTHROPOLOGY OF 15 X STR LOCI

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A total of 535 chromosomes from US European (132), SW Hispanics (155), African Americans (118), West Africans (primarily Ghana and Nigeria; 30), Ethiopia (34), Southeast Asia (32) and Siberia (34) were tested for 15 X chromosome STR loci (DXS8378, DXS9902, DXS6795 [LG1], DXS7132, DXS6803, DXS6789, DXS7424, DXS101, GATA172D05, DXS7130, GATA165B12 [LG2], HPRTB [LG3], GATA-31E08, DXS10147 and DXS7423 [LG4]), yielding 144 alleles using two multiplexes. The loci were individually analyzed by FST analysis across the seven populations, and aggregately analyzed using PCA followed by hierarchical cluster analysis. All probability values were corrected for multiple tests using the Bonferroni correction. FST analysis indicated that 11/15 loci had FST values >0.05, 9 of which were significant at the p=0.05 level, however only 4/15 were significant after Bonferroni correction. PCA analysis yielded six Eigen vectors, which accounted for 100% of the variance, and generated highly discriminating factor scores for the seven populations. Cluster analysis generated an anthropologically expected tree with African Americans clustering with West Africans on a deep root, East Asian and Europeans on a separate branch, with the Siberian and SE Asians separated by a relatively deep root. An unexpected result was Europeans, SW Hispanics (admixed) and Ethiopians clustering, indicating commonality with Europeans. These results support the use of these markers as tools in the investigation of the origins of modern human populations. Supported by grants from the Columbian College of Arts and Science (GWU) and NIJ grant #2011-DN-BX-K401

Suggested Reading

1 Nature Genetics (2010) 42: 830

2 Forensic Science International: Genetics (2011) 5: 415-421

Abstract number: ABS-189-ISABS-2013

CHARACTERIZATION OF REFERENCE STANDARDS WITH NEXTGEN SEQUENCING PLATFORMS

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Over the past 20 years the Applied Genetics group at the U.S. National Institute of Standards and Technology (NIST) has been providing DNA-based Standard Reference Materials (SRMs) for the human identity testing community. These forensic SRMs are required by the FBI DNA Advisory Board to calibrate DNA typing procedures performed in forensic laboratories. The available SRMs typically consist of genomic DNAs (~100 ng in 50 µL) that have been highly characterized for forensically relevant markers such as core autosomal and Y-chromosome short tandem repeat (STRs) in addition to mitochondrial genome sequence. To date, the characterization of the forensic markers of interest is a combination of either Sanger sequencing or fragment-based genotyping. With the emergence of ultra high-throughput or next-generation sequencing (NGS) technologies to forensic applications we are exploring extensive characterization of forensic DNA-based SRMs. The considerations for additional characterization of reference materials include: source of genomic DNAs, amount of DNA required, genetic markers to be characterized, analysis by multiple technology platforms, and the specific needs of the forensic community. This talk will review the past SRMs and identify requirements for the characterization of future forensic reference materials. Examples of NGS characterization of the mitochondrial sequencing SRMs 2392 and 2392-I will be presented.

Suggested Reading

1 Kline MC et al. (2011) STR sequence analysis for characterizing normal, variant, and null alleles. Forensic Sci Int Genetics 5: 329-332

2 Kayser M, de Knijff P (2011) Improving human forensics through advances in genetics, genomics and molecular biology. Nature reviews. Genetics 12: 179-192

3 Holland MM, McQuillan MR, O'Hanlon K (2011) Second generation sequencing allows for mtDNA mixture deconvolution and high resolution detection of heteroplasmy. Croat Med J 52: 299-313.

4 Bandelt H-J, Salas A (2011) Current Next Generation Sequencing technology may not meet forensic standards. Forensic Sci Int Genetics 6: 143-145

5 Gymrek M et al. (2012) lobSTR: A short tandem repeat profiler for personal genomes Genome Res 22:1154-1162

Abstract number: ABS-271-ISABS-2013

IMPROVED PREDICTION OF EYE AND SKIN COLOR BASED ON EIGHT SNPS

Hart K, Kimura S, Mushailov V, Budimlija Z, Caragine T, Prinz M, **Wurmbach E**
Office of Chief Medical Examiner of the City of New York, New York, Ney York, USA

Aim: The goal of this study was to improve the 7-plex system to predict eye and skin color by increasing precision and detailed phenotypic descriptions. **Methods:** Analysis of an eighth SNP, rs12896399 (SLC24A4), showed a statistically significant association with human eye color. This SNP was added to the 7-plex system (rs12913832 at HERC2, rs1545397 at OCA2, rs16891982 at SLC45A2, rs1426654 at SLC24A5, rs885479 at MC1R, rs6119471 at ASIP, and rs12203592 at IRF4). Further, instruction guidelines on the interpretation of genotypes were changed, based on the analysis of over 800 samples of various populations, the training set, to create a new 8-plex system. **Results:** Validation on over 200 newly collected samples, the test set, revealed improvements by determination of the blue eye and light skin colors and by increasing the number of positive descriptions substantially. It maintains a low error rate. **Conclusions:** The new 8-plex system for the prediction of eye and skin color significantly enhances its former version.

Translational Medicine Program

Abstract number: ABS-313-ISABS-2013

TCR GENE EDITING FOR TREATMENT OF HEMATOLOGICAL MALIGNANCIES

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Adoptive T cell therapy is an innovative therapeutic modality aimed at providing effective and long-lasting tumor-reactive T cells to cancer patients. Unfortunately, T lymphocytes able to recognize tumor cells with sufficient avidity are often deleted or tolerized because tumor antigens are usually self-antigens. New technological gene transfer tools allow today to enforce natural T cells, enabling to generate high numbers of gene-modified tumor-reactive T cells from virtually every cancer patient. Recently, T cells have been manipulated ex vivo with viral vectors coding for tumor specific Receptors or "suicide" genes to potentiate their efficacy and minimize toxicity. T cell receptor (TCR) gene-transfer into T cells is a precious tool for adoptive immunotherapy of cancer patients for whom natural tumor-specific lymphocytes cannot be isolated. Nevertheless, TCR-transferred T lymphocytes differ from their natural counterpart in carrying two different TCRs and this can result in efficacy defects and potential toxicity due to reduced expression of tumor-specific TCR and inappropriate pairing of TCR chains. To completely substitute T cell specificity, we recently developed the TCR gene editing approach, based on the combination of: i. Somatic knockout of the endogenous TCR genes (by transient exposure to α and β-chain specific Zinc Finger Nucleases-ZFN), and ii. Introduction of a tumor-specific TCR by lentiviral vectors (LV). TCR-edited cells express uniquely the tumor-specific TCR at high levels, can be easily expanded to near-purity and proved highly effective and specific in killing cancer cells. Challenges and advances recently made to potentiate adoptive immunotherapy with TCR edited lymphocytes will be discussed.

Suggested Reading

1 Provasi, Genovese et al. (2012) Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nat Med 18: 807-815; doi: 10.1038/nm.2700

2 Cieri et al. (2013) IL-7 and IL-15 instruct the generation of human memory stem T cells from naive precursors. Blood 121:5 73-584. doi: 10.1182/blood-2012-05-431718.

Abstract number: ABS-321-ISABS-2013

WILL CELLULAR IMMUNOTHERAPY FOR CANCER EVER BECOME STANDARD CLINICAL CARE?

Brenner M

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Despite more than two decades of clinical application, cellular immunotherapies for cancer have, almost without exception, failed to make the transition into licensed drugs that are standard of care for patients with malignant disease. This is in stark contrast to the success story of immunotherapy using monoclonal antibodies (MAb). At least part of the delay can be attributed to the dissimilarity between the business models needed to bring standard small molecule drugs/MAb to success and those required for cellular immunotherapies. Unlike small molecules, cellular immunotherapies are usually individualized medicines, intended to be curative rather than ameliorative. They have complex intellectual property, continuing high manufacturing costs, and require iterative cycles of pre-clinical and clinical development to fine-tune their safety and effector function.

Abstract number: ABS-341-ISABS-2013

MOVING CAR-MODIFIED T CELL THERAPY OF CANCER FORWARD IN THE CLINIC

Brentjens R

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T cells can be genetically modified to express artificial T cell receptors, termed chimeric antigen receptors (CARs) targeted to tumor-associated antigens. Previous work in preclinical models has demonstrated the potential of this approach in targeting T cells to systemically disseminated tumor and, in turn, eradicating it. More recently, we and others have successfully moved this technology into the clinic demonstrating early very promising responses with CD19-targeted CAR-modified T cells in patients with relapsed B cell malignancies including acute lymphoblastic leukemia and chronic lymphocytic leukemia as well as indolent B cell non-Hodgkins lymphomas. However, limitations to this adoptive T cell approach need to be addressed before it can be successfully optimized to B cell malignancies and further expanded to other cancers targeting other tumor-associated antigens. One such an approach is the design of "armored CAR" T cells additionally modified to express cytokines or co-stimulatory ligands to enhance in vivo cytotoxicity as well as persistence of CAR-modified T cells following infusion.

STEM CELL TREATMENT IN ALLOGENEIC BONE MARROW TRANSPLANTATION

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Graft v. host disease (GVHD) remains a critical co-morbidity accompanying allogeneic hematopoietic stem cell transplant (HSCT). Prophylactic immunosuppressive drugs can manage GVHD, but bring complications with increased infection and risk of relapse. When acute GVHD does arise, administration of high-dose steroids is the second line treatment, but many patients progress to steroid refractory disease with mortality above fifty percent. Adjunctive cell therapies have been developed to prevent or treat acute GVHD and balance the treatment-induced immunosuppression with the benefit of the graft v. leukemia effect. In 2004, Katarina Le Blanc demonstrated that administration of mesenchymal stromal (stem) cells (MSCs) effectively rescued a steroid-refractory acute GVHD patient. This observation stimulated numerous studies worldwide, sponsored both by academia and industry, in search of the clinical proof of concept for the therapeutic use of MSCs in mitigating GVHD. These studies have yielded mixed results supporting MSC potency, but also raising questions about dose size and dosing regimen. This presentation will review the studies using MSCs, including MultiStem®, for prophylaxis of acute GVHD with dose regimens spanning the first thirty days post HSCT. Clinical results will be presented with a forward-looking perspective.

Abstract number: ABS-173-ISABS-2013

ANALYSIS OF MIXTURES USING DEEP SEQUENCING OF HLA GENES WITH THE 454 GS FLX SEQUENCING SYSTEM: FORENSICS AND CLINICAL APPLICATIONS

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The clonal sequencing property of massively parallel next generation sequencing systems allows for the digital analysis of mixed samples by simply counting sequence reads corresponding to the component alleles. Previously, we have described the development of an amplicon sequencing method using the 454 GS FLX System and the Conexio Assign ATF 454 software for high resolution and high throughput genotyping of the HLA class I and class II loci. This system was used to analyze the blood of a Severe Combined Immunodeficiency (SCIDS) patient and estimate the proportion of maternal cells by determining the proportion of the total HLA-C allelic sequence reads corresponding to the non-transmitted maternal allele. More recently, we have carried out deep sequencing using the DPB1 and DQB1 exon 2 amplicons and modified the Conexio software to measure the background "noise", i.e. sequence reads generated by PCR and/or sequencing error. These sequences typically differed from the true alleles by one base and, in aggregate, represented approximately 2-3% of the total sequence reads (around 40,000 per sample). In mixtures of two cell lines (DPB1*01:01/*04:01 and DPB1*03:01/*04:02) in varying proportions, the minority HLA alleles could be detected at 0.5% with 1 ng DNA input (approximately 140 diploid genomes). Some possible clinical and forensics applications of this deep sequencing and digital analysis system will be discussed.

Suggested Reading

1 Bentley et al. Tissue Antigens, 2009
2 Holcomb et al., Tissue Antigens, 2011
3 Erlich, Tissue Antigens, 2012
4 Moonsamy et al., Tissue Antigens, 2013
5 Erlich et al., Diabetes, 2013

Abstract number: ABS-344-ISABS-2013

NANODIAGNOSTICS: ADVANCES IN POINT OF CARE MOLECULAR DIAGNOSIS OF CANCER BY THE USE OF NANOTECHNOLOGY

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Harvard University, Cambridge, Massachusetts, USA

Diagnostics, monitoring and treatment of cancer rely heavily on traditional and invasive methods of computed tomography guided biopsy and molecular investigation of primary or metastatic tissue. Advent of nanotechnology has provided the opportunity of advanced, sensitive and often non-invasive alternative of molecular interrogation of cancer. In particular, iron oxide nanoparticles coupled with a miniaturized nuclear magnetic resonance (μNMR) device have shown to accurately detect malignant cells in cancer tissue and in peripheral blood obtained from patients with metastatic cancer. Moreover, using a multimarker combination of cancer markers, μNMR is shown to readily detect individual circulating tumor cells (CTC) directly in whole blood without the need for primary purification. Comparative studies with traditional methods have demonstrated a superior ability of μNMR in the detection of CTCs in peripheral blood. μNMR method is sensitive and accurate and, combined with CTC analysis, could provide a fast, point of care cancer diagnostic tool while avoiding the need for invasive core biopsies.

GENOME-WIDE IDENITIFICATION OF PATHOGENIC MUTATIONS IN PATIENTS WITH NEUROLOGICAL AND DEVELOPMENTAL DISEASE

Goldstein DB

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One primary challenge in the interpretation of large-scale sequencing studies is the huge number of candidate variants that emerge. This occurs primarily because there are many functional variants in every sequenced genome and because our ability to prioritize variants based on bioinformatic criteria remains limited. Integrating functional characterization of identified mutations with careful genome interpretation can often provide compelling evidence implicating new disease-causing mutations and genes in phenotypically well-characterized patients. Here I report on progress in sequencing the data of an affected proband and proband's parents. In addition, I report on isolated but clearly genetic conditions including patients from hospitals in North Carolina and Israel. Next, I consider results from the Epi4K consortium focusing in particular on the analysis of the complete exome sequences of ~300 trios of patients with epileptic encephalopathies leading to secure identification of new disease causing pathways.

LIVER STEM CELLS FOR TREATMENT OF LIVER-BASED INBORN ERRORS OF METABOLISM - A PROMETHERA BIOSCIENCES PRACTICAL CASE

Halioua E

Abstract not provided!

Abstract number: ABS-323-ISABS-2013

ENHANCING IQ OF CHIMERIC ANTIGEN RECEPTOR-REDIRECTED T CELLS

Jensen M

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Recent conceptual as well as technological advances in the areas of molecular immunology, gene transfer, and cell processing have fostered increasingly sophisticated translational applications of adoptive T cell therapy for oncologic disease employing genetically-modified T-lymphocytes. My laboratory's work focus' on T-cell genetic modification for re-directing antigen specificity to tumors utilizing recent advances not only in the composition and specificity of receptor antigen recognition domains, but also the evolution of multifunctional cytoplasmic signaling domains developed for these chimeric antigen receptors (CARs) that provide dual activation and co-stimulatory signaling. My group is also investigating the context of adoptive transfer with respect to the conditioning of the recipient for enhanced T-cell engraftment and expansion, the grafting of CARs on to central memory T-cells having endogenous TCR specificities for viral epitopes to which the host has robust immunity, and, the provision tumor microenvironment survival capabilities. The increasingly broad array of genetic manipulations including not only transgene insertion, but targeted gene knock out using engineered targeted nucleases such as TALEN's and ZFN, as well as expression regulatory constructs provides for the creation of synthetic biology of orthogonal immune responses based on gene modified T cell adoptive transfer. The next decade of advances in this arena will depend on iterative bench-to-bedside back-to-the-bench translational studies capable of sustaining the evolution of these technologies in the context of clinical parameters relevant to the pediatric oncology patient population.

Abstract number: ABS-337-ISABS-2013

NEXT GENERATION SEQUENCING STUDIES OF HEREDITARY DISORDERS IN HIGHLY INBRED POPULATIONS

Kanaan MN

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Identification of inherited mutations has been an ongoing challenge in medical genetics. Using advanced targeted DNA capture and massively parallel sequencing technologies, in conjunction with homozygosity mapping relevant for consanguinous families, we are meeting this challenge for hereditary hearing loss, breast cancer and other genetic abnormalities. Our cohort consists of Palestinian Arab families of variable size at the onset of hearing loss and breast cancer. We constructed two custom design arrays of cRNA oligonucleotides containing 250 genes, responsible for both human and mouse deafness and a custom array containing 38 breast and ovarian cancer (BROCA) associated genes. We prepared paired-end libraries, followed by cluster amplification on v4 Illumina flow cells with our bar-coded multiplexed samples. A 2x72bp paired end recipe was used, resulting in a median base coverage of 300-572x and overall, 94.7% of our targeted bases covered by more than 10 reads, which was our cutoff for variant detection. We generated SNP and indel calls for our samples and filtered the variants against those of dbSNP131 and the 1000 Genomes project to identify private and rare variants. Novel genes and mutations were discovered. Most compelling, a number of mutations were found in genes previously known as involved only in mouse deafness. Protein structures were predicted to provide insight into the relationship of mutations with the predicted phenotypes. Characterization of the proteins encoded by these genes will enable a comprehensive understanding of biological mechanisms involved in the pathophysiology of these disorders.

Suggested reading

1 Brownstein Z et al. (2011) Targeted genomic capture and massively parallel sequencing to identify genes for hereditary hearing loss in Middle Eastern families. Genome Biol. 12: 9-15

2 Avraham KB, Kanaan M (2012) Genomic advances for gene discovery in hereditary hearing loss. J Basic Clin Physiol Pharmacol 23: 93-97

Abstract number: ABS-345-ISABS-2013

BIOLOGICAL TALES OF HUMAN DISEASE MUTATIONS

Oz-Levi D, Ben-Asher E, Olender T, **Lancet D**

Weizmann Institute of Science, Rehovot, Israel

Monogenic diseases, natural human knockouts, constitute a rich source of biological insight. With the groups of David Goldstein (Duke, USA) and Elon Pras (Sheba, Israel) we investigate >100 cohorts of Jewish origins, to shed light on novel disease mechanisms. We make use of our two databases: GeneCards, (www.genecards.org), with information on ~120,000 gene entries, including the largest assemblage of ncRNA genes (Bioinformatics 2013, 29:255-6), and newly developed integration of biological super-pathways; and MalaCards (http://www.malacards.org), the most comprehensive compendium of human diseases, with ~17,000 entries. Four deciphered diseases provide examples for the broad spectrum of biomolecular mechanisms uncovered by whole exome sequencing. The first is a recessive 1-base deletion mutation in TECPR2, an autophagy-implicated tectonin propeller domain protein, elucidated in five individuals with hereditary spastic paraplegia (Am J Hum Genet. 2012, 91:1065-72). This reveals for the first time a role for disrupted autophagy in Mendelian neurodegenerative diseases. The second is a missense mutation in the metabolic enzyme aspargine synthase (ASNS), discovered in families with severe microcephaly. Like other known microcephaly genes, ASNS is associated with cell cycle defects and its discovery implicate a new pathway in developmental brain disorders. The third case is a mutation in intractable diarrhea of infancy syndrome (IDIS), traced to a deletion of enhancer region active in the gastrointestinal tract. The last case implicates a splice site mutation in Talin1 (TLN1) in capillary leak syndrome. Further exome scrutiny will likely uncover a plethora of unexpected pathways, and contribute to a systems medicine overview of human disease.

Suggested reading

1 Belinky F et al. (2013) Non-redundant compendium of human ncRNA genes in GeneCards. Bioinformatics 29: 255-261

2 Rappaport N et al. (2013) MalaCards: an integrated compendium for diseases and their annotation. Database (Oxford): bat018; doi: 10.1093/database/bat018.

3 Oz-Levi D et al. (2013) TECPR2: A new autophagy link for neurodegeneration. Autophagy 9: 5, 1-2

4 Hasin-Brumshtein Y, Lancet D, Olender T (2009) Human olfaction: from genomic variation to phenotypic diversity, Trends Genet. 25: 178-184

Abstract number: ABS-200-ISABS-2013

HAND AND FACE TRANSPLANTATION: OVERVIEW AND OUTCOMES

Moran S

Mayo Clinic, Rochester, Minnesota, USA

Over the past decade, hand transplantation has become an established means of limb reconstruction for patients with severe injuries to the hand and forearm. Hand transplantation, like face transplantation, is a form of vascularized composite allotransplantation (VCA). Similar to solid organ transplantation, VCA consists of the transfer of living tissue from a donor to the recipient. The transplanted hand requires a vascular connection from the recipient for survival. The patient requires standard immunosuppression to prevent rejection. Unlike solid organ transplantation, nerve regeneration into the transplanted tissue is required for sensation within the skin and re-animation of muscles within the transplanted tissue. VCA should be differentiated from acellular allografts, which have been used for several years as non-vascularized structural grafts, such as tendon grafts and bone allografts used in orthopedic surgery. Since 1998 over 70 hand transplants have been performed successfully around the world, and there have been over ten cases of face transplantation. This presentation will provide a brief overview of the current state of hand and face transplantation and the development of Mayo Clinics hand and face transplantation programs.

Abstract number: ABS-320-ISABS-2013

CXCR4+ AND FLK-1+ IDENTIFY CIRCULATING CELLS ASSOCIATED WITH IMPROVED CARDIAC FUNCTION IN PATIENTS FOLLOWING MYOCARDIAL INFARCTION

Perez-Terzic C

Mayo Medical School, College of Medicine; Department of Molecular Pharmacology and Experimental Therapeutics, Division of Cardiovascular Diseases, Department of Medicine; Division of General Internal Medicine and Transplant Center, Department of Medicine, Center for Regenerative Medicine, Mayo Clinic, Rochester, Minnesota, USA

Biomarkers CXCR4/FLK-1 select cardiac-progenitors from a stem-cell pool in animal models. However, the translational value of these cells in ischemic heart disease is unknown. Here, flow-cytometry identified CD45-/CXCR4+/FLK-1+ cells in 30 individuals without ischemic heart disease and 33 first-time acute myocardial infarction (AMI) patients. Compared to controls, AMI patients had higher CD45-/CXCR4+/FLK-1+ cell load at 48 hours (baseline) and 3- and 6-months post-AMI (p=0.003,0.04,0.04, respectively). Cardiovascular risk factors as well as left-ventricular ejection fraction were not associated with the cell load. 2D-speckle-tracking strain echocardiography assessment of systolic function showed improvement in longitudinal-strain and dyssynchrony during follow-up associated with longitudinal increases in and higher baseline CD45-/CXCR4+/FLK-1+ cell load (r=-0.525,p=0.025; r=-0.457,p=0.29, respectively). In conclusion, CD45-/CXCR4+/FLK-1+ cells are present in adult human circulation, increased in AMI and associated with improved left-ventricular systolic function. Thus, CD45-/CXCR4+/FLK-1+ cells may provide a diagnostic tool to follow cardiac regenerative capacity and have the potential to be a prognostic marker for AMI.

Suggested Reading

1 Anversa P et al. (2006) Life and death of cardiac stem cells: a paradigm shift in cardiac biology. Circulation 113: 1451-1463. doi:10.1161/circulationaha.105.595181

2 Bergmann O et al. (2009) Evidence for cardiomyocyte renewal in humans. Science 324: 98-102. doi:10.1126/science.1164680

QUALITY CONTROL VALIDATION OF CELL EXPANSION SYSTEMS FOR ISOLATION AND CULTURE OF CELL THERAPY PRODUCTS

Pinxteren J

ReGenesys bvba, Heverlee, Belgium

MultiStem® cells are bone marrow derived nonhematopoietic adherent stem cells with a large expansion capacity and remarkable biological plasticity. They home and integrate into damaged tissues and modulate immunity, angiogenesis and other effects by cell-cell contact and paracrine regulation. Like mesenchymal stem cells (MSC), allogeneic MultiStem cells are non-immunogenic and immunosuppressive in vitro. However, MultiStem cells are different in phenotype, gene and protein expression and in higher proliferation capacity than MSC. MultiStem are currently in Phase II clinical trials for ischemic stroke and ulcerative colitis. Earlier Phase I trials for graft-v-host disease and acute myocardial infarction were successful. The clinical MultiStem dose requires the order of 200 million cells. The requirement for large cell numbers makes standard cell culture conditions expensive, labor intensive and prone to contamination. We have optimized a functionally closed automated cell expansion system for large-scale cell culture in the hollow-fiber bioreactor Quantum Cell Expansion System. We optimized this system for MultiStem culture, including stem cell isolation from bone marrow and clinical scale expansion. We could reach up to one billion cells after a six-day culture starting from ten million cells. Quality control showed that MultiStem harvested from the Quantum compared favorably to the cells generated by standard cell culture on plastic. Thus, the system can provide clinical cell doses strongly reducing labor without the need of cGMP-grade clean rooms. Recently we expanded and isolated cells similar to MultiStem from rat and horse. Currently, we are focusing on expanding cell products under xeno-free conditions.

DISEASE HETEROGENEITY AND ACCURACY OF DIAGNOSTICS FOR PERSONALIZED MEDICINE

Prendergast FG

Mayo Clinic, Rochester, Minnesota, USA

Patient and disease heterogeneity is the principal issue to be resolved for realization of personalized medicine. This is especially a problem for management of patients with cancer. In principle, we know what needs to be done, but the problem, in fact, is in most instances far more complex than is usually communicated. Resolution requires especially more genomic and epigenomic data gathered on a broader array of tissues, generated with even greater accuracy, and subjected to far more incisive computational analysis of the biological networks that drive pathogenesis.

Abstract number: ABS-190-ISABS-2013

NEANDERTHAL MAN'S PLACE IN NATURE

Rak Y

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Ever since its discovery, the Neanderthal man has been an enigma. Much of this stems from the clash between the eagerness to consider the Neanderthal a link in the modern human evolutionary lineage and the evolutionary difficulties posed by the Neanderthal skeleton's unique anatomy. The Neanderthal's fundamental boney facial architecture, his mandibular morphology (and what is implied by them on the biomechanics of his masticatory system) and the anatomy of its skull base, all emerge as part of a complex, highly derived morphology. These unique characteristics provide support for the contention that Neanderthals do not play a role (except for a shared past) in modern human natural history, either through the so-called "regional continuity" or through any other form of anagenetic progression. Instead, the Neanderthal lineage is a side branch that became extinct about 25,000 to 30,000 years ago.

Abstract number: ABS-182-ISABS-2013

THERMORESPONSIVE BIOPOLYMERS FOR TUMOR-SPECIFIC DRUG DELIVERY

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Severe adverse effects and normal tissue tolerance limit the current treatment of solid tumors as a fraction of an administered drug reaches the tumor, while the remainder harms healthy tissues. To expand the potential usefulness of current therapeutic approaches to solid tumors, we developed an externally triggered system for the selective delivery of therapeutics to these tumors.. Our proposed drug carrier, based on the thermally responsive biopolymer elastin-like polypeptide (ELP), is soluble at physiological temperature, but will undergo phase transition and aggregate in response to externally applied mild hyperthermia (40-41˚C). The coding sequence for our ELP was modified by adding the cell penetrating peptide (CPP) to promote tumoral and cellular uptake of the polypeptide, along with therapeutic peptides or small molecule drugs that inhibit cancer cell proliferation. The novelty and significance of our drug targeting system thus lie in the synergistic elements comprising the delivery system, along with its coordinated utilization of hyperthermia techniques and standard-of-care now utilized in the cancer clinic. This synergy and coordination can selectively induce intra-tumoral accumulation of a broad range of anti-cancer therapeutics. Moreover, the developed biopolymer carrier is innovative as it combines: passive targeting properties of macromolecular carriers, facilitated by enhanced permeability and the retention effect; active drug targeting to the tumor site, catalyzed/activated by an external trigger; and an efficient, intracellular tumor drug delivery approach, mediated by a CPP. This novel approach can be integrated into current systemic therapies.

Suggested Reading

1 Walker L et al. (2012) Cell penetrating peptides fused to a thermally targeted biopolymer drug carrier improve the delivery and antitumor efficacy of an acid-sensitive doxorubicin derivative. Intl J Pharmaceutics 436: 825-832, PMID:22850291

2 Moktan S et al. (2012) Thermal targeting of an acid-sensitive doxorubicin conjugate of elastin-like polypeptide enhances the therapeutic efficacy compared to the parent compound. Mol Cancer Ther. 11: 1547-1556. PMID:22532601

3 Bidwell G.L III, Perkins E, Raucher D (2012) A thermally targeted c-Myc inhibitory polypeptide inhibits breast tumor growth. Cancer Lett. 319:136-143, PMID: 22261328

4 Moktan S, Raucher D (2012). Anticancer activity of proapoptotic peptides is highly improved by thermal targeting using elastin-like polypeptides. Intl J Peptide Res Therap, in press.

5 Bidwell GL III, et al. (2013) Thermally targeted delivery of a c-Myc inhibitory polypeptide inhibits tumor progression and extends survival in a rat glioma model. PLoS ONE, in press

Abstract number: ABS-324-ISABS-2013

PHARMACOLOGICAL APPROACH TO SPINAL CORD INJURY REGENERATION

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Spinal cord trauma creates a complex wound that manifests with a cascade of secondary responses, including inflammation and neurodegeneration, the latter of which is largely refractory to repair. A growing understanding of spinal cord injury (SCI) pathophysiology has resulted in the development of specific clinical management strategies, but there remains no drug treatment that effectively improves outcome. An important therapeutic tactic is to minimize secondary injury occurring at acute and more chronic stages, thereby preventing pathogenesis and promoting an environment favoring endogenous repair and the efficacy of growth promoting interventions and rehabilitation. Among neurotoxic factors deregulated in SCI are serine proteases of the thrombolytic, fibrinolytic and kallikrein families either as a result of elevations in endogenous cells, secretion by infiltrating immune cells or extravasation. We recently discovered these enzymes exert their cellular effects by cleaving and thereby activating G-protein coupled receptors termed Protease Activated Receptors (PARs). As cell surface receptors, PARs endow cells with the ability to respond, or to over respond, to the rapidly changing proteolytic microenvironment that occurs with CNS trauma, inflammation and blood brain barrier breakdown. Current efforts are focused on testing the hypothesis that PARs regulate unique cellular responses in the traumatically injured spinal cord including neuron degeneration, astrogliosis and demyelination, and therefore that PARs can be therapeutically targeted to prevent neural injury and promote repair. If this hypothesis is correct, PARs may serve as targets for the development of new pharmacotherapies to promote repair and regeneration in cases of SCI and other debilitating neurological conditions.

Suggested Reading

1 Vandell AG et al. (2008) Protease activated receptor dependent and independent signaling by kallikreins 1 and 6 in CNS neuron and astroglial cell lines. J Neurochem 107: 855-870.

2 Scarisbrick IA et al. (2012) Kallikrein 6 is a novel molecular trigger of reactive astrogliosis. Biol Chem 393: 355-367.

3 Scarisbrick IA et al. (2012) Kallikrein 6 regulates early CNS demyelination in a viral model of multiple sclerosis. Brain Pathol 22: 709-722.

4 Yoon H et al. (2013) Kallikrein 6 signals through PAR1 and PAR2 to promote neuron injury and exacerbate glutamate neurotoxicity. J Neurochem, in press

5 Burda JE et al. (2013) A critical role for PAR1 in KLK6-mediated oligodendrogliopathy. GLIA, in press

HIP DECOMPRESSION BY BONE MARROW CONCENTRATE IN EARLY OSTEONECROSIS OF FEMORAL HEAD

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Mayo Clinic, Rochester, Minnesota, USA

Aim: To describe our surgical procedure for the treatment of osteonecrosis of the femoral head using a minimally invasive technique. **Methods:** Currently we use a combination of an outpatient, minimally invasive iliac crest bone marrow aspirations and decompressions of the femoral head to treat osteonecrosis of the femoral head at our institution. Following the decompression of the femoral head, adult mesenchymal stem cells obtained from the iliac crest are injected into the area of osteonecrosis. Patients are then discharged from the hospital to home using crutches to assist with ambulation. **Results:** Seventy-seven hips have undergone minimally invasive decompression augmented with concentrated bone marrow at our institution. Sixteen hips (21%) have progressed to further stages of osteonecrosis, ultimately requiring total hip replacement. Significant pain relief was reported in 86% of patients (n=60), while the rest of patients reported little or no pain relief. There have been no significant complications in any patient undergoing this procedure. **Conclusion:** The use of a minimally invasive decompression augmented with concentrated bone marrow results in significant pain relief and halts the progression of disease in a majority of patients who undergo the procedure.

Abstract number: ABS-334-ISABS-2013

DEVELOPING A NEURAL STEM CELL THERAPY FOR STROKE DISABILITY: THE CLINICAL PATHWAY

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ReNeuron Limited, Guildford, UK

Human neural stem cell (hNSC) line, CTX0E03, was clonally derived from early expansion of fetal cortical hNSCs following retroviral insertion of a c-mycERTAM fusion gene that permits controlled expansion in the presence of growth factors and 4-hydroxytamoxifen. The CTX0E03 cell line has been banked and is used in the GMP (Good Manufacturing Practice) manufacture of CTX Drug Product (CTX-DP), an advanced therapy medicinal product currently in clinical development. Preclinical studies with CTX DP in MCAo (Middle Cerebral Artery Occlusion) rat stroke models have shown dose-related behavioral recovery in sensorimotor function and evidence of cell survival, limited migration, striatal angiogenesis and endogenous neurogenesis. A Phase I dose escalation safety trial (the Glasgow PISCES trial) in stable, chronic stroke patients (6 months to 5 years after stroke) has now completed recruitment and updated data, recently presented at the European Stroke Conference will be reported. No safety issues attributable to the CTC-DP have been seen and some improvements in neurological function warrant further investigation in a Phase II efficacy trial. In this talk, I will describe how ReNeuron has developed a suitable protocol that will allow an ethically sound, cost-effective and clinically meaningful efficacy endpoint to be established in a Phase II trial for a stem cell therapy for stroke disability.

Abstract number: ABS-339-ISABS-2013

MULTIPLE LARGE GENES TARGETED BY GENOMIC INSTABILITY IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

Smith DI

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Extremely large genes pose a considerable problem for cells because—to make a primary RNA transcript from one of these genes—it takes so long that it can actually interfere with DNA replication. Thus, it is not too surprising that a number of the largest human genes actually reside within common fragile sites (CFSs), large regions of profound genomic instability found in all individuals. We are studying oropharyngeal squamous cell carcinomas (OPSCC), cancers that form in the base of the tongue and the tonsils. We utilized RNAseq to sequence the transcriptomes of twelve OPSCCs. We examined the expression of transcripts for the forty largest human genes, which span more than one megabase of genome sequence and found that in eight OPSCCs expression for many of these genes was decreased. In the remaining four OPSCCs we did not detect decreased expression of any of these genes. Several patients with tumors demonstrating decreased expression for many large genes had metastatic disease. We hypothesize that tumors with decreased expression of many large genes are also characterized by much more genomic instability. Since a number of the largest human genes are actually spanned by a CFS region, this would suggest a link between increased genomic instability and decreased expression of multiple large CFS genes. The decreased expression of many of these genes together should have a profound effect on the resulting cellular phenotype.

Suggested Reading

1 Laborde RR et al. (2012) Transcriptional Profiling by sequencing of oropharyngeal cancer. Mayo Clin Proc 87: 226-232.

2 Smith DI et al. (2006) Common fragile sites, extremely large genes, neural development and cancer. Cancer Lett 232: 48-57.

REGENERATIVE MEDICINE: AT THE CORE OF HEALTHCARE TRANSFORMATION

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Mayo Clinic, Rochester, Minnesota, USA

The pandemic of chronic diseases, compounded by the scarcity of usable donor organs, mandates radical innovation to address the growing unmet needs of individuals and populations. Beyond life-extending measures that are often the last available option, regenerative strategies offer transformative solutions in treating degenerative conditions. By leveraging newfound knowledge of the intimate processes fundamental to organogenesis and healing, the emerging regenerative armamentarium aims to boost the aptitude of human tissues for self-renewal. Regenerative technologies strive to promote, augment, and reestablish native repair processes restituting organ structure and function. Multimodal regenerative approaches incorporate transplantation of healthy tissues into damaged environments, prompt the body to enact a regenerative response within damaged tissues, and/or use tissue engineering to manufacture new tissue. Stem cells and their products demonstrate a unique aptitude to form specialized tissues and/or promote repair signaling providing active ingredients of regenerative regimens. Concomitantly, advances in materials science and biotechnology have unlocked additional prospects for growing tissue grafts and engineering organs. Translation of regenerative principles into practice demonstrates feasibility and safety in the clinical setting. Regenerative medicine and surgery are thus poised to transit from proof-of-principle studies towards clinical validation, and ultimately standardization paving the way for next generation individualized management algorithms.

Abstract number: ABS-330-ISABS-2013

PATTERNS OF Y-CHROMOSOME DIVERSIFICATION

Underhill P

Stanford University, Stanford, California, USA

Exposing the emergence of genetic diversity in contemporary populations and its flow across the geographic landscape provides one record of pre-historic and historic population movements. Subsequent in situ genetic differentiation provides insights into the origins of regional populations. Although recovering human history perspectives from a single genetic locus must be evaluated cautiously, the lower effective population size of the Y chromosome compared to diploid components of the genome coupled with a robust and well-resolved phylogeny (gene tree) constitutes a molecular narrative offering an independent perspective regarding human migratory history. The sensitivity of haploid genomes to genetic drift and founder effects often results in a strong correlation with geography at both inter or intra continental scales. Intrinsic to the phylogeny is time depth with the deeper bifurcations reflecting the early periods of human evolution followed by more recent molecular innovations consistent with post-glacial demographic radiations. The earliest levels of Y-chromosome genetic differentiation within Eurasia subsequent to the initial "Out of Africa" event by anatomically modern humans will be discussed. This level of phylogenetic unification extends our knowledge of the actual evolutionary relationships within the more internal framework of the worldwide phylogeny and provides specific genetic evidence of an initial common denominator between the original peoples of the Near East and Europe. Examples of phylogenetically and geographically distinctive subsets of chromosomes are described that have characteristics consistent with isolation and subsequent indigenous innovation.

Suggested Reading

1 Underhill PA, Kivisild T (2007) Use of Y chromosome and mitochondrial DNA population structure in tracing human migrations. Annu. Rev. Genetics 41: 539-564.

2 Jobling MA, Tyler-Smith C (2003) The human Y chromosome: an evolutionary marker comes of age. Nature Rev Genetics 4: 598-612

3 Underhill PA et al. (2010) Separating the post-Glacial coancestry of European and Asian Y chromosomes within haplogroup R1a. Eur J Human Genetics 18: 479-484

4 Chiaroni J, Underhill PA, Cavalli-Sforza LL (2009) Y chromosome diversity, human expansion, drift, and cultural evolution. PNAS 106: 20174-20179

5 Hammer MF et al. (2009) Extended Y chromosome haplotypes resolve multiple and unique lineages of the Jewish priesthood. Human Genetics 126: 707-717

Abstract number: ABS-332-ISABS-2013

GENETICS OF SLAVIC-SPEAKING PEOPLES: PATRILINEAL, MATRILINEAL AND AUTOSOMAL PORTRAITS

Villems R

Estonian Biocentre, Tartu, Estonia

Genes and languages and, in particular genes and language families, is one of the traditional fields of interdisciplinary inquiry for quite a few decades. Linguists, as well as population geneticists, like to build trees. And it is perhaps worthwhile to indicate that their toolboxes for construction of phylogenies are not so different and became recently even quite similar to those we use in population genetics. In my talk, I will focus on Balto-Slavic speaking peoples. This group of languages within the Indo-Hittite macro-family of languages is well understood by linguists, and, what is also important to notice, different linguistic schools (almost) agree on the topology of this tree, starting from the split between its Baltic (Latvian, Lithuanian and extinct Prussian) and Slavonic branches some 3000-3500 YBP. Our joint study with research groups and colleagues from many countries covers 16 populations-two Baltic and 14 Slavic. While our Balto-Slavic mtDNA and Y-chromosomal data sets cover about 6000 and 7000 samples, respectively, I will focus on high-coverage autosomal genotyping of the Balto-Slavic populations in the context of their neighboring populations over the entire region of South, Central and East Europe, the Caucasus, Central Asia and West Siberia and will discuss the found patterns in terms of their genetic history.

Abstract number: ABS-153-ISABS-2013

STEMNESS ARISEN

Vuk-Pavlović S

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Recent evidence for induction of stem cell characteristics in somatic cells by epithelial-mesenchymal transition, by induced activation of a small number of transcription factors, or by modification of microenvironment has invalidated the once held notion of differentiation as a strictly unidirectional deterministic process. Together with the broad interest elicited by current clinical trials testing different cellular preparations dubbed "stem cells," this necessitates reconsideration of the very definition of the stem cell (1). As a result, the focus has shifted from consideration of "stem cells" as a class of cells to "stemness" as a property that can be acquired, conferred and/or retained by cells. What is stemness that allows a cell (population) to divide, maintain the size of its pool and differentiate at the same time? How is it defined, induced, maintained? A recent view posits "stemness as a cell default state" (2). It is based on experimental evidence compatible with a deterministic model of stemness maintained as long as maintained is the intrinsic/inherent inhibition to differentiation. Another model invokes stochastic gene and protein expression that drives transitions among deterministic attractors characterizing stemness and the possible differentiation states (3). Most recent developments interrogate the factors that define all levels of cell differentiation (i.e., attractors) as properties emergent within complex biological systems.

Suggested Reading

1 Lander AD (2009) J Biol 8: 70 http://jbiol.com/content/8/8/70

2 Casanova J (2012) EMBO Reports, 13: 396-397

3 MacArthur BD, Ma'ayam A, Lemischka IR (2009) Nature Rev Mol Cell Biol 10: 672-681

TREATING CONGESTIVE HEART FAILURE BY AUTOLOGOUS ADHERENT PROGENITORS

de Waele P

Cardio BioSciences, Mont-Saint-Guibert, Belgium

Our company is developing the proprietary cardiopoiesis platform based on fundamental research and technology from Mayo Clinic (Rochester, Minnesota, USA). This novel platform is designed to drive the differentiation of multipotent stem cells to the cardiac programme. The most advanced cell therapy product candidate is autologous C3BS-CQR-1, intended for heart failure. Its purported actions are direct—repairing cardiac tissue through proliferation, engraftment and terminal differentiation of transplanted cells—and the beneficial indirect paracrine mode through secretion of factors by the transplanted cells. Preclinical data show that hearts of rodents with ischemic heart failure can be anatomically "repaired" or reconstructed by stem cells from cardiac patients yielding important benefits for heart function and survival. A phase II controlled randomized clinical trial led to positive outcome—an increase of 25% in left ventricle ejection fraction ($p<0.0001$) and a statistically significant increase in exercise capacity measured by the six-minute walking distance test (+77m change at 6 months versus baseline in comparison to the control group; $p<0.01$). Six months after treatment, the cell therapy group had a 7 percent absolute improvement in ejection fraction over baseline versus a non-significant change in the control group. This improvement in ejection fraction is dramatic, particularly given the length of time between ischemic injury and cell therapy. This success triggered the authorization to start the Phase III CHART programme (Congestive Heart Failure Cardiopoietic Regenerative Therapy). The company also developed C-Cath®, an intra-myocardial delivery catheter designed to enhance myocardial therapeutic agent retention.

GUCY2C AT THE INTERSECTION OF OBESITY AND COLORECTAL CANCER

Waldman SA, Lin JE, Colon-Gonzalez F, Snook A, Valentino M, Kim G, Blomain E, Hyslop T

Department of Pharmacology and Experimental Therapeutics, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

While there is an association between body mass and intestinal malignancies, mechanisms that link obesity and colorectal tumorigenesis remain undefined. Here, we explore the hypothesis that obesity and colorectal cancer are linked through dysregulation of hormone axes mediated by GUCY2C, the receptor for guanylin in the colorectum and uroguanylin in small intestine. These hormones are the most commonly lost gene products in colorectal cancer, and their universal loss early in neoplasia is required for tumorigenesis. GUCY2C regulates intestinal homeostasis, and its silencing by hormone loss accelerates proliferation, increases DNA damage, and reprograms cellular metabolism, increasing colorectal cancer in mice. Beyond its role in cancer, GUCY2C and uroguanylin comprise a gut-brain endocrine axis that regulates appetite, body mass and metabolism. Elimination of this endocrine axis in mice results in hyperphagia, obesity, and the metabolic syndrome. Recent studies revealed that GUCY2C hormone expression in colon is eliminated by diet-induced obesity. Hormone expression is reversibly modulated by ingested calories, rather than by the pathophysiological milieu of obesity. Moreover, enforced expression of GUCY2C hormone by intestinal cells eliminates tumorigenesis induced by obesity. Thus, ingested calories contributing to obesity recapitulate mechanisms underlying sporadic colorectal cancer by suppressing hormone expression, silencing GUCY2C, and disrupting epithelial homeostasis. They define a mechanism linking diet and obesity to colorectal cancer and identify silencing of the GUCY2C tumor suppressor as a link between reversible risk factors like calories and molecular mechanisms underlying cancer development. They offer strategies for countering these risks, including calorie restriction and oral hormone therapy.

Abstract number: ABS-317-ISABS-2013

UTILIZING PRIOR INFORMATION IN A BAYESIAN DESIGN TO IMPROVE THE POWER OF GENETIC ASSOCIATION STUDIES

Zidovetzki R[1], Jacob C[2], Armstrong D[1,2]

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A novel Bayesian multistep design was utilized to study genetic association of systemic lupus erythematosus (SLE). This method selects genes to include in an association study on the basis of their prior likelihood of association with a disease, thereby increasing the overall power of the study by reducing the multiple testing correction. To determine the prior likelihood of association with SLE, we developed programs, which use expert information and existing databases to select and prioritize genes. The first step selected ~1,000 genes (containing ~10,000 SNPs) which were genotyped in 251 SLE trios (patient and both parents). Two novel genes, SELP and IRAK1 were found to be associated with SLE with False Discovery Rate (FDR)<=0.05. Additionally, we identified 17 "noteworthy" genes with FDR<=0.8 which were investigated in a follow-up case/control study. The case/control study confirmed significance of the most of these genes, resulting in identification of a number of novel SLE-associated genes. We are currently working to identify causative mutations from this study. In the case of NCF2, computational modeling indicated that the H389Q mutation affected interaction of NCF2 with its partner in ROS production. Introducing this mutation into a cell system led to a reduction in ROS production, indicating that H389Q is an SLE-causative mutation. This methodology is now being adapted in a Bayesian approach to the whole genome sequencing where the prior likelihood of a gene's association is used to calculate the posterior probability resulting in a sharp increase in the power of the study.

Suggested Reading

1 Jacob CO et al. (2007) Identification of novel susceptibility genes in childhood-onset systemic lupus erythematosus using a uniquely designed candidate gene pathway platform. Arthritis Rheum. 56: 4164-4173.

2 Armstrong DL, Jacob CO, Zidovetzki R (2008) Function2Gene: a gene selection tool to increase the power of genetic association studies by utilizing public databases and expert knowledge. BMC Bioinformatics 9:311.

3 Armstrong DL et al. (2009) Identification of new SLE-associated genes with a two-step Bayesian study design. Genes Immun 10: 446-456

WORKSHOPS

Workshop by Croatian Academy of Legal Sciences: Medicine and the Law — Medical Expertise and Admissibility of Evidence Obtained Through Analysis in Court Proceedings

Abstract number: ABS-299-ISABS-2013

INTERNATIONAL LEGAL STANDARDS CONCERNING THE USE OF DNA ANALYSIS WITHIN CRIMINAL JUSTICE SYSTEM

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Suppression of crime demands the use of modern and effective methods. One of these methods is DNA analysis. Notwithstanding the importance of the facts that could be determined by method, use of DNA analysis has to correspond to fundamental rights of the defendant in criminal proceedings. Therefore, this overview of the international legal standards concerning DNA analysis within the framework of the criminal justice system includes relevant legal documents (i.e. EU Council Framework Decision 2008/977/JHA of 27 November 2008 on the protection of personal data processed in the framework of police and judicial cooperation in criminal matters, COE Recommendation on use of DNA analysis within the framework of the criminal justice system) as well as recent jurisprudence of international courts (i.e. ECHR, S and Marper v. UK) that established legal standards important for balancing between aforementioned values and interests.

Abstract number: ABS-303-ISABS-2013

MEDICAL EXPERTISE IN FAMILY LAW PROCEEDINGS - FROM RELATIVITY TO ABSOLUTE VALUES

Milas Klarić I

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In family proceedings, medical expertise is commonly used in two legal institutes. The first one relates to paternity and maternity proceedings. Because these are civil proceedings, forcing the parties to participate in the expert evaluation is not possible. But, when it comes to expertise, given the recent achievements in medical science, the result is almost 100 percent certain. A major advance in Croatian family legislation followed after Mikulic vs. Croatia case before the European Court of Human Rights. On the other hand, for the application of medical expertise in the proceedings for deprivation of legal (business) capacity, although it can be the backbone of the proceedings, there are no clear criteria. The proceedings often lead to uncertain interdependence between real opportunities of medical experts and judges within the legally defined powers and, very often, to violation of human rights of persons with disabilities.

Abstract number: ABS-296-ISABS-2013

LEGAL ISSUES FOR RESEARCHERS AND MEDICAL PRACTITIONERS: IT IS NOT JUST SCIENCE ANYMORE

Henning PJ

Wayne State University Law School, Detroit, Michigan, USA, and University of Zagreb Faculty of Law, Zagreb, Croatia

This presentation will not be about the science of applied biology. Instead, it will look at issues that are increasingly important to scientists and doctors in practice: government regulation of the funding of scientific research, practice of medicine, and promotion of pharmaceuticals and medical devices. Specifically, the presenter will look at how the government uses the law to police how grants are properly accounted for, means by which potential misconduct is investigated, and statutes that have been used to regulate and punish companies for misconduct, both domestically and internationally. The goal is to provide a brief overview of how governmental regulation - and lawyers - have come to play an increasingly important role in the scientific research endeavor and the practice of medicine.

USE OF INFORMATION OBTAINED THROUGH DNA ANALYSIS IN CRIMINAL PROCEEDINGS

Đurđević Z

Faculty of Law, University of Zagreb; Croatian Academy of Legal Sciences, Zagreb, Croatia

In a recent decision, Croatian Constitutional Court contested the provisions of the Criminal Procedure Code of 2008 on the use of data obtained through DNA analysis. Article 186 of the CPC made no distinction between the types of personal data pertinent to the level of intrusion into personal integrity and privacy on one hand and the gravity of the criminal offense on the other. These provisions were also not aligned with the EU Framework Decision on the protection of personal data processed in the framework of police and judicial cooperation in criminal matters (2008). Therefore, in 2013 the legislator has amended the CPC by introducing the provisions that meet the standards concerning the purpose of collecting personal data, data processing, data verification, and the prohibition of the use of certain data.

Abstract number: ABS-297-ISABS-2013

CRIMINAL RESPONSIBILITY FOR DISCLOSURE OF A MEDICAL SECRET

Munivrana Vajda M

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This presentation will focus on the issue of professional secrecy, with special emphasis on medical secrets. Health care professionals, as well as members of some other professions, are under a duty to keep as secret information about personal or family life entrusted to them in the course of their occupation. A breach of this duty entails criminal responsibility with the maximum punishment of imprisonment of one year. The duty to keep a secret, however, is not absolute. Disclosure of personal information may be warranted by reasons of public interest or the interest of a third party, which prevails over the interest of keeping the secret. In certain circumstances health care workers may even be under an obligation to report possible abuse, and thus reveal confidential data. The presentation will analyze potential situations and interests which may give rise to such an obligation or which may justify breach of confidentiality.

Abstract number: ABS-298-ISABS-2013

DNA AND PRIVACY PROTECTION BY CRIMINAL LAW

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Since the beginning of DNA sequencing, forensic science encounters legal problems that can only be addressed following understanding of DNA sample gathering and by respecting right to privacy of individuals. Every human being has unique DNA sequence, with the exception of identical twins, and such data can be used for identification purposes- it can be used for convicting the guilty and as well as for acquitting of innocent (K. Rooker, The Impact of DNA Databases on Privacy, University of Dayton School of Law, 2000). Biological evidence is used to produce DNA profiles that are kept in databases, which may violate privacy rights. For example, to "produce information in relation to health, paternity, and other personal issues.« (N. Wilker, S. Stawski et al., DNA on Trial: Genetic Identification and Criminal Justice, DNA Data Banking and the Public Interest, P. Billings, ed., 1992, p. 146). Insurance companies, employers and governmental agencies are interested in such information, which can lead to genetic discrimination (limit the access to health care, employment, governmental services etc.; Ibid.). The problem of DNA databases is that "once a technological program like DNA identification gets established for pariah group such sex offenders, it is inevitable that there will be pressure to extend it to other groups and also to allow access to increasing number of individuals and institutions« (E. Shapiro & M. Weinberg, DNA Data Banking: The Dangerous Erosion of Privacy, 38 Cleveland State Law Review 455, 1990, p. 476). Authors will analyse the legislative framework of criminal law regarding protection of privacy of DNA data.

Workshop: Communication Skills in Science and Medicine

Abstract number: ABS-327-ISABS-2013

IMPORTANCE OF COMMUNICATION SKILLS IN MEDICINE

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Personalized medicine has the potential to fundamentally change the way health care is practiced and delivered. However, its success will depend on the ability of health professionals, scientists, medical educators, pharmaceutical companies, insurance companies, and policy makers to collaborate, and to create an integrated framework that meets the health care needs of the people. We must not forget that the most important part of personalized medicine is the patient, the person. As health care in general is a very complex system, reflecting social changes, in recent decades great attention has been paid to the quality of communication in science and medicine. Communication is the most widely used clinical skill in medical practice, which includes all participants of the health system. Training in communication skills in science and medicine is essential for a long-term theoretical, practical, individual, and team work. Communication and relationships have an impact on patients' experience of care, improve patients' adherence to treatment regimens, clinical outcomes, quality of care, and patients' safety, contribute to teamwork and cultural sensitivity, and reduce medical malpractice risk. Experience and talent are not enough to ensure optimal communication. Only good communication can provide and establish good relationship between the health professional and patient, It is very important for health professionals to use their communication skills to provide successful medical treatment and care, to establish and build good relationship with their patients, and to be aware of uniqueness of every patient. Human relationship is what matters most!

Suggested Reading

1 Đorđević V, Braš M, Miličić D. Introduction. In: Đorđević V, Braš M, Miličić D. Person in medicine and healthcare: from bench to bedside to community. Zagreb: Medicinska naklada. 2012. p. 43.

2 Mezzich JE. Building person-centered medicine through dialogue and partnerships: perspective from the international network for person-centered medicine. Int. J. Pers. Cent. Med. 1:10-13, 2011

Abstract number: ABS-328-ISABS-2013

PERSON-CENTERED MEDICAL INTERVIEW

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We are witnessing an unprecedented development of medical science and personalized medicine that promises to revolutionize health care and significantly improve patient health outcomes. However, technological superiority must not make us lose sight of the physical, psychological, social, and spiritual totality of the patient. The core of the medical profession has always been and will be the relationship between the health professional and the person seeking assistance. Due to the recent advances in neuroscience, we are now able to describe and discuss the biological mechanisms that underlie the health professional-patient relationship. We now know that different physiological and biochemical mechanisms take part in complex functions, like trust, empathy, and compassion, which are all very important elements in the health professional-patient relationship. Medical interview is a complex process of obtaining information for the purpose of making a diagnosis and it is an extremely important factor in establishing the relationship between health professionals and patients . The essential elements of the integrated patient-centered and physician-centered interview are to build a relationship, open the discussion, gather information, understand the patient's perspective, share information, reach agreement, and provide closure. Therefore, the Zagreb model of Person-centered medical interview is developed by the authors (Đorđević, Braš, Brajković), focused not only on the disease or illness but on patient's quality of life in the context of health and disease, used for the patients with cancer and patients with chronic pain syndrome. The Zagreb model of person-centered medical interview is an important bridge between personalized and person-centered medicine.

Suggested Reading

1 Kurtz SM, Silverman JD, Draper J. Teaching and learning communication skills in medicine. Oxford: Radcliffe Medical Press, 1998

2 Đorđević V, Braš M, Brajković L. Person-centred medical interview. Croat. Med. J. 53:310-313, 2012

Abstract number: ABS-329-ISABS-2013

COMMUNICATION SKILLS IN SCIENCE AND MEDICINE

Brajković L

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Communication is an integral part of any relationship with patients and their families, and represents the key to the success of the medical team. Communication and relationship have been demonstrated to have an impact on patients' experience of care, to improve patients' adherence to treatment regimens, clinical outcomes and quality, patient safety, teamwork, cultural sensitivity, and to reduce medical malpractice risk. Having good communication skills is essential for doctors to establish good doctor patient relationship. With the increase in demand from patients who value doctors who are patient centred, together with the rise of consumerism in medicine, health service research on doctor patient relationship has become an important area of interest for healthcare professionals. Better doctor patient communication was shown to be associated with better emotional and physical health, higher symptom resolution, and better control of chronic diseases that included better blood pressure, blood glucose and pain control. More studies showed that the degree of patient-centred communication was associated with less discomfort, less concern and better mental health in patients. It is necessary to mention that experience and talent are not enough to ensure optimal communication doctor - patient - family. Experience alone can be a poor teacher in communication skills and observation of 'bedside manner' is an inefficient way of teaching communication skills. Successful educational interventions require multipronged strategies including building up knowledge, demonstration, feedback, reflection, and self-assessment, repeated practice in safe and simulated environment. Specific teaching and learning methods are required in communication skill training.

Suggested Reading

1 Kurtz SM, Silverman JD, Draper J. Teaching and Learning Communication Skills in Medicine. Oxford: Radcliffe Medical Press, 1998.

2 Brajković L, Braš M, Đorđević V, Cvek M. Role of communication skills in person-centered medicine. In: Đorđević V, Braš M, Miličić D. Person in medicine and healthcare: from bench to bedside to community. Zagreb: Medicinska naklada, 2012

Workshop:
Adapting Clinical Trials to Modern Cancer Therapy

Abstract number: ABS-333-ISABS-2013

DENDRITIC CELL VACCINATION IN ACUTE MYELOID LEUKEMIA

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We have reported on the induction of complete and molecular remissions in AML patients, following vaccination with dendritic cells (DC), electroporated with mRNA encoding the full-length sequence of the Wilms' tumor protein WT1 (PNAS 107:13824-13829, 2010). In addition to the original construct with the full-length WT1 (construct 1), we also used 2 other constructs, one with a Sig-DC-LAMP MHC class II-skewing signal and a deletion of the WT1 nuclear localization signal (construct 2) and one similar to it, but codon-optimized (construct 3). DC electroporated with mRNA derived from constructs 1, 2 and 3 were used to vaccinate respectively 13, 6 and 10 AML patients at very high risk of relapse. In those 3 groups, the clinical response rate, as measured by a normalization of WT1 mRNA tumor marker levels in blood and/or marrow, occurred in respectively 7/13, 1/4 and 0/6 patients. Globally, 8/29 patients have not relapsed yet. Of those 8 patients, 5 had an increased WT1 mRNA tumor marker level which normalized following DC vaccination, 3 of them now more than 5 years after the start of DC vaccination and most probably cured. Mono- or poly-epitope WT1-specific tetramer-positive T cells were detectable in all evaluable patients, with the highest frequency in patients who remained in complete remission after DC vaccination. In conclusion, WT1 mRNA-transfected DC vaccination is emerging as a non-toxic and effective strategy to prevent relapse in AML. Contrary to expectations, the original full-length WT1 construct without MHC class II-skewing signal has demonstrated the highest clinical activity so far.

Abstract number: ABS-249-ISABS-2013

PERSONALIZED MODELING FOR EFFICIENT PLANNING OF COMBINATIONAL HORMONE THERAPY FOR PROSTATE CANCER PATIENTS

Elishmereni M[1], Kheiffetz Y[1], Vuk-Pavlović S[2], Kohli M[2], Agur Z[1]

[1]Institute for Medical BioMathematics, Bene Ataroth, Israel, [2]Mayo Clinic, Rochester, Minnesota, USA

Aim: Over the years, the therapeutic arsenal for treating prostate cancer (PCa) has grown to contain several agents, from hormone-based and steroidal drugs, to diverse chemotherapeutics and novel immunotherapies. However, the historic treatment paradigm for patients with advanced PCa, i.e. initiation of hormone therapy (with the option for secondary hormone therapy) followed by chemotherapy, often proves to be inefficient. Better planning of treatment schedules and combinations is required for improving patient responses. The goal of our study was to develop a biomarker-based mathematical/statistical model for intelligent design of combined therapies for PCa patients. **Methods:** A semi-mechanistic mixed-effects model for PCa was formed based on a mathematical model of vascular tumor growth and its susceptibility to a collection of drugs. The model was implemented, analyzed and simulated on a Monolix platform. Model parameter estimation was performed using an extensive Mayo Clinic dataset of ca. 500 patients with different stages of PCa, treated by various hormonal and chemotherapeutic drugs; Individual tumor growth dynamics and biomarkers were provided for each patient. **Results:** By analyzing the model in conjunction with the clinical dataset, we were able to identify biomarkers that influence biologically relevant model parameters. The model retrieved individual patient profiles of tumor dynamics (embodied by progression of PCa biomarker prostate-specific antigen), validating it retrospectively. **Conclusions:** The model can be applied to make accurate predictions of tumor progression and survival, and is intended for the design of complex single/combinational therapeutic regimens on a personalized basis.

Suggested Reading

1 Agur Z, Vuk-Pavlović S (2012) Personalizing immunotherapy: Balancing predictability and precision. Oncoimmunology 1: 1169-1171.

2 Kohli M, Tindall DJ (2010) New developments in the medical management of prostate cancer. Mayo Clin Proc 85: 77-86.

Abstract number: ABS-179-ISABS-2013

NEW STATISTICAL TOOLS FOR NOVEL APPROACHES TO CLINICAL TRIALS IN PERSONALIZED MEDICINE

Gasparini M

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Clinical trials have been a success story for the applications of statistics to high-impact problems for science and society. With the recent move towards personalized medicine, statisticians have been devising new tools for the analysis of modern clinical trials, such as random effects models to account for patient-to-patient variation, a stricter integration between PK/PD modeling and the analysis of clinical response, more flexible designs for adaptive multi-step clinical trials, new tools for evidence synthesis and new methods to handle multiplicity issues in genomics and in general multivariate problems. This talk is an attempt to describe these developments to an applied biological audience.

Suggested Reading

1 Spiegelhalter, Abrams, Myles (2004) Bayesian Approaches to Clinical Trials and Health-Care Evaluation

2 Whitehead (2003) Meta-Analysis Of Controlled Clinical Trials

3 Gabrielsson and Weiner (2007) Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications

4 Gilks WR, Richardson S, Spiegelhalter DJ, eds (1998) Markov chain Monte Carlo in practice

5 Cheung YK (2011) Dose Finding by the Continual Reassessment Method

Abstract number: ABS-340-ISABS-2013

IMPROVING THE EFFICACY OF IMMUNOTHERAPY BY DYNAMIC PERSONALIZATION: THE PROSTATE CANCER CASE STUDY

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Insufficient therapeutic response to cancer drug therapy in general, and to immunotherapy in particular, has motivated the development of a new approach—the dynamic personalization—the dynamic modification of personalized treatment. Thus, we have developed a method to predict and enhance the individual response to immunotherapy by using personalized mathematical models constructed in the early phase of treatment. Our approach includes an iterative in-treatment evaluation of patient-specific models, model-based simulations of subsequent response to ongoing therapy, and suggestion of potentially more effective patient-specific modified treatment. Using a mathematical model of prostate cancer immunotherapy, we applied our model to data obtained in a clinical investigation of an allogeneic whole-cell therapeutic prostate cancer vaccine. Simulations, based on personalized models, suggested that an increase in vaccine dose and administration frequency would stabilize the disease in most patients. Following prospective validation Dynamic Personalization will hopefully be implemented in clinical trials in immunotherapy ultimately resulting in a more efficacious immunotherapy.

USE OF BIOMARKERS IN PERSONALIZED THERAPY OF PROSTATE CANCER

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Rapid evolution of molecular technology to identify genetic markers and lead to dissecting inherent variance of individual cancers has had a tangible impact on clinical trial designs in oncology and patient care. Several cancer types now are treated based on molecular predictive biomarkers and not tumor histology alone. Advanced stage prostate cancer including castrate resistant prostate cancer (CRPC) is an ideal tumor type and stage suited for developing predictive biomarkers as there are now multiple treatment options available to treat this stage; at the same time this stage harbors tremendous somatic heterogeneity. Incorporation of the advances in cancer therapeutics underlies the change of treatment from empiric and clinic-pathological based to a more "personalized" approach based on tumor genome its variations. The goal is to individualize cancer therapy to enhance efficacy and minimize adverse effects and toxicity for each patient's unique tumor. While there is increasing recognition and incorporation of molecular factors in targeted cancer therapeutics, delivering these individualized treatments is in its early development and brings with it unique challenges and opportunities to integrate molecular pathology, cancer pharmacogenomics and pharmacogenetics with novel clinical trial design. This talk will summarize the current knowledge underlying the treatment of advanced prostate cancer and provide examples of the current status and future directions of molecular cancer therapeutics based on emerging "omic" technologies.

Workshop:
Glycoscience in
Personalized Medicine

INVESTIGATING THE RELATIONSHIP BETWEEN IGG N-LINKED GLYCANS AND COLORECTAL CANCER SURVIVAL IN A LARGE POPULATION-BASED COHORT IN SCOTLAND

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We aimed to identify IgG N-glycans biomarkers with discriminative power to predict survival in patients with colorectal cancer and so help inform treatment. CRC is the 2nd most cause of cancer deaths in the UK with 5-yr survival of 55% for men and women. The growing repertoire of treatments available for CRC, including new chemotherapy approaches, combined with challenging benefit:toxicity ratios and cost, means that it is crucial to target interventions to patients most likely to benefit. TNM and Duke's pathological staging does not fully categorise poor/good prognosis tumours within stage groupings, whilst current biomarkers (e.g., tumour markers, CRP) do not have adequate discriminative power for use in clinical practice. Hence, identifying novel biomarkers that further refine pathology-based prognostic information offers much potential for clinical and public health benefit. We report on progress made in a project to investigate IgG N-glycans as a novel biomarker of survival outcome in CRC.

Abstract number: ABS-174-ISABS-2013

PROTEIN GLYCOSYLATION IN PERSONALIZED MEDICINE

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Nearly all proteins are modified by covalent addition of complex oligosaccharides called glycans. Glycan parts are equally important for structure and function of proteins as polypeptide parts, but contrary to polypeptides, glycans are not defined by the firm genetic template. Instead, glycans are a product of complex dynamic interaction between (i) genetic polymorphisms, gene expression and regulation of hundreds of genes and (ii) past and present environmental factors. Due to their structural complexity and technological limitations the knowledge about glycans is lagging significantly behind the knowledge about proteins or DNA, but the situation is changing rapidly. First population studies of the glycome revealed significant inter-individual variations in protein glycosylation. Genome wide association studies of the glycome are mapping the complex network of genes, which regulate protein glycosylation. Large-scale studies of glycome in several major diseases are underway and they are starting to reveal the role of protein glycosylation in the personalized disease risk and disease course. We know that we are all different, but we still treat nearly all patients with the same therapy. Differences in glycosylation are responsible for a large part of human phenotypic variation and the time has come to include this part of human biology into personalized medicine.

Suggested Reading

1 Zoldoš V, Horvat T, Lauc G (2013) Glycomics meets genomics, epigenomics and other high throughput omics for system biology studies. Curr Opin Chem Biol 17: 34-40

2 Lauc, G. et al. (2013) Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. PLoS Genet 9: e1003225

3 Thanabalasingham G. et al. (2012) Mutations in HNF1A result in marked alterations of plasma glycan profile, Diabetes, published online doi: 10.2337/db12-0880

4 Pučić M et al. (2011) High throughput isolation and glycosylation analysis of IgG—variability and heritability of the IgG glycome in three isolated human populations. Mol Cell Proteomics 10: M111.010090

5 Gornik O, Pavić T, Lauc G (2012) Alternative glycosylation modulates function of IgG and other proteins—implications on evolution and disease. BBA Gen Subjects 1820: 1318-1327

HOW DOES INTRAVENOUS IMMUNOGLOBULIN THERAPY MODULATE IMMUNITY?

Nimmerjahn F

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Intravenous immunoglobulin preparations (IVIG) comprise pooled IgG antibodies from the serum of thousands of donors. Initially they were used as IgG replacement therapy in immunocompromised patients. Since the discovery that IVIG therapy can ameliorate immune thrombocytopenia (ITP) more than thirty years ago, the use of intravenous immunoglobulins has been extended to a wide variety of autoimmune and inflammatory diseases. Despite the broad efficacy of IVIG therapy, its modes of action largely remain unclear. In this talk, recent insights into the molecular and cellular pathways that are involved in IVIG-mediated immunosuppression will be discussed, with a particular focus on IVIG as a therapy for IgG-dependent autoimmune diseases. Most importantly, some insights into the role of the specific pattern of IgG glycosylation and its impact on the anti-inflammatory activity of IVIG will be provided.

TOWARDS PERSONALIZED MEDICINE BY GLYCAN BIOMARKERS DISCOVERED BY GLYCOBLOTTING-ASSISTED HIGH THROUGHPUT SERUM GLYCOMICS

Nishimura S-I

Division of Drug Discovery Research, Faculty of Advanced Life Science, Hokkaido University, Sapporo, Japan

Despite growing recognition of the importance of glycan modifications in biological processes, researchers had no facile way to manipulate and work with the glycans that impart so much function to proteins. Carbohydrates are largely similar chemically, making purification procedures difficult and derivatization a necessity. Yet, the methods that do exist are laborious or inefficient, and the modifications are application-specific. The aim of this study is to establish a standard protocol for the rapid and large-scale human serum glycomics to construct the human disease relevant glycome database. Advent of a PCR-like key technology for glycan-specific enrichment protocol based on the simple nucleophilic addition reactions between hydrazide/aminooxy- and ketone/aldehyde functional groups, the "glycoblotting method" allowed for the first time high throughput and quantitative glycomics (1). We demonstrated that glycoblotting using BlotGlyco beads is the only method that allows large-scale clinical glycomics of human whole serum glycoproteins (96 samples/plate), because it requires very little material (<100 μL) and, when combined with the automated SweetBlot system and MALDI-TOFMS, takes only 24 hours to complete (2). Recent work in collaboration with clinical teams indicated that dynamic alteration of the human-serum glycan profile could identify promising diagnostic biomarkers in hepatocellular carcinoma (3, 4) and pancreatic cancer (5).

Suggested Reading

1 Nishimura et al. (2004) High-throughput protein glycomics: combined use of chemoselective glycoblotting and MALDI-TOF/TOF mass spectrometry. Angew Chem Int Ed Engl. 44: 91-96

2 Nishimura, S. (2011) Toward automated glycan analysis. Adv. Carbohydr. Chem. Biochem. 65: 219-271

3 Kamiyama et al. (2013) Identification of novel serum biomarkers of hepatocellular carcinoma using glycomic analysis. Hepatology, in press

4 Miyahara et al. (2013) Serum glycan as a prognostic marker in patients with advanced hepatocellular carcinoma treated with sorafenib. Ibid, in press.

5 Nouso et al. (2013) Clinical utility of high-throughput glycome analysis in patients with pancreatic cancer. J Gastroenterol, in press.

Abstract number: ABS-314-ISABS-2013

GENOME-WIDE ASSOCIATION STUDIES OF HUMAN PLASMA N-GLYCOME

Lauc G[1], Huffman J[2], Pucić M[1], Polašek O[3], Gornik O[4], Wilson J[5], Hayward C[2], Rudd P[6], Campbell H[5], **Rudan I**[5]

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To identify genetic loci associated with human plasma protein glycosylation and IgG glycosylation, we quantitated N-linked plasma protein glycans and IgG glycans using ultra-performance liquid chromatography (UPLC) in more than 2,000 individuals from four European discovery populations. Meta-analysis of genome-wide association study (GWAS) results identified polymorphisms at six loci (FUT8, FUT6/FUT3, HNF1A, MGAT5, B3GAT1 and SLC9A9) that were associated with plasma protein glycosylation, and nine genome-wide significant loci (B4GALT1, MGAT3, ST6GAL1, B4GALT1, FUT8, MGAT3, IKZF1, IL6ST-ANKRD55, ABCF2-SMARCD3, SUV420H1, and SMARCB1-DERL3) associated with IgG glycosylation. Most of those genes have not been previously implicated in protein glycosylation. However, they have been strongly associated with levels of acute phase proteins (e.g. fibrinogen, CRP), autoimmune and inflammatory conditions (e.g., systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, diabetes type 1, multiple sclerosis, Graves' disease, celiac disease) and/or haematological malignancies (acute lymphoblastic leukaemia, Hodgkin lymphoma, nodular sclerosis, and multiple myeloma). Follow-up functional experiments in HNF1a, HNF4a, fucosyltransferases and haplodeficient Ikzf1 knock-out mice confirmed the functional significance of observed associations. We explored biomarker potential of affected N-glycans in MODY and Systemic Lupus erythematosus and demonstrated substantial discriminative power in a ROC-curve analysis. Our study shows that it is possible to identify genetic loci that control glycosylation of human plasma proteins using GWAS and develop new biomarkers for human diseases based on plasma glycosylation profiling.

Suggested Reading

1 Lauc G et al. (2013) Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. PLoS Genet 9: e1003225.

2 Lauc G. et al. (2010) Genomics meets glycomics-the first GWAS study of human N-Glycome identifies HNF1 as a master regulator of plasma protein fucosylation. Ibid 6: e1001256.

3 Huffman JE et al. (2011) Polymorphisms in B3GAT1, SLC9A9 and MGAT5 are associated with variation within the human plasma N-glycome of 3533 European adults. Hum Mol Genet 20: 5000-5011

TWIN STUDIES OF IGG N-GLYCANS: DISSECTING THE GENETIC AND EPIGENETIC CONTRIBUTIONS OF THE GLYCOME

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Objective: To determine the extent by which genetic and epigenetic factors contribute to circulating levels of N-glycosylated immunoglobulin G in humans using twins. Twins are the perfect natural experiment to separate genetic and environmental influences and estimate contributions reliably. We performed the first ever glycomics twin study in a group of adult UK twins. **Methods:** IgG N-glycan levels in plasma were derived using the UPLC analysis in 220 monozygotic and 306 dizygotic twin pairs from the TwinsUK cohort. Using a classical twin study design, we assessed the additive genetic, common and unique environmental components defining the variance in 76 N-glycans. Epigenome-wide association analysis using the Illumina 27k chip was carried out on all N-glycans with a heritability under 35%. **Results:** 51 of the 76 glycans (67%) studied have an additive genetic component of 50% or greater, meaning that at least 50% of the variance in their levels is determined by genetic factors. On the other hand, 12 glycans had a low genetic contribution (h^2<0.35). Epigenome-wide significant hits ($p < 2 \times 10[-6]$) were found for four of the 12 glycans mapping to two independent probes. Probe cg08392591 maps to a CpG island 5′ from the ANKRD11 gene, a p53 activator on chromosome 16. Probe cg26991199 maps to SRSF10 gene on chromosome 1 and is involved in regulated RNA splicing and in particular is involved in regulation of splicing of mRNA precursors upon heath shock. **Conclusions:** Our data indicate that majority of variation in IgG N-glycan levels can be explained by genetic factors; in those with a low genetic contribution, epigenetic factors also play an important role. Glycans have a great potential for understanding genetic pathways and mechanisms.

Abstract number: ABS-308-ISABS-2013

EPIGENETIC REGULATION OF PROTEIN GLYCOSYLATION IN HEALTH AND DISEASE

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Posttranslational modification to proteins by glycosylation is an important source of variability in human populations. In addition to genetic predetermination of the individual glycome, the environment plays an important role in its changes during lifetime. Epigenetic mechanisms are proven to be mediators between genes and environment, and epigenetic regulation of glyco-genes might explain both the temporal stability of the glycome in homeostasis and specific glycan changes in disease. We demonstrated the importance of epigenetic deregulation of genes involved in protein glycosylation in several recent studies. Epigenetic silencing by cytosine methylation in the promoter of HNF1A gene (a transcription factor previously shown to be a master regulator of plasma protein antennary fucosylation) was found to associate with branching of N-glycans in the plasma glycome. The same type of changes in plasma N-glycome was found to occur as a consequence of inactivating mutation in this gene, which causes HNF1A-MODY subtype of monogenic diabetes. We further demonstrated that epigenetic changes, induced by epigenetic inhibitors, affect the membrane N-glycome of HeLa cells, and that the N-glycome profiles can be reverted after the removal of inhibitors. Glycans on the cell membrane are essential elements of tumor cell's metastatic potential and are also an entry point for nearly all pathogenic microorganisms. Since some epigenetic inhibitors are used as therapeutic drugs, our study opens a new line of research in the application of these drugs as anticancer and antimicrobial agents.

Suggested Reading

1 Zoldoš et al., Epigenetics, 2012
2 Horvat et al., BBA General Subjects, 2012
3 Horvat et al., PLoS One, 2013
4 Zoldoš et al., Glycoconjugate Journal, 2013
5 Zoldoš, Horvat, Lauc, Curr Opin Chem Biol, 2013

YOUNG INVESTIGATOR AWARDS PRESENTATIONS

Young Investigator Award

Abstract number: ABS-280-ISABS-2013

ONCOLYTIC VACCINIA VIRUS EXPRESSING DISIALOGANGLIOSIDE MIMOTOPE MEDIATES STRONG CYTOTOXIC EFFECT ON MURINE NEUROBLASTOMA, GLIOMA AND MELANOMA CANCER CELL LINES IN VITRO

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With its broad tropism and great replicative potential, vaccinia virus is today considered as a new promising tool for cancer therapy, both as an oncolytic virus and a novel pharmacophore. In this study we aim to increase the potency of Vaccinia Virus Western Reserve (VVWR) by arming it with a mimotope that mimics disialoganglioside (GD2). Vaccinia replication and subsequent cell lysis are specifically restricted to cancer cells by thymidine kinase deletion. We have assessed killing efficacy in three murine cancer cell lines in vitro: neuroblastoma (NXS2), glioma (GL261) and melanoma (B16-F10). VVWR mediated oncolysis starts two days post infection and continues up to eight days post infection, where the killing efficacy was 100%. Six days post infection we observed a cytotoxic effect on more than 50% of cells in GL261, NXS2 and B16-F10 at 0.01, 0.1 and 1 evg per cell, respectively. We have furthermore investigated the effect of intratumoral VVWR injection (108 evg) in vivo, using the NXS2 subcutaneous tumor model. We have observed tumor-specific vaccinia replication; therefore additional immunological studies are ongoing in order to determine whether the immune response is activated upon GD2 mimotope expression.

Young Investigator Award Abstract number: ABS-194-ISABS-2013

RELATION OF TOUCH DNA FROM DIFFERENT SURFACES WITH DONOR AGE AND GENDER

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DNA can be transferred from palms to handled items. Different factors influence this process. The aim of this study was to investigate the influence of handling time, item texture, donor age and gender on quantity of DNA available from the touched items. The dependence of donor age and gender on touch DNA quantity deposits left by participants on items of different texture (paper, plastic, plastic coated metal) was also analyzed. 60 participants (30 men and 30 women) were included in this study. They were holding or rubbing items with their fingers, palm and a side of the palm of dominant hand in different time intervals. Items were swabbed with moistened cotton swab. Genomic DNA was isolated using Chelex. DNA concentration was determined by real-time polymerase chain reaction. The results showed that amount of participants' DNA left on touched items was not correlated with the time interval. On contrary, it was dependent on item texture. The highest amount of touch DNA was transferred on plastic coated metal and the least on paper. In addition, participants between 35 and 44 years of age left the greatest amount of touch DNA, while participants between 25 and 34 years of age left the least. Moreover, man left higher DNA amount on handled items when compared to women. In conclusion, the property of leaving DNA on handled items is individual. Further research is needed to understand the process of DNA transfer from donors to handled items and factors that have an influence on it.

Young Investigator Award Abstract number: ABS-133-ISABS-2013

A GENOME-WIDE RANKING OF GENIC INTOLERANCE TO FUNCTIONAL VARIATION FACILITATES PREDICTING DISEASE CAUSING GENES AND INTERPRETING PERSONAL GENOMES

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Aim: A challenge for interpreting personal genomes is identifying which genes are relevant to disease in any given individual. Genes carrying relatively fewer or relatively more common functional variants in healthy individuals may be judged respectively more or less likely to cause certain kinds of disease. We sought to develop a simple score to rank genes according to their tolerance to have relatively more or less functional genetic variation than expected based on the apparently neutral variation found in the gene. **Methods:** We describe a score that utilizes information from 6503 whole exome sequences available through the NHLBI Exome Sequencing Project. We illustrate utility of this intolerance score through various OMIM backdrops including "haploinsufficiency", "recessive", and others. **Results:** We show that genes responsible for Mendelian diseases are significantly enriched for genes scored more intolerant to functional genetic variation than genes that do not cause any known disease (P<10-16). We further dissect this into distinct disorder classes, showing that OMIM genes linked to Developmental disorders have the lowest tolerance scores on average (observed 19.54 percentile, expected 50.00). Additional disorder classes enriched for intolerant genes include Cancer (p=2x10-7), Neurological (p=4x10-7) and Skeletal (p=5x10-4) disorders. On the other hand, the disorder class most often linked to genes with high tolerance scores, and thus possibly reflecting a positive selection mechanism, is Immunological disorders (average 70.44 percentile). **Conclusion:** We show that use of a tolerance ranking system greatly facilitates interpreting personal genomes and identifying high impact mutations contextually through the gene in which they occur.

Young Investigator Award Abstract number: ABS-131-ISABS-2013

IMPROVED ANALYSIS OF LONG STR AMPLICONS FROM DEGRADED SINGLE SOURCE AND MIXED DNA

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DNA profiles from degraded samples often suffer from information loss at the longer STR loci. Sensitising the reactions, either by performing additional PCR cycles or increasing the CE injection settings, carries the risk of over-amplifying or overloading the shorter fragments. We explored whether profiling of degraded DNA can be improved by preferential capturing of the longer amplified fragments. To this aim, a post-PCR purification protocol was developed that is based on AMPure XP beads that have size-selective properties. A comparison was made with an unselective post-PCR purification system (DTR gel filtration) and no purification of the PCR products. Besides a set of differently and serially degraded single source samples, unequal mixtures of degraded DNAs were analysed, in order to extract more genotyping information for the minor contributor without overloading the major component at the shorter amplicons. Purification by the AMPure protocol resulted in higher peak heights especially for the longer amplicons, while DTR gel filtration gave higher peaks for all amplicon sizes. Both purification methods presented more detected alleles, with the AMPure protocol performing slightly better, on average. In conclusion, the in-house developed AMPure protocol can be employed to improve STR profile analysis of degraded single source and (unequally) mixed DNA samples.

SUBMISSIONS SELECTED FOR PODIUM PRESENTATIONS

Selected for Podium Presentation Abstract number: ABS-232-ISABS-2013

EVALUATION OF DIRECT PCR AMPLIFICATION USING SWABS AND WASHING REAGENTS

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The objective of this study was to generate DNA profiles from body fluids deposited on different swab substrates. These samples were pretreated with purification reagents or extraction buffers prior to amplification with direct and non-direct amplification kits. Saliva samples from living donors and blood samples from deceased individuals were deposited on Pur-Wraps® sterile cotton tipped applicator, Pur-Wraps® Foam Swab, MiniPax®, PurFlock Ultra Flocked swab, FAB-swab, Fitzco SPIN-EZE™ Push-off™ swab, and Bode SecurSwab™. These devices were kept at room temperature for varying lengths of time ranging from one day to approximately one year. 1.2mm punches were obtained from each substrate and washed with PunchSolution™, SwabSolution™, Prep-n-Go™ Buffer, ECS™ wash buffer, and FTA™ reagent and amplified. Autosomal STR loci and the amelogenin gender locus were amplified using PowerPlex® Fusion reagent kit from Promega Corporation and AmpFlSTR® Identifiler® Direct, Identifiler® Plus and Identifiler® amplification kits from Applied Biosystems®-Life Technologies (AB). In addition, male body fluids were amplified using PowerPlex® Y23 System from Promega Corporation. Substrate types, amount of reagents needed, and, if necessary, amplification parameters were varied in this study to detect autosomal and Y-STR DNA profiles. Analysis of the amplified product was performed by capillary electrophoresis injection on the AB 3130xl Genetic Analyzer. The generated DNA profiles were analyzed using GeneMarker® HID Software Version 2.2.0 from SoftGenetics®. Autosomal and Y-STR profiles were generated successfully from the swab substrates containing blood and saliva. Concordant profiles were obtained within and between all amplification kits.

Selected for Podium Presentation Abstract number: ABS-180-ISABS-2013

WTCCC3 AND GCAN: A GENOME-WIDE ASSOCIATION STUDY OF ANOREXIA NERVOSA

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Anorexia nervosa (AN) is a complex and heritable eating disorder characterized by the maintenance of dangerously low body weight. We established the Genetic Consortium for Anorexia Nervosa which is an unprecedented worldwide collaboration combining existing DNA samples of AN patients into a single resource. As part of the Wellcome Trust Case Control Consortium we conducted the largest genome-wide association study of AN to date combining 2,907 AN cases, originating from 15 different countries of European ancestry, and 14,860 ancestrally matching controls. Individual association analyses were carried out in each ancestry stratum and then meta-analysed across all 15 strata. Seventy-six SNPs were taken forward for in silico and de novo replication in another 15 datasets of European ancestry and from Japan. Global meta-analysis across discovery and replication datasets, comprising a total of 5,551 AN cases and 21,080 controls, was then performed. Suggestively associated SNPs were rs9839776 (p=3.01x10-7) within SOX2OT and rs17030795 (p=5.84x10-6) within PPP3CA in the main analysis and rs1523921 (p=5.76x10-6) located between CUL3 and FAM124B and rs1886797 (p=8.05x10-6) located near SPATA13 in Europeans only. In comparing the discovery to the replication results, 76% of the effects were in the same direction, an observation highly unlikely to be due to chance (P = 4x10-6). This suggests that many true findings exist but that our sample, the largest yet reported, was underpowered for their detection at the genome-wide level and that accrual of large genotyped AN case-control samples should be an immediate priority for the field.

Selected for Podium Presentation Abstract number: ABS-175-ISABS-2013

HOW PHARMACOGENETICS MIGHT HELP TO PREDICT POSTOPERATIVE PAIN AND RESPONSE TO PATIENT CONTROLLED ANALGESIA BY MORPHINE: A CASE REPORT

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We illustrate the role of genetic variability in postoperative acute pain and analgesic response with a case of a 47 years old woman, who underwent hysterectomy-adnexectomy. **Aim:** a total dose of morphine consumed in the first 24 postoperative hours and the number of pain episodes with intensity >4 (Numeric Rating Scale, NRS) was analyzed in relation to single nucleotide polymorphisms (SNPs) alleles in mu-opioid receptor (OPRM1) gene. **Methods:** this case was enrolled after obtaining approval from ethical committee and written informed consent. Morphine (0.15mg/Kg i.v.) was infused 45mins before the end of surgery, followed by i.v. morphine via Patient Controlled Analgesia (PCA) for 48hs (each bolus: 1mg, lock-out 5mins, max dose 20mg in 4hs). We assessed postoperative pain using NRS from 0 (no pain) to 10 (most severe pain) after 3-6-12-24-36-48hs. We genotyped OPRM1 SNPs rs1799971 (A118G) and rs563649 by FRET-based SNP genotyping. **Results:** this case was the only patient, out of 202, carrying TT genotype for rs563649 and AA genotype for A118G. She consumed 22 mg of morphine in 24hs, that was more than in any patient with AA genotype for A118G (20.04±14.9) and more than average morphine dosage of all patients with NRS>4. 3 times in the first 24hs (21.52±16.08); she had a delay emergence from anesthesia (Sedation Scale, SS = 2) and she complained nausea and vomiting 36hs after surgery. **Conclusion:** our case suggests that OPRM1 genotype rs563649 may be informative of postoperative pain scores, PCA morphine consumption and incidence of opioid side effects.

Selected for Podium Presentation Abstract number: ABS-310-ISABS-2013

LARGE TANDEM, HIGHER ORDER REPEATS (HOR) AND REGULARLY DISPERSED REPEAT UNITS CONTRIBUTE SUBSTANTIALLY TO DIVERGENCE BETWEEN HUMAN AND CHIMPANZEE Y CHROMOSOMES

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Comparison of human and chimpanzee genomes has received much attention recently, because of paramount role for understanding evolutionary step distinguishing us from closest living relative. In order to contribute to Y chromosome evolutionary history, we study and compare tandems, HORs and regularly dispersed repeats in human and chimpanzee Y chromosomes, using robust Global Repeat Map (GRM) algorithm. We find a new type of long-range acceleration, Human Accelerated HOR Regions (HAHORs). In peripheral domains of 35mer human alphoid HORs we find riddled features containing ten additional repeat monomers. In chimpanzee we identify 30mer alphoid HOR. We construct alphoid HOR schemes showing significant human-chimpanzee difference, revealing rapid evolution after human-chimpanzee separation. We identify and analyze over twenty other large repeat units, most of them reported here for the first time: chimpanzee and human ~1.6 kb 3mer secondary repeat unit (SRU) and ~23.5 kb tertiary repeat unit (for ~0.55 kb primary repeat unit (PRU)); human 10848, 15775, 20309, 60910, and 72140 bp PRUs; human 3mer SRU (for ~2.4 kb PRU); 715mer and 1123mer SRUs (for 5mer PRU); chimpanzee 5096, 10762, 10853, 60523 bp PRUs; chimpanzee 64624 bp SRU (for 10853 bp PRU) and so on. We show that substantial human-chimpanzee differences are concentrated in large repeat structures, at the level of as much as ~70% divergence, sizably exceeding previous numerical estimates for some selected noncoding sequences. Smeared over the whole sequenced assembly (25 Mb) this gives ~14% human-chimpanzee divergence. This is significantly higher estimate of divergence between human and chimpanzee than previous estimates.

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ANTIGEN TARGETING TO DENDRITIC CELLS AS THERAPEUTIC APPLICATION IN THE FIGHT OF CANCER

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Dendritic cells (DCs) are important cells for the presentation of antigens. In dependency on the surroundings, DCs are capable of presentation of antigen in an immature or mature state. Therefore, immune responses are tightly regulated by DCs, as T cells recognizing peptide MHC-complexes on immature DCs undergo deletion or anergic responses whereas T cells recognizing pMHC complexes on mature DCs undergo proliferative responses leading to T cell memory. Our lab is focusing on understanding how DC subpopulations regulate these T cell responses and orchestrate the immune system in vivo. We have developed antigen-targeting antibodies to direct antigens in vivo to different DC subpopulations. Thereby, we could show that antigen-loaded CD11c+CD8- DCs induce pronounced CD4 helper T cell responses whereas antigen loaded CD11c+CD8+ induce prominent CD8 but also CD4 T cell responses leading to protective immune responses. Especially interesting candidate receptors for antigen targeting are C-type lectin receptors and Fc receptors - both subclasses are highly efficient endocytosis receptors. Here, we demonstrate that although antigens might be internalized into other antigen presenting cells, only DCs are important for the initiation of effector T cell responses in vivo. Importantly, we are now able to show that not only efficiently cross-presenting CD11c+CD8+ DCs are capable of inducing protective immune responses in an experimental murine melanoma model but also CD11c+CD8- DCs.

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SNP ANALYSIS OF DDAH2 GENE IN CARDIOVASCULAR DISEASE

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Objectives: This study aims to detect dimethylarginine dimethylaminohydrolase (DDAH) type 2 gene polymorphisms among Egyptian patients, to explore functional correlations of these profiles with serum asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), L-arginine, and C-reactive protein (hsCRP), and to assess their association with risk of cardiovascular disease (CVD) in young susceptible individuals. **Methods:** 100 Egyptian male CVD patients (35-50 y) were recruited for the study from National Heart Institute, Cairo, Egypt. They were classified according to severity of coronary insufficiency, as verified by coronary angiography, into: a) patients under conservative medical treatment (Med, n=12), b) patients directed for PCI (PCI, n=41), c) patients directed for GABG operation (CABG, n=36), and d) patients suffering from acute myocardial infarction (AMI, n=11). Age and sex-matched controls (n=100) were included from the general population. **Results:** Data revealed a complete association between two polymorphisms (SNP1, -1151 C/A, rs805304 and SNP2, - 449 C/G, rs805305) of the DDAH2 gene. The A allele / AA genotype for SNP1 and G allele / GG genotype for SNP2 were significantly associated with CVD in the Egyptian patients and their frequency is correlated with severity of coronary insufficiency. No significant association between serum levels of biochemical parameters and carriage of specific DDAH2 genotype. Patients having AMI showed higher serum levels of ADMA, SDMA, and hsCRP; and lower serum L-arginine and L-arginine/ADMA than chronic patients. **Conclusion:** DDAH2 gene polymorphisms are correlated with the early incidence of CVD in Egyptians. This study was supported by the Science and Technology Development Fund grant No. 2951.

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RECONSIDERING THE PARADIGM OF CANCER IMMUNOTHERAPY BY COMPUTATIONALLY AIDED REAL-TIME PERSONALIZATION

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Although therapeutic vaccination often induces markers of tumor-specific immunity, therapeutic responses remain rare. An improved understanding of patient-specific dynamic interactions of immunity and tumor progression, combined with personalized application of immune therapeutics would increase the efficacy of immunotherapy. Here, we developed a method to predict and enhance the individual response to immunotherapy by using personalized mathematical models, constructed in the early phase of treatment. Our approach includes an iterative real-time in-treatment evaluation of patient-specific parameters from the accruing clinical data, construction of personalized models and their validation, model-based simulation of subsequent response to ongoing therapy, and suggestion of potentially more effective patient-specific modified treatment. Using a mathematical model of prostate cancer immunotherapy, we applied our model to data obtained in a clinical investigation of an allogeneic whole-cell therapeutic prostate cancer vaccine. Personalized models for the patients who responded to treatment were derived and validated by data collected before treatment and during its early phase. Simulations, based on personalized models, suggested that an increase in vaccine dose and administration frequency would stabilize the disease in most patients. Together, our findings suggest that application of our method could facilitate development of a new paradigm for studies of in-treatment personalization of the immune agent administration regimens (P-trials), with treatment modifications restricted to an approved range, resulting in more efficacious immunotherapies.

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FAMILIAL SEARCHING COMBINING AUTOSOMAL AND Y CHROMOSOMAL STRS AND SURNAMES

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Introduction of new legislation in the Netherlands in 2012 allows familial searching in the DNA-database and in DNA-dragnets. This concept was used in an attempt to identify a relative of the perpetrator of rape and murder of a young Dutch woman in 1999. We have followed four lines of investigation to identify a relative of the perpetrator: 1. Autosomal STR based Familial search in the Dutch DNA database. The autosomal DNA profile of the perpetrator was compared to autosomal DNA profiles of 140,000 suspects/convicts using CODIS7 and Bonaparte software. Likelihood ratios (PI and SI) were calculated; 2. Local Y STR screening. A group of approximately 420 males with birthplace or place of residency in the area of the committed crime was selected from the Dutch DNA database. The Y STR DNA profile of the perpetrator was compared to Y STR profiles observed in this group; 3. Screening rare surnames and Y STRs. A group of approximately 260 males that did not live in the direct area of the committed crime but who had rare surnames that reside in the area was selected from the Dutch DNA database. The Y STR DNA profile of the perpetrator was compared to Y STR profiles observed in this group; 4. Voluntary large scale Y STR based familial search amongst several thousands male individuals in the area. Results of each of the four lines of investigation will be presented at the conference.

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NEXT GENERATION SEQUENCING METHODS FOR FORENSIC ANALYSIS

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The combination of Short Tandem Repeat (STR) markers and Capillary Electrophoresis (CE) detection methods has established DNA testing at the center of modern criminal investigations. Although the methods have become more sophisticated, the fundamentals of the systems have changed little in the last decade. The increasing need for more comprehensive results from ever decreasing amounts of input material are placing strain on the fixed capability of CE platforms and current interpretation methods. In a quest to resolve current limitations, forensic scientists worldwide are now investigating the value of Next Generation Sequencing (NGS) for forensic applications. Illumina's sequencing by synthesis (SBS) technology offers a massively parallel approach for simple and accurate sequencing of large numbers of PCR amplicons. This offers new opportunities for taking methods currently confined to the research arena and transferring them to routine forensic analysis. In this paper, we will examine the limitations of current DNA testing methods and the specific benefits of the sequencing by synthesis approach for forensic analysis. We will also discuss the types of applications, which may be enabled using next generation sequencing methods and consider how interpretation methods for even the most complex of samples may be improved.

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RAPID HUMAN IDENTIFICATION: EVALUATION OF THE RAPIDHIT200

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The forensic science community has been effectively using traditional methods of STR analysis for more than twenty years, through PCR amplification and gel or array-based capillary electrophoresis. Emerging technologies offer the ability to advance the speed and mobility of the STR analysis process. One such platform is the RapidHIT200 system from IntegenX, which combines the cell lysis, DNA purification, quantification, amplification, and capillary electrophoresis steps into one bench-top unit that generates an STR profile from cheek swabs in less than 90 minutes. The instrument is currently capable of providing investigative leads through the analysis of reference samples. When employed in police stations, rapid testing results can be compared to local databases of unsolved casework, or can be compared, if acceptable in the relevant jurisdiction, to state and federal databases. We have evaluated the RapidHIT to determine if the instrument is ready to be adopted by the forensic community. Contamination and reproducibility studies illustrated the platform's ability to outperform traditional bench top methods when dealing with freshly collected (pristine) samples. Analysis of baseline noise resulted in an analytical threshold (AT) of 900-1500 RFU for detection of true allelic peaks, with typical peak heights well over 12,000 RFU. Peak height ratios for profiles at different levels of intensity were used to determine a stochastic threshold (ST) to guard against falsely reporting homozygote profiles. Finally, a study was conducted to assess how the RapidHIT200 handles aged samples, to determine potential impacts of storing samples prior to rapid DNA analysis.

ABSTRACTS OF POSTER PRESENTATIONS

Genome-based Applications in Forensic Science

Presentation number: FG1 Abstract number: ABS-122-ISABS-2012

GENETIC VARIATION IN AN IMMIGRANT POPULATION IN UK COMPARED WITH ITS POPULATION OF ORIGIN FOR FORENSIC DNA DATABASE UTILIZATION PURPOSES BY USING 15 STR LOCI

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Allelic frequencies and forensic parameters for autosomal Short Tandem Repeats (STR), as well as genetic structure were determined in two populations to answer the question, can we use origin population database for an immigrant population for forensic application? A total of 209 samples, 96 British Pakistanis as an immigrant population from UK cities and 113 from Pakistani cities were PCR-typed for 15 autosomal STR by using AmpFlSTR® Identifiler Applied Biosystem Kit. Powerstat®V12 software was used for data interpretation. GENPOP 4.0.10 linkage disequilibrium and Arlequin version 3.5 P values for HWE equation were calculated. The genotype of each locus was in Hardy-Weinberg equilibrium for 15 STRs in both populations after Bonferoni correction except: D8S1179 and D18S51 for the Pakistani population, and D19S433 and D5S818 for the British Pakistani population. Average Power of Discrimination for British Pakistanis was 0.93 as compared to 0.94 of origin population. The FST between these two populations was 0.02288. Immigration of Pakistanis to the UK has affected the allelic frequencies, number of alleles per loci and power of discrimination for different loci. The results have showed that these two populations are slightly different genetically, and a separate DNA database for each population would be of importance for forensic applications. A slightly increased rate of homozygosity in the British Pakistanis may suggest more consanguinity and autosomal recessive disorders compared to the Pakistani Population. This could be due to the UK Pakistanis having specific migration patterns and geographical distribution in the UK, and maintaining more selective inbreeding.

Presentation number: FG2 Abstract number: ABS-136-ISABS-2013

MOLECULAR IDENTIFICATION OF FORENSICALLY IMPORTANT FLIES (DIPTERA) ON THE BASIS OF PARTIAL CHARACTERIZATION OF COII GENE

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Precise species identification of every insect sample collected from criminal scenes play an essential role in the accurate estimation of minimum postmortem interval (PMImin). The morphological similarity poses a great challenge for forensic entomologists. DNA-based method can be used as a supplemental means of morphological method. Chosen molecular marker must be not only easily sequenced, but also contains enough variation to generate unique identifiers at either the species or population levels. In the present study, we demonstrate for the first time the utility of the 635-bp-long cytochrome oxidase II gene (COII) in identification of forensically important Diptera (21 species) belonging to the main families of forensic importance (Calliphoridae, Sarcophagidae and Muscidae) with some species originating from 2 geographical areas (China, Egypt). This region was amplified using polymerase chain reaction (PCR) followed by direct sequencing of the amplification products. Nucleotide sequence divergences were calculated using the Kimura two-parameter (K2P) distance model and a neighbour-joining (NJ) phylogenetic tree generated. No overlapping between intraspecific and interspecific variations. All examined specimens were assigned to the correct species and formed distinct monophyletic clades. The species from 1 geographical location formed firstly separated clade then joined with similar species from other location with high bootstrap support. The investigated species have been clustered in NJ tree in accordance with their taxonomic classification. Thus the 635-bp-long COII region has been shown suitable for clear differentiation and identification of forensically relevant Diptera.

Presentation number: FG3 Abstract number: ABS-124-ISABS-2013

ISOLATION OF HIGHLY PURE MALE DNA FROM SEXUAL ASSAULT CASES USING A NUCLEASE TO DESTROY CONTAMINATING VICTIM DNA

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The profiling of sperm DNA present on vaginal swabs taken from rape victims is a proven tool for identification of rapists. Large amounts of the victim's epithelial cells contaminate the sperm present on swabs, however, and complicate this process. The standard method for obtaining pure sperm DNA from a vaginal swab is to digest the epithelial cells with Proteinase K and then physically separate the victim's solubilized DNA from the sperm by pelleting sperm heads and repeatedly washing the sperm pellet. The sperm pellet washing steps are labor intensive, difficult to automate, and result in sperm loss. An alternative approach that does not require washing is to digest with Proteinase K, pellet the sperm, and then destroy the residual victim's DNA with the nuclease DNase I. Five functioning crime labs have tested this method on semen spiked female buccal swabs in a direct comparison with their standard methods. In every case, the nuclease method outperforms the standard method currently used by these labs. In most labs the nuclease method is able to provide a clean male profile from buccal swabs spiked with only 1,500 sperm. The nuclease method also provides superior male STR profiles when using timed post-coital swabs and forensic samples taken from casework.

Presentation number: FG4 Abstract number: ABS-309-ISABS-2013

NOVEL GLOBAL REPEAT MAP (GRM) ALGORITHM: DIRECT MAPPING OF SYMBOLIC DNA SEQUENCE INTO FREQUENCY DOMAIN

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We have developed very robust novel GRM algorithm (http://www.hazu.hr/grm//tools.html) for identification and analysis of complex repeats. The main feature of GRM is its ability to identify a broad variety of repeats of unbounded length that can be arbitrarily distant in sequences. The use of complete set of complete K-string ensemble enables direct mapping of symbolic DNA sequence into frequency domain, with straightforward identification of repeats as peaks in GRM diagram strongly reducing the noise with respect to all available algorithms. GRM could be considered as powerful generalization of Tandem Repeat Finder framework to the frequency domain. Thus we obtain very efficient, robust and highly automatized repeat finding tool and computation is very fast. GRM diagram for a whole chromosome is computed within minutes on desktop computer. The method is robust to substitutions and insertions/deletions and to complexities of sequence pattern. It is an effective tool for inherent de-noising frequency diagrams in detecting repeat units. We present several case studies of GRM use, in order to illustrate its capabilities: GRM diagram for human chromosome 7 and identification of alpha satellite tandem repeats and higher order repeats (HORs), identification of Alus and Alu tandems in human chromosome 7, increasing resolution of GRM repeat identification by segmenting genomic sequence into smaller subsegments, complex HOR pattern based on ~2.4 kb repeat monomer in human Y chromosome, etc. GRM algorithm is convenient for use, in particular for large repeat units, highly mutated and/or complex repeats, and global repeat maps for large genomic sequences (chromosomes and genomes).

Presentation number: FG5 Abstract number: ABS-213-ISABS-2013

INFLUENCE OF UVC RADIATION ON BLOOD, SALIVA AND SEMEN SAMPLES

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The major threat in forensic laboratories is a sample mix-up and contamination of forensic evidence with foreign DNA, which could lead to identification of mixed DNA profiles, inhibition of PCR reactions or wrong DNA genotyping. The aim was to study in real conditions the true possibility of working surfaces contamination in the laboratory and the anti-contamination effects of commercial cleaning agents and ultraviolet C (UVC) radiation on naked DNA, diluted and non-diluted blood, semen, saliva samples and controls. The doses of UVC radiation, which would cause a complete loss of DNA profile in biological samples were also investigated. Buccal swab sample, blood, saliva and semen were obtained from a single male donor. Genomic DNA was extracted using Chelex. DNA was amplified using the AmpFlSTR® NGM™ PCR Amplification Kit. Statistical data analysis was performed using Excel. DNA concentration, UVC dose, sample volume, radiation time, number of correctly detected alleles on genetic loci and number of correctly detected alleles in four loci groups were tested. To our knowledge, the rate of UVC induced DNA damage of semen stains was not analyzed so far. The results of this study pointed out the inefficiency of commercial cleaning agents for working surfaces decontamination, when dealing with non-diluted blood and semen. High UVC doses are necessary for complete decontamination of working surfaces with inflicted non-diluted blood and semen controls. It is recommended to carefully and thoroughly clean working surfaces with commercial agents followed by minimal 16 hour UVC exposure (dose approximately 4380 mJ/cm2) for complete and successful decontamination.

Presentation number: FG6 Abstract number: ABS-255-ISABS-2013

COMPARISON BETWEEN POLYCYANO UV AND CYANOACRYLATE IN FINGERPRINT MARKS DETECTION

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The goal of detection is induction and fixation of fingerprint marks on objects connected to the perpetrator of the offense. The aim of this study was comparative crime scene fingerprint marks analysis by cyanoacrylates and PolyCyano UV for fast and accurate personal identification. The fingerprint marks detection using cyanoacrylate and PolyCyano UV methods was performed on 1000 different non-porous surfaces (glass, PVC, lacquered wood, metal, etc.) from crime scene cases. The methods are carried out in cyanoacrylate chambers, under strictly defined and controlled conditions, such as temperature and moisture. Also, time of exposition is also important and dependent on chamber size. Fingerprint marks induced by cyanoacrylate are white colored and easily detectable on dark surfaces, opposite to fingerprint marks on light surfaces. Therefore, the forensic white light or color enhancer is necessary for their visualization. The advantage of cyanoacrylate method is the persistence of fingerprints; however, additional treatment to induce fingerprints marks is needed. Opposite to that, PolyCyano UV method requires shorter induction time for fingerprint marks without additional treatment. Thus, induced fingerprints are visible under UV light by using the appropriate UV filter. This method is particularly suited for colorful backgrounds, due to fluorescent properties of PolyCyano UV. Moreover, PolyCyano UV fluorescence is decreasing over time. Due to this fact, photographs should be processed as soon as possible. In conclusion, further research is needed to improve the PolyCyano UV method for better crime scene fingerprint marks detection.

Presentation number: FG7 Abstract number: ABS-127-ISABS-2013

CASE REPORT: DNA IDENTIFICATION OF A WORLD WAR II VICTIM

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In the Netherlands about 700 individuals are still recorded missing since World War II. For many the fate is known, but their exact burial location remains unknown. Nowadays the Netherlands Forensic Institute deploys several forensic techniques to aid identification, i.e., anthropological analysis with combinations of autosomal, Y chromosomal and mitochondrial DNA profiling. In 1943, the members of the Dutch resistance movement killed the 23-year old Pieter Hoppen because they thought he collaborated with the German occupying forces. In 2008, a documentary filmmaker discovered that Pieter Hoppen was in fact not a collaborator. One of the members of the Dutch resistance movement decided to clear his conscience and revealed the burial site. In 2009 personnel from the Dutch military recovered skeletal remains from a young male at the designated site. To confirm that the remains belonged to the Pieter Hoppen, a search was started for DNA reference material. In a case file, the Red Cross recovered an envelope and letter, written in 1947 by the father or brother of Pieter Hoppen to request help in finding their relative. A Y chromosomal DNA profile from the envelope matched the Y chromosomal DNA profile from the skeletal remains, giving strong support for the hypothesis that the skeletal remains belonged to Pieter Hoppen. His remains were reburied at the Field of Honor in Loenen.

Presentation number: FG8 Abstract number: ABS-194-ISABS-2013

RELATION OF TOUCH DNA FROM DIFFERENT SURFACES WITH DONOR AGE AND GENDER

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DNA can be transferred from palms to handled items. Different factors influence this process. The aim of this study was to investigate the influence of handling time, item texture, donor age and gender on quantity of DNA available from the touched items. The dependence of donor age and gender on touch DNA quantity deposits left by participants on items of different texture (paper, plastic, plastic coated metal) was also analyzed. 60 participants (30 men and 30 women) were included in this study. They were holding or rubbing items with their fingers, palm and a side of the palm of dominant hand in different time intervals. Items were swabbed with moistened cotton swab. Genomic DNA was isolated using Chelex. DNA concentration was determined by real-time polymerase chain reaction. The results showed that amount of participants' DNA left on touched items was not correlated with the time interval. On contrary, it was dependent on item texture. The highest amount of touch DNA was transferred on plastic coated metal and the least on paper. In addition, participants between 35 and 44 years of age left the greatest amount of touch DNA, while participants between 25 and 34 years of age left the least. Moreover, man left higher DNA amount on handled items when compared to women. In conclusion, the property of leaving DNA on handled items is individual. Further research is needed to understand the process of DNA transfer from donors to handled items and factors that have an influence on it.

Presentation number: FG9 Abstract number: ABS-311-ISABS-2013

START/STOP CODON LIKE TRINUCLEOTIDES EXTENSIONS IN PRIMATE ALPHA SATELLITES

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Centromeres remain »the final frontier« in unexplored segments of genome landscape in primate genomes. They are characterized by 2-5 Mb arrays of rapidly evolving alpha satellite (AS) higher order repeats (HORs). Alpha satellites, as specific noncoding sequences, may be significant in the light of postulated regulatory role of noncoding sequences. Using the Global Repeat Map (GRM) algorithm we identified species-specific alpha satellite HORs in NCBI assemblies of chromosome 5: a 13mer in humans, 5mer in chimpanzees, 14mer in orangutans and 3mers in macaques. The suprachromosomal family (SF) classification of alpha satellite HORs and the surrounding monomeric alpha satellites is performed; specific segmental structure was found for major alpha satellite arrays in chromosome 5 of primates. In the framework of our novel concept of start/stop Codon Like Trinucleotides (CLTs) as a "new DNA language in noncoding sequences", we find characteristics and differences of these species in CLT extensions, in particular the extensions of stop-TGA CLT. We hypothesize that these are regulators in noncoding sequences, acting at a distance, and that they can amplify or weaken the activity of start/stop codons in coding sequences in protein genesis, increasing the richness of regulatory phenomena.

Forensic DNA Phenotyping

Presentation number: FG10 Abstract number: ABS-144-ISABS-2013

FINGER DERMATOGLYPH STUDY IN THREE WEST ALGERIAN POPULATIONS

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Nowadays the study of dermatoglyphs has a great importance in judicial and criminal researches. The human dermatoglyphic traits present variations within and between populations. The purpose of our study was to determine the frequencies of the patterns on fingers (arch, ulnar and radial loop, double loop, and whorl) and identify the possible differences in three ethnic groups of West Algeria. Dermatoglyphic prints were collected from 54 randomly healthy Northwest Algerians from Oran area, 39 Reguibates - a tribe of Arabic origin - and 44 Zenata, a Berber population from Southwest Algeria. The frequencies of the digital figures, whorl, ulnar loop, radial loop, double loop and arch obtained in these three populations were, respectively: 38,46%, 48,9%, 2%, 2%, 8,4% for Oran area, 41%, 46,66%, 1,11%, 4,62%, 5,7% for Reguibates and 26,1%, 55,6%, 5,6%, 3,38% and 8,6% for Zenata. A complete analysis of the results may allow us to characterize these three populations and to compare them with populations worldwide.

Presentation number: FG11 Abstract number: ABS-216-ISABS-2013

GENETIC CHARACTERIZATION OF CENTRAL CROATIA POPULATION USING MENTYPE® ARGUS X-8 KIT

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The new hot target on forensic crime scene is the X chromosome. The aim was to analyze 8 X-linked short tandem repeat (STR) markers in central Croatian population and to evaluate forensic efficiency of used X-STR markers. We carried out a statistical analysis of the data from previously performed genetic analyses collected during routine forensic work by the Forensic Science Centre ''Ivan Vučetić''. The Mentype® Argus X-8 PCR amplification kit was used for typing 99 unrelated healthy women and 78 men from central Croatia. Haplotype frequencies were calculated only in male samples. Arlequin 3.5 software was used to assess Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD), observed (Ho) and expected heterozygosity (He). Power of discrimination (PD) for males and females, polymorphism information content (PIC), power of exclusion (PE), mean exclusion chance (MEC) for deficiency cases, normal trios and duos were determined using online database ChrX-STR.org. In female samples deviations from HWE (P>0.00625) for each locus were not found. LD test performed both on female and male samples, revealed no significant association between markers (P>0.00178). In 78 men, 37, 30, 35 and 30 haplotypes were found for four linkage groups. Locus DXS10135 was the most polymorphic (PIC=0.9306). PD varied from 0.6922 to 0.9345 in male and from 0.8447 to 0.9918 in female samples. Combined PD reached 99.999999% in males and 99.9999999999% in females. To our knowledge, this is the first population study based on X-STR analysis in Croatia. Further studies are needed to get an overview of the X-STR variability in Croatia.

Presentation number: FG12 Abstract number: ABS-262-ISABS-2013

DENTAL DNA FOR HUMAN IDENTIFICATION

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For forensic purposes victims can be identified by classic methods such as visual recognition by a relative, fingerprints, and dental radiographs analysis. However, in cases of burned victims or in post-mortem decomposition, classic methods are ineffective. In these situations, teeth are the only elements that can be used for identification purposes because they resist time and physico-chemical attack. We proposed to develop, a molecular protocol for human identification from the teeth. Two methods of preparation of the tooth and four kits of DNA extraction were tested on six teeth isolated from living persons and 13 teeth isolated from victims who had been dead since two years and 13 years. Our results showed that the use of dental pulp and QIAmp blood and Tissu extraction kit provided only for current teeth a sufficient quantity and a good quality of DNA which was successfully amplified for mitochondrial DNA and 16 nuclear STR (Short Tandem Repeat). The genetic profile obtained from the dental DNA was authenticated by comparison with the genetic profile obtained from the blood of the same person. The PrepFiler BTA forensic Kit is the only kit which under specific conditions makes it possible to obtain from ancient teeth a mitochondrial DNA profile and a nuclear profile with some STR. We developed for in Tunisia a rapid and effective molecular protocol for human body identification in forensic using dental pulp obtained by longitudinal section of the tooth and PrepFiler BTA forensic Kit for DNA extraction.

Presentation number: FG13 Abstract number: ABS-201-ISABS-2013

VALIDATION OF AMPFLSTR® SGM PLUS® AMPLIFICATION KIT AT LOW DNA CONCENTRATIONS

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Internal validation of new method is a mandatory step before its implementation into the everyday casework. Touch DNA analysis has become very popular, and scientists are nowadays struggling with very low DNA concentrations to generate a full DNA profile. The aim of this study was to validate AmpFlSTR® SGM Plus® PCR amplification kit at low DNA concentrations. Genomic DNA from buccal swab and epithelial cells deposited on clothes was isolated using Chelex. Genomic DNA content of each sample was determined by quantitative real-time polymerase chain reaction using Quantifiler® Human DNA Quantification Kit. AmpFlSTR® Control DNA 007, buccal swab and touch DNA were diluted as follows: 0.06 ng/μL, 0.04 ng/μL, 0.02 ng/μL and 0,01 ng/μL. Each sample was amplified in pentaplicate using the AmpFlSTR® SGM Plus® PCR amplification kit. Statistical data analysis was performed using Excel. Results were presented as mean value of DNA concentrations and loci number. In all AmpFlSTR® Control DNA 007 dilutions, a full DNA profile was detected. In buccal swab samples, a full DNA profile was not detected at a concentration of 0.01 ng/μL. Moreover, in touch DNA samples a full DNA profile was detected at a concentration of 0.06 ng/μL. Partial DNA profiles were identified in other concentrations tested. The results of this study showed a lower sensitivity of validated PCR kit when amplifying low amounts of DNA. The more sensitive kits with a greater number of loci should be used in forensic casework due to their higher sensitivity and improved STR performance for forensic casework.

Forensic DNA Databases

Presentation number: FG14 Abstract number: ABS-283-ISABS-2013

DIRECT PCR OPTIMIZATION

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Lately, with the aim of easier, faster and cheaper analysis of the referent samples, collected for various purposes and primarily for the creating forensic DNA databases as well as rapid paternity testing, the procedures that completely bypass the step of DNA extraction from this type of specimens are optimized. This contributes significantly to saving time needed for samples processing, which is ultimately the primary goal. Namely, the direct PCR model entails the amplification of direct STR markers from the buccal mucosa swabs and blood samples collected by some of the different types of collectors. In Total, 28 buccal swab samples were processed. The PowerPlex® 18D System was applied, which allows the direct amplification of 18 STR loci, 13 CODIS loci, and Penta E, Penta D, D2S1338, D19S433, as well as of amelogenin, and certainly enhances the power of individual discrimination due to extended range of loci. The total volume of the individual reaction mixture was 25 µL. The amplification of samples was performed using the PCR thermocycler, according to the manufacturer's recommendations. Detection of the results was performed on an ABI PRISM® 310 genetic analyzer. Given that the two Promega kits share 16 STR loci, it was possible to examine the accuracy of the detected alleles using PowerPlex® 18D by comparing them to the alleles detected using PowerPlex® 16 multiplex of the same persons, thus determining the optimal method of collecting and processing this type of biological traces by the use of direct amplification kit.

Presentation number: FG15 Abstract number: ABS-295-ISABS-2013

POLYMORPHISM OF SIX NEW Y CHROMOSOME STR LOCI (DYS576, DYS481, DYS549, DYS533, DYS570, DYS643) IN REFERENT SAMPLE OF BOSNIA AND HERZEGOVINA

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Y STR markers are useful in establishing paternal linkages, kinship testing and familial searching, also finds its application in studying migration pattern of populations. Y STR haplotyping is particularly important for forensic analysis in case of rape, especially in those cases where more than one male was involved. New kit PowerPlex® Y23 enables the coamplification and 5-dye detection of 23 loci, 6 new loci(DYS576, DYS481, DYS549, DYS533, DYS570, DYS643) in comparison to Applied Biosystems and 11 new loci in comparison to previous PowerPlex Y kit which included 12 STR loci. Aim of this study was to indicate the significance of increasing the number of STR loci in forensic analysis. This study was conducted on referent sample of 100 unrelated males from Bosnia and Herzegovina. Genomic DNA was extracted from buccal swab using salting out method as well as QiagenDNeasy™ Tissue Kit. PCR amplifications have been carried out in PE GeneAmp PCR System Thermal Cycler. Y Chromosomal 23 STRs alleles were typed using ABI 310 Genetic Analyzer. Estimation of haplotype frequencies, haplotype diversity and average gene diversity were done using Arlequin Software V. 3.5. Ninety-eight unique haplotypes were detected, and one appeared two times. The most polymorphic locus is DYS418 on which we detected 14 alleles. Furthermore on this DYS418 locus we detected allele 33 which was not incorporated in allelic ladder provided by PowerPlex® Y23 kit. The least polymorphic loci in our study were DYS389I, DYS391, DYS437 and DYS393.

Presentation number: FG16 Abstract number: ABS-290-ISABS-2013

NON-PATERNITY AND GENETIC INCONSISTENCIES IN CASES OF DISPUTED FATHERHOOD IN DNA LABORATORY IN OSIJEK, CROATIA

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Uncertainty of paternity nowadays can be easily resolved with DNA testing. The method of DNA profiling used for determining whether two individuals have a biological parent-child relationship in Forensic DNA laboratories is based on PCR and the use of highly polymorphic short tandem repeats (STRs). The aim of this study is to establish (1) the rate of non-paternity in a sample from paternity testing laboratory, (2) association of non-paternity event with other available data and (3) type of inconsistencies between the obtained genotypes. The study is based on review of archive material from 248 cases of paternity testing during the 13 years (2001-2013) in Laboratory for DNA analysis in Medical school in Osijek, Croatia. Paternity testing was in 64.1% of cases ordered by the court (child as a plaintiff) and in 14.5% of cases requested privately by the father. Mothers` maiden name was found in 67.7% children, and in 12.5% of children had fathers` last name. Paternity testing was false in 13.3% of cases. Children with false paternity test were younger than children with positive paternity test (Me 0.41 years vs. Me 1.00 years; p=0.049). Six mutations were observed within 3 480 allelic transfers at 15 STR-loci. Our non-paternity rate of 13.3% in population sample was among the lowest of published data from other paternity testing laboratories.

Presentation number: FG17 Abstract number: ABS-155-ISABS-2013

AN EVALUATION OF DIRECT PCR AMPLIFICATION

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The objective of this study was to generate DNA profiles from body fluids deposited on different substrates via direct amplification. These substrates were not subjected to purification reagent or extraction buffer prior to amplification. Saliva samples from living donors and blood samples from deceased individuals were deposited on Fitzco proPRIMEi Indicating Micro, Fitzco 705 Micro, Fitzco Blood Direct Cards #1 and #2, Fitzco Collection Card, Fitzco CEP swab, Whatman EasiCollect, FTA® Indicating Micro, Fitzco ProPRIME Direct, and Bode DNA Collector. These ten devices were kept at room temperature for varying lengths of time ranging from one day to approximately one year. 1.2mm punches were obtained from each substrate and amplified with reagents contained in each amplification kit. Autosomal STR loci and the amelogenin gender locus were amplified using PowerPlex® 21D, PowerPlex® 18D, PowerPlex® 16 HS, and PowerPlex® 16 Systems from Promega Corporation, and AmpFlSTR® Identifiler® Direct, Identifiler® Plus and Identifiler® kits from Applied Biosystems®-Life Technologies (AB). In addition, male body fluids were amplified using PowerPlex® Y23 System from Promega Corporation and AmpFlSTR® Yfiler™ kit from AB. Substrate types, amount of reagents needed, and, if necessary, amplification parameters were varied in this study to detect autosomal and Y-STR DNA profiles using the nine amplification kits. Analysis of the amplified product was performed by capillary electrophoresis injection on the AB 3130xl Genetic Analyzer. Autosomal and Y-STR profiles were generated successfully from most of the substrates after storage at room temperature for approximately one year.

Presentation number: FG18 Abstract number: ABS-246-ISABS-2013

FORENSIC AUTOSOMAL DATABASE IN BELARUS: PATERNITY CASES, CRIMINALISTIC AND POPULATION GENETIC DATA SETS

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Establishment of forensic databases is necessary for forensic DNA analysis to eliminate or include suspect person, to detect probability of paternal exclusion, or, in DNA identification tasks. Forensic utilization of paternity data sets (93 regions, N=2550), criminalistic data sets (Belarus territory, N=9626) and population genetic data sets (11 local populations, N=1040) in Belarussian forensic database is considered. Population genetic data set consists of both ethnic Belarussians and mixed origin individuals. Population genetic approach has been used for analysis of paternity and criminalistic data sets. The results of analysis were compared with those received for population data set, including ethnic Belarussians and mixed origin individuals, the ethnicity of each individual was assigned by self-definition. Genetic subdivision and differentiation of Belarussian population was investigated taking in consideration historical and ethnographic territorial peculiarities. The strategy of utility of these three data pools (paternity, criminalistic and population genetic data sets) for Belarussian forensic autosomal database is demonstrated.

Presentation number: FG19 Abstract number: ABS-276-ISABS-2013

RAPID HUMAN IDENTIFICATION: EVALUATION OF THE RAPIDHIT200

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The forensic science community has been effectively using traditional methods of STR analysis for more twenty years, through PCR amplification and gel or array-based capillary electrophoresis. Emerging technologies offer the ability to advance the speed and mobility of the STR analysis process. One such platform is the RapidHIT200 system from IntegenX, which combines the cell lysis, DNA purification, quantification, amplification, and capillary electrophoresis steps into one bench-top unit that generates an STR profile from cheek swabs in less than 90 minutes. The instrument is currently capable of providing investigative leads through the analysis of reference samples. When employed in police stations, rapid testing results can be compared to local databases of unsolved casework, or can be compared, if acceptable in the relevant jurisdiction, to state and federal databases. We have evaluated the RapidHIT to determine if the instrument is ready to be adopted by the forensic community. Contamination and reproducibility studies illustrated the platform's ability to outperform traditional bench top methods when dealing with freshly collected (pristine) samples. Analysis of baseline noise resulted in an analytical threshold (AT) of 900-1500 RFU for detection of true allelic peaks, with typical peak heights well over 12,000 RFU. Peak height ratios for profiles at different levels of intensity were used to determine a stochastic threshold (ST) to guard against falsely reporting homozygote profiles. Finally, a study was conducted to assess how the RapidHIT200 handles aged samples, to determine potential impacts of storing samples prior to rapid DNA analysis.

Presentation number: FG20

DNA DATABASE AND ITS CONNECTION WITH THE PRISON SYSTEM IN CROATIA

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DNA analysis is routinely used in criminology to obtain information in detection of offenses, paternity proofing and establishing identity of unidentified bodies or their parts. To be useful, DNA databases should contain large numbers of processed and stored genetic profiles of suspects and offenders. By the end of 2011, the Croatian DNA database contained more than 30000 DNA profiles of offenders and perpetrators; based on statistics, a large number of them are or will be repeat offenders. According to the Ministry of Justice data for 2011 and 2012, the number of repeat offenders was 43,88% and 42,59% of total prisoners, respectively. Legal regulation of DNA databases in Croatia is moving toward full compliance with acquis communitaire of the European Union, but is aided also by Croatian laws on molecular genetic analysis that are fully compliant with the regulations of the European Union. The next step should be the harmonization of legislation at the international level aimed at the global fight against terrorism and organized crime. DNA databases must be designed for most efficient access and use of data in the shortest possible time.

Genetic Analysis of Forensic Non-Human Material

Presentation number: FG21 Abstract number: ABS-199-ISABS-2013

CYTOCHROME OXIDASE I GENE: UTILITY OF SHORT VERSUS LONG FRAGMENTS IN IDENTIFICATION OF FORENSICALLY SIGNIFICANT DIPTERA

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Molecularly based methods are considered an important tool within the identification toolkit for accurate evaluation of a chosen genetic marker. Up-to-date, there are no molecular data for forensically important fly species from Egypt, either by using short or long fragment of the mitochondrial cytochrome oxidase I (COI) gene. Moreover, there are no data about long fragment of COI from China because all studies from china extracted from short fragments as being simple, cheap and reproducible. Thus, assessment will be valuable to assess applicability of 2 genetic markers of different length (short, long) in identification on species and population levels. Analysis of specimens belonging to 18 dipterous species has been performed. Both markers were amplified by polymerase chain reaction (PCR) followed by direct sequencing. Nucleotide sequence divergences were calculated using the Kimura two-parameter (K2P) distance model and neighbour-joining (NJ) phylogenetic trees generated. Although both tested markers displayed overlapping between intra and interspecific variations but long marker outperforms short one in completeness of monophyletic separation with high bootstrap support. Moreover, NJ tree based on long fragment clustered species more in accordance with their taxonomic classification than in short fragment. Thus, identification based on long COI fragment is more reliable and safer than that based on short fragment.

Presentation number: FG22 Abstract number: ABS-121-ISABS-2012

POSTMORTEM DECAY CHANGES AND DETERMINATION OF TIME OF DEATH IN BIRDS OF PREY

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Research on post mortal changes in dead birds of prey with the aim of determining the time of death and tracking decay changes was conducted. Since previously conducted research are often not applicable to wild animals, while in the case of birds of prey there has been no research on post mortal dynamics, we aimed to determine: 1) the applicability of models based on mammal decomposition on birds of prey; 2) the phases of decomposition and their length; 3) the gradation of certain decomposition degrees according to body mass loss during the decay process. The research was conducted on 21 specimens of dead birds of prey during 12 months. The specimens were exposed to weather conditions. The decay cages were equipped with autonomous weather stations to monitor the temperature of air and ground, relative moisture, speed and directionality of wind and atmospheric pressure. During the research no bloating of the body was recorded, which regularly occurs in mammals as a second stage of the decomposition process. Therefore, models based on mammal decomposition research are not applicable to birds of prey. Regularity of body mass loss regardless of weather conditions can be used as additional tool in estimating of time of death in birds of prey.

Presentation number: FG23 Abstract number: ABS-267-ISABS-2013

USE OF DIFFERENT PRESERVATION METHODS TO PRESERVE DNA IN MUSCLE AND BONE TISSUE SAMPLES

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Following mass fatality incidents, DNA profiling is essential for identification and re-association of fragmented, burnt or decomposed corpses that would be very difficult or impossible by traditional means such as fingerprinting and odontology. However, successful DNA recovery depends on the collection and preservation of biological material obtained from deceased individuals and the availability of reference samples. The aim of this research was to determine the efficiency of different preservation methods, to preserve DNA in muscle and bone tissue samples stored under different conditions for a period of 6 months. Goat muscle and bone tissue samples (1 gram each) were preserved in triplicate using 50% and 100% ethanol, and without any preservative solution at room temperature for 6 month. The samples were also oven dried at 70 ^{O}C for 3 hrs and preserved at room temperature for 6 months. Similarly, samples were also preserved at 5 ^{O}C and -20 ^{O}C. DNA extraction was carried out using DNeasy® Blood and Tissue kit. Primers were designed using C-MYC and recombination activation gene to amplify 131 bp, 290 bp, 305 bp and 506 bp amplicons. Successful PCR amplification of both muscle and bone tissue samples were obtained from all preservation methods except samples which were preserved at room temperature without any preservative solution, where severe DNA degradation was observed. The results suggested that the ethanol, low temperature storage and oven dried tissues can be used to preserve DNA in both muscle and bone tissue samples until 6 months.

Presentation number: FG24 Abstract number: ABS-177-ISABS-2013

CASE REPORT OF A FATAL DOG ATTACK

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The case of a fatal dog attack on a 40-year-old woman who was found dead on the floor in her garage together with her dogs is presented. Besides the body an artificial penis was found and suspicion of torture and sexual abuse of dogs arise. The aim of the genetics study was to identify human and canine DNA on the object from the crime scene. The pubic hair and bloodstain were found on the object and the swab of surface of the object was taken for canine DNA typing. Genomic DNA was extracted using EZ1 DNA Investigator Kit (Qiagen) on Biorobot EZ1 device (Qiagen). The nuclear DNA of the samples was quantified using the Quantifiler Human DNA Quantification Kit (AB). STR typing of human autosomal DNA was performed using the AmpFlSTR Identifiler PCR Amplification Kit (AB) and canine autosomal DNA using the Canine Genotypes, Panel 1.1 kit (Finzymes Diagnostics). The fluorescent labelled products were separated on an automatic ABI 3130 Genetic Analyzer (AB). The genetic profiles were determined using the Data Collection v 3.0 and GeneMapper ID v3.2 (AB) computer software. STR profiles obtained from pubic hair and bloodstain matched the profile of death victim and STR profile from the swab of the evidential object indicated mixed profile of different dogs which was in agreement with STR profile of at least two examined dogs involved in attack. The results show that victim and her dogs were in contact with the object from the crime scene.

Presentation number: FG25 Abstract number: ABS-316-ISABS-2013

FORENSICALLY USEFUL LARGE CARRION BEETLES IN CROATIA, SILPHIDAE (COLEOPTERA, INSECTA) - TAXONOMIC OVERVIEW WITH DISTRIBUTION NOTES

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Forensic entomology deals with biology and ecology of insects, mainly in estimation of the post-mortem interval, but also provides evidence of body movement when the insect recovered from the corpse is found outside its normal geographical distribution. Here we list the large carrion beetles (Silphidae) present in Croatia with ID photos and figures of each species together with notes on their distribution and biogeography. Our latest data were assembled with the help of the literature and collections of the Museums of Natural History in Split and Zagreb. According to the latest world catalogue, there are 21 species recorded in Croatia. Most are present in Croatian museum collections and can help the taxonomic verification of collected crime evidence. Most of the large carrion beetles present in Croatian fauna are also widely distributed across Europe, except for the few Mediterranean species such as *Silpha olivieri* (Bedel, 1887) that is adapted for warmer and dryer climate. Therefore, biogeography should be taken into account when analysing insect succession and temperature dependence in carrion decomposition as well as geographical origin of the carcasses. In cases when early developmental maggot stages are difficult for identification by morphological analysis, DNA can be extracted and COI barcoding regions can be sequenced. That would enable the molecular identification of maggots and provide the information for NCBI database. Since entomology has not been fully integrated into the official forensic practice in Croatia, we hope this information will further stimulate taxonomic and related research to advance forensic entomology.

Forensic and Comparative Genetics

Presentation number: FG26 Abstract number: ABS-185-ISABS-2013

AN INNOVATIVE PLAN FOR PROCESSING AND ANALYZING PROPERTY CRIME EVIDENCE FOR A LARGE METROPOLITAN POLICE FORCE

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In response to local crime data and citizen satisfaction surveys the Kansas City Police Department (KCPD) developed and executed a plan to train selective police officers to investigate, document, process and collect evidence from non-violent crimes involving personal and business property. Members of the Kansas City Police Department, Forensic Experts from the Kansas City Police Crime Laboratory and personnel from the Jackson County Missouri Prosecutor's Office were responsible for the training, evidence analysis and data collection from the project. A database for tracking a number of components specific for these investigations was created. The components examined included types of evidence collected and analyzed, Automated Fingerprint Identification System (AFIS) hit percentages, Combined DNA Index System (CODIS) hit percentages and case dispositions. Prior to implementation of this plan the KCPD's clearance rate for such crimes was approximately one-half of the national average. After the plan became fully operational the KCPD's clearance rate had more than doubled and is now above the national average for similar metropolitan police agencies in the United States.

Presentation number: FG27 Abstract number: ABS-254-ISABS-2013

REMOVAL OF PCR INHIBITORS BY POWDERED ACTIVATED CARBON

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Bloodstains are common forensic samples, which can often be problematic for genotyping. Besides hematin naturally occurring in bloodstain extracts, components of the substrate on which forensic evidence is found can affect DNA amplification as powerful polymerase chain reaction (PCR) inhibitors. The aim of this study was to overcome the PCR inhibition by using powdered activated carbon (PAC) purification. We evaluated the effectiveness of PAC-purification at 37˚C with 60 minutes contact time vs. ultrafiltration using Amicon®Ultra-4 unit (100K) in removing hematin and soluble humic substances. Genomic DNA from bloodstains on peat soil substrate and from blood without substrate was extracted using Chelex®100 and purified by PAC or Amicon®Ultra-4. In addition, for some samples purification was not performed. Real-time PCR was used for DNA quantity estimation and AmpFlSTR®SGM Plus® kit for amplification. Moreover, concentrations of residual inhibitors were determined by UV/VIS spectrometry and compared to unpurified samples. PAC more effectively removed hematin, without DNA loss and provided high DNA quality. Our results show that purification significantly decreased humic substances content. Conversely, both purification methods significantly increased average DNA yield and provided equivalently good DNA quality. In conclusion, this study provides the first insight into a new application of PAC as a well-known adsorbent, based solely on temperature and contact time without introducing any reagents that may interfere with subsequent downstream analysis and may provide the basis for its further application in forensic casework.

Presentation number: FG28 Abstract number: ABS-154-ISABS-2013

SUITABILITY OF BLOOD AS A RELIABLE SOURCE OF BIOLOGICAL SPECIMEN FOR INDIVIDUALIZATION USING DNA PROFILING TECHNIQUE

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The role of forensic genetics in the establishment of identity is to compare samples recovered from the crime scene with the suspect. Blood is universally accepted as a good source of material for DNA profiling. Forensic scientist can encounter the problem of mixed or completely mismatched DNA profiles of a single individual when the source of the analyzed biological material is a person who is a genetic chimera. Such genetic peculiarity may prevent the association of the perpetrator with the stain left at the crime scene or lead to false paternity exclusions. In the present study we evaluated the presence of donor cells within the recipient's blood using sixteen STR markers (fifteen autologous and one sex marker) in twenty-five different bone marrow transplant or hematopoietic stem cell recipients. Donor chimerism was observed in all recipients.

Presentation number: FG29 Abstract number: ABS-167-ISABS-2013

POLYMORPHISMS OF 15 STR LOCI IN THE TURKISH POPULATION OF BOSNIA AND HERZEGOVINA

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Allele frequencies of 15 STRs included in the PowerPlex 16 System (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX and FGA) were calculated from the referent sample of 100 unrelated individuals of both sexes from the Turkish population living in Bosnia and Herzegovina. Buccal swabs, as a source of DNA, were collected from volunteers who gave the informed consent. DNA extraction was performed using QIAamp DNA Micro kit. Two ng of DNA template was used to amplify 15 STR loci by PCR multiplex amplification which was performed by using the PowerPlex 16 kit (Promega Corp., Madison, WI, USA) according to the manufacturer's protocol. The amplifications were carried out in a PE Gene Amp PCR System thermal cycler (Applied Biosystems) and capillary electrophoresis was carried out in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) in accordance with the manufacturer's recommendations. The frequency of each locus was calculated from the numbers of each observed genotype. Deviation from Hardy-Weinberg equilibrium and observed heterozygosity were calculated. Data were analyzed by using Microsoft Excel workbook template - Powerstats V12 and the power of discrimination (PD), power of exclusion (PE), as well as other population genetic indices for the 15 STR loci were calculated. Obtained results contribute to existing Turkish DNA database, as well as insight of differences and similarities in comparison to population of Bosnia and Herzegovina.

Presentation number: FG30 Abstract number: ABS-170-ISABS-2013

EARLY VALIDATION STUDIES ON THE RAPIDHIT™ 200 HUMAN DNA IDENTIFICATION SYSTEM

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Rapid DNA testing represents the next major breakthrough in how DNA will contribute to law enforcement, intelligence, homeland security, and defense missions. The IntegenX RapidHIT™ System is designed to enable safe and easy deployment outside of the laboratory, and operation by non-scientists. Producing full DNA profiles in 90 minutes, the RapidHIT System enables DNA-based intelligence and valuable leads to be obtained quickly at the start of investigations. Validation of Rapid DNA presents some significant challenges however. The measurement uncertainty approach used by many laboratories can be difficult to apply to a fully enclosed 'black box' system, and use outside of the laboratory may require adherence to additional standards (e.g., ISO17020) not familiar to the forensic geneticist. Developmental validation studies performed on the RapidHIT System demonstrate that traditional validation approaches can be used, provided that factors such as the scope of the intended application, the end user, and the environment are carefully considered. Results from the developmental validation conducted at IntegenX, and also validation data from early users, encompassing sensitivity, reproducibility, precision, and concordance are presented. These results demonstrate that the RapidHIT is 'fit for purpose' as an intelligence tool.

Presentation number: FG31 Abstract number: ABS-181-ISABS-2013

EXAMINATION OF DNA RECOVERY POTENTIAL FROM FIRED SHELL CASINGS

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As the sensitivity of forensic DNA analysis has grown, so has the demand for testing. Along with this demand, has been an expansion into the types of samples for which DNA testing is often requested. Unfortunately, despite the implications of the portrayal of forensic DNA analysis in television and film, limits still exist for which forensic DNA analysis is possible, useful and warranted. Additionally, constraints on limited resources available for DNA testing, such as personnel, supplies and funding, further limit the amount of testing that may be allowed for samples deemed unlikely to be successful. One such sample type request frequently encountered in this laboratory is for DNA testing of the spent or fired shell casings. It has been the experience of this examiner that this sample type rarely, if ever, leads to sufficient DNA for analysis. This study sought to exam this belief through the analysis of the samples, which previously would not have been tested, as well as through experimental samples generated in the test firing of firearms within the laboratory. It is hoped this study will reaffirm the case management decisions utilized within this laboratory regarding sample selection especially as it relates to prioritization of resources. Additionally, it is hoped this study will provide examiners much needed scientific support when questioned about these matters during testimony in the courts.

Presentation number: FG32 Abstract number: ABS-219-ISABS-2013

QUALITY ASSESSMENT OF DNA EXTRACTED FROM DIFFERENT TISSUES BY TWO METHODS

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Aim: In this study we compare two methods for extraction of DNA, Phenol Chlorophorm and QIAAamp® DNA Mini kit to find out which is a better method from tissues in a referent samples with different period of decomposition. **Materials and Methods:** The tissues: spleen, pancreas, lungs, liver, hearth and muscle, 20-30 mg, were decomposed in controlled conditions for a period of 6 months. Degradation was performed in 3 controlled conditions: room temperature, in refrigerator at +4 ^{O}C and ambient temperature. Temperature was measured with digital thermometer TESTO, and notes on temperature variations were taken every 2 hours. Quantification of extracted DNA was performed with Quantifiler ABI on 7500 RealTime PCR ABI. Comparison of the two protocols for extraction of DNA were statistically analysed with T-test. Quality of extracted DNA in all tissues which were decomposed in 3 controlled conditions and taken for analyse in 5 periods during 6 months were statistically analysed with AMOVA. **Results:** Our results show that bigger amount of extracted DNA we get with Phenol Chlorophorm method, but with QIAAamp® DNA Mini kit, the extracted DNA can also be used for further PCR reaction. **Conclusion:** Phenol Chlorophorm method is slow, more expensive, cancer-causing and big amount of extracted DNA can make problem during PCR amplification. Comparative results for extracted DNA of separated tissue samples show that they all give almost the same amount of DNA. Because of that, in forensic DNA analyses we should take in consideration individual state of the tissues and ambient conditions.

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A DATABASE SEARCHING MODEL APPLIED IN ANTHROPOLOGIC RESEARCH

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In this paper we have proposed to build a connection among the fields of bioinformatics, anthropology and artificial intelligence on the base of our research in this area. In our domain of research (anthropology), we must have an important database for analysis. Our objective was to create a universal database-searching model, virtually applicable for every arbitrary data. So, we have created a pattern that offers the possibility to extend a genetic algorithm like an optimization method and to turn it into an approximation method. In the first stage of the method, we applied for each records of the database the method of the polynomial approximation with genetic algorithms (PAGA), which results in the creation of an algebraic polynominal for each of the records. In the second stage we process the input data with the PAGA method, which means we also create an algebraic polynominal that represent the input data. The search for the input data in the database means that we need to find all the associated polynominals, which approximate best the polynominal representing the input data. If the difference doesn't satisfy the minimal required, we consider this data absent in the original database and we could save this data set like a new record. The application is to support the anthropological research through the intelligent interrogation of some individual-data versus a database-community to interpret more efficiently the affiliation of those data. This database-searching model we use to identify the geographic area from where an individual proceeds.

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DIGIT RATIO IN MEN OF TWO ETHNIC GROUPS IN POPESTI, ROMANIA

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The length of fingers and the digit ratio are studied in population and forensic genetics, too. Some authors suggested that the digit ratio between the ring finger and forefinger is determined during early fetal development and that it is influenced by sex hormones. If this is true, fingers may provide a permanent and easily visible, historic marker of important hormonal events during a critical time of fetal development. The effective size for digit ratio between the sexes varies as a function of geography and race. We measured the length of forefinger, middle finger and ring digit in men of two ethnic groups in of Popesti, Bihor County, Romania. We measured the fingers of right and left hands of 200 male Hungarians and of the same number of male Romani (Gypsies). We also calculated digit ratios 2D:4D, 2D:3D and 3D:4D and compared the groups. We calculated the F distribution and Z test. While each ethnic group is genetically very homogenous (as consanguinity is common), statistical analysis of our data demonstrated significant differences between them. Previously we created a database with digit measurements of individuals from different areas of Bihor County and neighbouring counties that allows us to compare different groups of diverse localities and identify the geographic area from which an individual originates.

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COMPARISON OF DIGIT RATIO IN FEMALES OF TWO ETHNIC GROUPS AND TWO LOCALITIES IN ROMANIA

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Study of inheritance in humans necessitates the appearance of particular methods of genetic analysis. We are studying the sexually dimorphic ratio of the length of the forefinger and the ring finger. Females typically have forefinger and ring fingers of about the same length. The effect size for digit ratio between the sexes varies as a function of geography and race. We present the digit ratio in Hungarian and Romani females in the regions of Stei and Oradea. We measured the length of the forefinger, middle finger and ring finger of the right and the left hands of 200 women for each group. We also calculated the digit ratio: 2D:4D, 2D:3D and 3D:4D and compared them by the F distribution and Z test. Statistical analysis demonstrated significant differences between the females of the two groups. Also, the variation coefficient of all length and digit ratio is smaller in Stei than in Oradea because the population of Stei is more genetically homogenous. Previously we created a database with digit measurements from different areas of Bihor County and neighbouring counties. Combined with the present data, the database will facilitate the comparison of different groups from diverse localities and can provide an additional resource for the study of population genetics and forensic applications.

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DIGIT RATIO VALUES IN TWO GROUPS OF MALES IN MARGHITA AND ORADEA, ROMANIA

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Some authors suggest that fingers provide a permanent and easily visible marker of important hormonal events during a critical time of fetal development. Generally, the length of male forefinger (index finger) is 96 percent of the length of the ring finger resulting in the average ratio of 0.96. Males generally have a digit ratio below 1.00, the low digit ratio. We measured the length of forefinger, middle finger and ring finger in Hungarian and Romani men of Marghita and Oradea in Bihor County, Romania. We measured the fingers of right and left hands of 200 men of each group. We also calculated the digit ratios 2D:4D, 2D:3D and 3D:4D and compared them by F distribution and Z test. Statistical analysis demonstrated the significant differences between the two groups. The group of Marghita is more genetically homogenous than the group of Oradea. The explanation for these results may be the genetic content of the population. The cause of variation found in sexually dimorphic traits, such as digit ratio, is certainly puzzling, and it is a fertile area for future research.

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IDENTIFICATION OF SKELETAL REMAINS - DNA DATA EXCHANGE

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The requirement for identification of skeletal remains in Slovakia results from the need to clearly identify victims murdered in the 90's, an important step in evidence development. Analysis of skeletal remains is increasingly used in other types of crime and disaster (e.g., mining disaster) victim identification. We studied 369 samples of skeletal remains recovered from different locations in Slovakia between the years 2005 and 2012 and performed the total of 480 DNA extractions resulting in identification of 245 bodies/body parts. We used silica based DNA extraction as the method of choice; overall success rate was 83,3%. For teeth, this rate was 91,2%, for femurs 87,9%, and for other parts of skeleton it was 72,3%. These results clearly show that success depends on the type of sample, post mortem interval, site status and status of skeletal remains. DNA profiles from human skeletal remains were compared with reference samples or - in the case of unidentified remains - were uploaded and compared to the national DNA database. Nowadays DNA profiles from unidentified remains are compared daily with DNA profiles in operational databases of the countries signatories of the Prüm Convention. In addition, we present the first match of our data with the data in Austrian national database which led to the identification of an unknown scull.

Presentation number: FG38 Abstract number: ABS-203-ISABS-2013

PRELIMINARY STUDY ON INFERRING A SUBPOPULATION ORIGIN OF UNKNOWN MALE SUSPECT FROM Y CHROMOSOME HAPLOGROUP - BAYESIAN NETWORK APPROACH

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Genetic inference on the population origin of an unknown offenders or victims doubtlessly are very useful at the investigation stage. The aim of the study was to use the possible stratification of Polish population, based on Y chromosome haplogroup distribution, to infer the origin of unknown male suspect. Y chromosome haplogroup was used as a polymorphic marker, because of its greater ability, than the autosomal markers, to detect population structure. Previously published data on allele frequencies of the AmpFlSTR Yfiler loci in 10 subpopulations from Poland: mixed Southern, Highlanders, Gypsies, Pomerania, Upper Silesia, Podlasie and from North-Eastern Poland occupations of different origin such as Belarusian, Lithuanians, Tatars and Old Believers was applied. To graphically represent the relationships between the observed data a simple Bayesian network was developed, enabling inference as to the probability of individual's subpopulation origin. Almost all of the tested pairs of subpopulations revealed the value of δc higher than 0.3, what indicates that the adopted way of using Y-chromosome haplogroups as a polymorphic "marker" is a good measure of subpopulation differentiation. The FST coefficient ranged from 0.004 among Upper Silesians and Polish Highlanders to 0.309 (Oldbelievers and Byelorusians), respectively. The obtained for the model area under the ROC curve amounted 0.826 and the percent of cases correctly classified: 89.44. The study provides a promising way to infer the most likely geographic origin of the genetic profile based on typically used in forensic practice Y chromosome markers.

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A DETAILED ANALYSIS OF ALCOHOL PHARMACOKINETICS IN HEALTHY KOREAN MEN

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To determine blood alcohol concentration (BAC) by extrapolation, an understanding of basal pharmacokinetics is indispensable. This study provides a detailed pharmacokinetic analysis of alcohol in 42 healthy Korean men. Among several factors, the ingested dose of alcohol and the allelic variation of aldehyde dehydrogenase 2 (ALDH2) are significant factors influencing the pharmacokinetic parameters, particularly in the absorption and elimination phases. The change in the alcohol dose ingested influenced the maximum concentration (Cmax), the time to reach Cmax (Tmax), the absorption rate constant (K01), the area under the concentration-time curve (AUClast), and the hourly elimination rate. The 487Glu (ALDH2*1) to 487Lys (ALDH2*2) amino acid conversion in ALDH2 resulted in changes in Cmax (ALDH2*1: 0.03 ± 0.01 g/dL [± SD] vs ALDH2*2: 0.05 ± 0.004 g/dL [$p < 0.01$]), AUClast (ALDH2*1: 4.48 ± 2.19 g/dL•min vs ALDH2*2: 7.52 ± 1.26 g/dL•min [$p < 0.05$]), and the BAC elimination rate (ALDH2*1: 0.05 ± 0.02 g/L/h vs ALDH2*2 0.09 ± 0.01 g/L/h [$p < 0.05$]). Moreover, the comparison of BAC and breath alcohol concentration (BrAC) by Bland-Altman plot showed good agreement, suggesting that the measurement of BrAC can be a good alternative for the determination of BAC, particularly in the post-absorption phase. These results provide fundamental information about the pharmacokinetics of alcohol and the determination of BAC in forensics.

Presentation number: FG40 Abstract number: ABS-292-ISABS-2013

WHO TOUCHED THAT? APPLYING CLASSIC STR MULTIPLEX SYSTEMS TO TOUCH DNA

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The aim of this study was to test the possibilities of touch DNA processing using standard STR multiplex kits: PowerPlex® 16 and AmpFLSTR® Identifiler® Plus. For the purpose of the study, a total of 18 swab specimens were processed: 8 door knobs, 5 office chair armrests, 3 light switches, 1 laptop key and 1 mouse button. In order to obtain maximum DNA yield, isolation was conducted according to Qiagen protocols, Investigator Kit® for the PowerPlex® samples and Micro Kit® for the Identifiler® samples. Additionally, samples processed using PowerPlex® kit were put through speed-vac and 32 PCR cycles. Likewise, the Identifiler® samples were further purified and concentrated using Microcon® filters. We observed partial mixture profiles, as well as allele dropout, suggesting the amount of DNA was within the area of low copy number DNA. A single sample, one of the armrests, contributed a full DNA profile. As for the mixed DNA profiles that were also detected, it was very hard to ascertain whether alleles were not in fact allele drop-ins or misinterpreted stutters. Most of the profiles were unsuitable for statistical analysis and were inconclusive when compared to reference samples. Nevertheless, some were fairly informative and could prove useful as part of a more complex investigation. The completely generated DNA profile was successfully connected to a donor profile.

Molecular Anthropology

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POPULATION STRUCTURE OF THE ISLAND OF RAB ESTIMATED BY THE ANALYSIS OF CARDIO-RESPIRATORY TRAITS

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Physiological traits are good indicators of the microevolutionary influence on the biological structure of the population. The aim of this research was to study the population structure of the island of Rab (Croatia) by analysis of physiological (cardio-respiratory) traits. The sample collected in 2002 is comprised of 600 adult examinees from five island's settlements. Cardio-respiratory traits (lung volumes - FVC, FEV1, lung flows - PEF, MEF25%, MEF 50%, MEF 75%, and arterial blood pressure - systolic, diastolic) were analyzed by multivariate biostatistical methods in order to determine the degree of heterogeneity among the populations of the island's settlements and the pattern of their variation. Analyses showed statistically significant inter-population differences for almost all investigated cardio-respiratory traits. Physiological variability among the populations corresponds to that previously established for morphological traits of the head and body and most likely reflects genetic differences, given the fact that populations have been exposed to homogenous physical environment of the relatively small island. Our findings point to the maintained reproductive isolation between the Rab settlements and the results are in line with the geographical, historic and migration data.

Presentation number: AG2 Abstract number: ABS-132-ISABS-2013

MATERNAL GENETIC PROFILE OF A NORTHWEST ALGERIAN POPULATION

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The North African population gene pool based on mitochondrial DNA (mtDNA) polymorphisms has been shaped by the back-migration of several Eurasian lineages in Paleolithic and Neolithic times. Recent influences from sub-Saharan Africa and Mediterranean Europe are also evident. The presence of East-West and North-South haplogroup frequency gradients strongly reinforces the genetic complexity of this region. However, this genetic scenario is beset with a notable gap, which is the lack of consistent information for Algeria, the largest country in the continent. To fill this gap, we analyzed a sample of 240 unrelated subjects from a northwest Algeria cosmopolitan population. mtDNA sequences analysis was performed on the regulatory hypervariable segment I region (HVSI). Haplogroup diagnostic mutations were analyzed using PCR-RFLPs and/or SNaPshot multiplex reactions. Of all North African populations, Eurasian lineages are the most frequent in Algeria (80%) while sub-Saharan Africa origin accounts for the remaining (20%). Within them, the North African genetic component U6 and M1 count for 20%. Indeed, the U6 haplogroup, highly distributed in Northwestern African populations, show a high frequency in Algeria (11.83%), while, the M1 frequency (7.1%) raises an anomalous peak in its decreasing Northeast - Northwest gradient. Moreover, the high frequency of HV subgroups (38.33%) point to direct maritime contacts between the European and North African western sides of the Mediterranean. Besides, the most common western H subgroups, H1 (47.8%) and H3 (10.1%), represent 60% of H lineages. These frequencies and HV0 (7.5%) lie well within the observed Northwestern to Northeastern African decreasing gradients.

Presentation number: AG3 Abstract number: ABS-143-ISABS-2013

ABO BLOOD GROUPS POLYMORPHISM IN WEST ALGERIAN POPULATIONS

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To determine the phenotypic, the allelic frequencies and polymorphisms of the ABO blood groups gene in three ethnic populations of West Algeria, samples of randomly healthy voluntaries were phenotyped by standard serologic techniques for the ABO blood groups and genotyped for exons six and seven of ABO locus by the multiplex ASPCR method. Sampling was done on 283 North-West Algerians from Oran area, 137 Reguibates a tribe of Arabic origin and 95 Zenata Berber populations from Southwest Algeria. The phenotype frequencies of A, B, AB and O obtained in these three populations were respectively: 33,56%, 18,72%, 2,12%, 45,58% for Oran area, 24,09%, 7,3%, 16,79%, 51,82% for Reguibates and 18,95%, 25,26%, 2,10%, 53,68% for Zenata. The obtained results concerning the frequencies of the phenotype A, B and O show no significant differences from Oran area to the Zenates population. However, the frequencies in the Reguibates population compared with those obtained in Oran area revealed significant differences. The ABO genotypes of 82 samples were determined. We identified five classic alleles in this population: ABO* A101 (0,067) , ABO* A102 (0,1049), ABO* B101 (0,1111), ABO* OO1 (0,3580), ABO* OO2 (0,3456) and three B alleles were B101 alleles excluded. This is the first study reporting the detailed preliminary distribution of ABO genotypes in West Algerians.

Presentation number: AG4 Abstract number: ABS-247-ISABS-2013

INCREASED AUTOIMMUNE DISEASE PREVALENCE IN SARDINIAN POPULATION EXPLAINED BY RUNS OF HOMOZYGOSITY AND GENOMIC REGIONS UNDER POSITIVE SELECTION

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Aim: The objective of this study was to provide a plausible explanation of a few increased autoimmune disease prevalences, like diabetes-type 1 and multiple sclerosis, in the Sardinian population. To perform that we studied Runs of Homozygosity (RoH) and genetic regions showing signatures of positive selection. **Methods:** About 1.2M Single Nucleotide Polymorphisms genotyped in 1080 Sardinian individuals were used to investigate the genetic population structure, as well as to estimate RoH and Extended Haplotype Homozygosity regions. **Results:** Using four different methods - fixation index (Fst), inflaction factor (λ), multidimensional scaling (MDS) and ancestry estimation - we were able to highlight, as expected from a genetic isolate, the high internal homogeneity of the island. Comparing Sardinia to mainland Italy through several classes of RoH we have shown that the genome of the Sardinians has globally a higher percentage and a greater mean length of RoH than other Italians from mainland. Several short RoH, characteristic of Sardinia, were identified. In addition, we observed that some genomic regions showing signs of positive selection. Both types of genomic regions harbor genes that may be correlated with the prevalence of type 1 diabetes and multiple sclerosis. Several of these loci could be also linked to the spread of malaria, a disease that was endemic in Sardinia until the 1940s. **Conclusion:** We confirmed the high genetic homogeneity of Sardinia and, we showed that the high prevalence of certain diseases may be explained by the shared ancestry combined with the action of evolutionary forces.

Presentation number: AG5 Abstract number: ABS-188-ISABS-2013

MITOCHONDRIAL DNA VARIABILITY IN MACEDONIANS AND MACEDONIAN ROMANY: IMPLICATION TO THE ROLE IN HUMAN FORENSIC IDENTIFICATION

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In this study we have analyzed a mitochondrial DNA diversity in a sample of 140 unrelated autochthonous Macedonians and 159 Macedonian Romany, representing different ethnical groups residing within the same country of Republic of Macedonia. Based on high-resolution analysis of both coding and non-coding regions of mitochondrial genomes, to each of the sample haplogroup and subhaplogroup affiliation was determined. Population of Macedonians represent quite typical European population, with highest frequency of haplogroup H (42.85%), followed by hg U (17.86%), T (11.43%), J (7.85%) and K(3.57%). The other, less frequent haplogoups, also take part in shaping the maternal genetic pool of this population. On the other hand Macedonian Romani, as an example of endogamous population of Indian origin, shows significant departure from haplogruop composition and frequencies when compared to Macedonians. The highest frequency represent M5a1 haplogroup (38.36%), untypical for any European population, but very common among Indian and Romany populations. Second most frequent haplogroup is hg H (28.64%), but only two haplotypes of hg H20 and H7a1 encompass one half of this haplogroup's diversity. Hg X2 (mostly represented by X2e1), present with less than 1% in typical European population makes more than 10% of maternal gene pool of Macedonian Romany population. This data could have their potential role in human identification as an additional tool in forensic science community.

Presentation number: AG6 Abstract number: ABS-291-ISABS-2013

IMPACT OF MOTHER'S NUTRITION DURING PREGNANCY AND LACTATION, AND NUTRITION OF OFFSPRING ON THE DEVELOPMENT OF PCOS

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Aim: Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder associated with abdominal obesity and ovulatory dysfunction with displayed metabolic changes. Small number of key genes together with environmental factors seems to be responsible for PCOS development. The aim of the study was to detect association between the type of mother's nutrition during pregnancy and lactation, and nutrition of offspring on the development of PCOS. **Methods:** 10 female Sprague Dawley rats were randomly divided in 2 groups. One group was fed with standard laboratory chow (CD), and the other one with high content of saturated fatty acids (HFD). After period of coupling and lactation offspring of the both groups were randomly divided in 2 groups - CD and HFD group. We got 4 groups of offspring genetically similar, but exposed to different intrauterine and postnatal nutrition environments. The weight of offspring was measured once a week. They were sacrificed with 36-40 weeks, after which ovarian morphology and biochemical serum samples were studied. **Results:** Offspring fed HFD of the mother fed HFD had the most frequent changes in ovarian morphology, they had PCOS. They also had metabolic abnormalities, including higher body weight and changes in lipid status. **Conclusion:** The type of nutrition will significantly increase incidence and intensity of the development of PCOS in offspring of mothers fed with high content of saturated fatty acid.

Presentation number: AG7 Abstract number: ABS-152-ISABS-2013

POPULATION—GENETIC ANALYSIS OF SEVEN QUALITATIVE TRAITS IN MAGLAJ MUNICIPALITY, BOSNIA AND HERZEGOVINA

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Genetic structure of a population can be analyzed by studies of qualitative properties controlled by one gene or a small number of genes, each with two alleles, where the type and frequency of alleles have great significance for further analysis of the similarities and differences within and among local populations. This article provides a population-genetic analysis of phenotypes in the population of the municipality Maglaj by six monogenic qualitative characteristics (shape of earlobes, hairiness, middle phalanx, flexibility of lateral tongue edges, extensiveness of the distal and proximal joint of the thumb and flexibility of the distal phalanx of the little finger) and a sexually conditioned property (digital index). In this paper we analyzed the genetic structure of four populations of the rural municipality Maglaj (Kosovo, Novi Šeher, Jablanica, Moševac), one isolated local population (Ravna) and the urban population area of Maglaj. The survey covered a total of 440 students (213 girls and 227 boys). The main objectives of this paper are estimates of intragroup and intergroup variations in the municipality Maglaj, based on examination of the complex of qualitative phenotypic traits (in a sample of 440 respondents) aged 11-18 years. Genetic heterogeneity among the various populations is determined by analysis of complex genetic distance. Assessment of the degree of heterogeneity of the studied set of local populations by individual phenotypic traits was performed using analysis of Wahlund variance. Statistical significance of the differences between the sexes and the areas was determined by t-test%.

Presentatio number: AG8 Abstract number: ABS-202-ISABS-2013

INFLUENCE OF POPULATION SUBSTRUCTURING ON STATISTICAL FORENSIC PARAMETERS OF GENETIC STR MARKERS IN SOUTHEASTERN EUROPE

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Population substructuring due to organization into small groups has probably been a characteristic demographic feature during the vast majority of human population history, and persists today to a greater or lesser extent in many rural areas. As some of the few persisting isolates among contemporary Southeastern European (SE European) human groups, isolated Croatian islands are among the most suitable models for theoretical analyses differentiation and substructuring. In this study the data obtained by the analysis of 9 autosomal STR loci of microsatellite DNA in Croatian island and Slovenian populations were integrated with the published data from other Croatian islands and wide SE European region. The sample was defined hierarchically and the influence of isolation of the Croatian insular populations on substructuring of the population of Croatia, as well as the influence of the regional population groups on substructuring of South-Eastern Europe was detected. Due to the decreased influence of endogamy, lower genetic differentiation was detected at the higher level of grouping of SE European populations (FST=0,002) than at the level of sub-populations of Croatia (FST = 0,005). Finally, the influence of substructuring of the aforementioned levels on the calculation of forensic statistical parameters was determined. It was found that the isolation of Croatian insular populations affects the value of forensic parameters. This led to the modeling of adequate sampling of the entire population when creating the DNA database of genetic STR markers that would reflect all characteristics of its subpopulations.

Presentation number: AG9 Abstract number: ABS-233-ISABS-2013

COSMOPOLITAN CENTRAL ASIA: TAJIK MTDNA TRACES THE EAST-WEST MOVEMENT OF ANCIENT NOMADS

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Tajikistan is a country in the mountains of southeast Central Asia. Due to its isolation, mtDNA variation in the Tajiks has been fragmentary studied on a limited number of samples. In 1997 saliva samples were collected from unrelated Tajiks across Tajikistan. After long-term preservation DNA was extracted from 2 mm FTA discs. Due to degradation mtDNA was amplified using the primary and secondary PCRs with nested primers in the multiplex format. The origin of 91 mitochondrial genomes from Tajikistan traced from western Eurasia (62.6%), eastern Eurasia (25.3%), south Asia (11.0%), and North Africa (1.1%). Significant population structure in the distribution of these mtDNA lineages was revealed within the regional groups in Tajikistan. The mtDNA variation was compared between the Tajiks and 45 populations of Eurasia. Pairwise Fst comparisons and the correspondence analysis revealed non-significant differences between the Tajik and Uzbek populations. Although both nations speak languages belonging to different linguistic groups, this result corresponds to their cultural and economic proximity. Surprisingly, after the Uzbeks, the Tajik mtDNA pool most closely resembles to the Ossetians, an Indo-Iranian people from the North Caucasus. The Tajiks also display intensive gene flow and admixture with some other populations of Central Asia and the Iranian Plateau living along the centers and crossroads of the earliest civilizations and belonging to different linguistic groups including the Uyghur, Kazakh, Karakalpak, Turkmen, Pathans, Iranian Arabs, and Gilaki. This study demonstrates an impact of ancient nomad migrations and invasions on the distribution of mtDNA variation in Eurasia.

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STABLE ISOTOPE ANALYSIS OF PRESUMPTIVE REMAINS OF EARLY MEDIEVAL MEMBERS OF THE ARAGON ROYAL DINASTY: INSIGHTS ON THEIR DIETARY HABITS

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Stable isotope analysis of bone provides information on an individual's diet, with the carbon isotope ratio 13C/12C (d13C) indicating how much marine protein there was in the diet, as compared to terrestrial protein, and the nitrogen isotope ratio 15N/14N (d15N), reflecting the level of animal protein that an organism consumes and therefore, its trophic level in an ecosystem. The aim of this study was to gain knowledge about the dietary habits of eighteen medieval skeletons (dated between 600 and 1300 years BP) pertaining to or buried along with the first kings of Aragon (Northern Spain) by means of the analyses of carbon and nitrogen stable isotope ratios of bone collagen. All samples produced collagen yields with a C:N ratio between 3.2 and 3.4. The d13C values ranged from -19 to -17.7 ‰ and d15N values, from 8.6 to 12.9 ‰, which in general suggests a significant input of animal proteins. Moreover, dietary variation was proven in this royal lineage along the centuries with a progressive increase of protein intake over time. The diet revealed by isotope analyses of bone collagen of this early medieval upper class group is consistent with what could be expected on the ground of historical and archaeological records. A mixture of terrestrial and freshwater fish protein intake appears as the most likely explanation for the observed isotopic values.

Presentation number: AG11 Abstract number: ABS-183-ISABS-2013

GENETIC LANDSCAPE OF NORTH EURASIA REVEALED BY GENOME-WIDE SNPS AND Y- AND X-CHROMOSOMAL LINEAGES

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Research of genetic diversity in humans provide the keys to evolutionary history of our species and has fundamental implications for genetic dissection of common diseases, forensic science and anthropology. We have characterized the genetic variation in North Eurasia using genome-wide SNP set and Y- and X-chromosomal haplogroups. 36 populations under study represent Central and Eastern Europe, Caucasus, Central Asia, Volga-Ural region, North Asia, Siberia and Eastern Asia. Autosomal SNP genotyping was performed with the Illumina BeadChips. Y genetic diversity was analyzed with UEP and STR markers. Single linkage disequilibrium block in ZFX locus was used to estimate X-chromosomal variability. General structure of North Eurasian genetic landscape strictly corresponds to geography. Principal component conversion of genome-wide variability into two-dimensional space precisely projects on the geographical map. Basic components of the Eurasian gene pool are characterized by the clinal longitudinal variability. Next level of the spacious differentiation has the latitudinal vector. Y- and X-chromosomal data produced the patterns of population relationships, similar to genome-wide SNPs, but the level of Y-chromosomal differentiation is several times higher than those for autosomal and X-chromosomal SNPs. Multidimensional analysis of Y- and X-chromosomal variability confirms the clinal patterns of genetic variability and reveals the same basic genetic components as genome-wide data. Phylogenetic and phylogeographic analysis of non-recombining lineages provide further details on the origin of Western-Eurasian, Proto-Uralic, Eastern-Eurasian and Paleo-Asiatic components in North Eurasian gene pool. Our data emphasize that genetic structure has to be taken into account for medical and forensic genetic applications.

Presentation number: AG12 Abstract number: ABS-187-ISABS-2013

MATERNAL GENETIC VARIATION OF THE SLOVENIAN POPULATION IN A BROADER EUROPEAN CONTEXT AND COMPARED TO ITS PATERNAL COUNTERPART

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Slovenia is a European country situated at the crossroads of main European cultural and trade routes. It is geographically more linked to Central Europe, but history draws it closer together to its ex-Yugoslavian, Southeast European (SEE) neighbors. Slovenian maternal heritage has not been analyzed since 2003 and our aim was to analyze SNP markers of 97 Slovenian mtDNAs in high resolution to see where this population fits according to its maternal genetic variation. We compared the Slovenian sample with the neighboring SEE populations, as well as with other published European population datasets. Also, we compared the obtained mtDNA variation results with the available Slovenian Y chromosome data to see how these two uniparental marker systems correspond to each other. In the PC plot based on mtDNA haplogroups frequencies, Slovenian population has an outlying position mostly due to the increased prevalence of J (14.4%) and T (15.4%) clade and especially because of the abundance and diversity of J1c samples in Slovenia, represented with 8 haplotypes and in a percentage of >11%. Although in an outlying position, Slovenian mtDNA variation still shows a certain degree of affinity to SEE. On the contrary, Slovenia's paternal genetic heritage yielded results that correspond to the population's geographic location and groups Slovenian population considerably closer to Central European countries, based on increased prevalence of Northern/Central European R1a-M198 and decreased frequency of Balkan-specific I2a2-M423. Such differences in maternal and paternal marker systems could indicate that Slovenian genetic variation was influenced by sex-biased demographic events.

Presentation number: AG13 Abstract number: ABS-282-ISABS-2013

AN ASIAN TRACE IN THE GENETIC HERITAGE OF THE EASTERN ADRIATIC ISLAND OF HVAR

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The Island of Hvar is situated in the central eastern Adriatic, and its relatively small rural population has been reproductively isolated thought history. Therefore, founder effects, genetic drift and inbreeding have had significant role in the shaping of current genetic diversity of Hvar Islanders. We analyzed Y-chromosome SNP markers of 412 Hvar islanders in high resolution, with the aim to investigate the current paternal genetic diversity. We found a relatively high frequency (6.1%) of unrelated male samples belonging to the Q*-M424 haplogroup, which is unusual for European populations. Interestingly, a previous study showed 9 individuals from Hvar with mitochondrial haplogroup F, which is almost absent in Europe. Both findings could indicate a certain connection with Asian populations, where these haplogroups are most common. This might be a result of several migratory events in the history, one of which could be linked to the ancient Silk Road, the other a consequence of the arrival of the Slavs, following the Avars, to the eastern Adriatic in the 6th century or due to the expansion of the Ottoman Empire in 16th to 18th century. The presence of these rare mitochondrial and Y-chromosome lineages are an example of founder effect and random genetic drift which, in this small island with a high degree of isolation and endogamy, had a strong impact on shaping the genetic diversity of the population.

Presentation number: AG14 Abstract number: ABS-243-ISABS-2013

GENETIC PORTRAIT OF THE BESERMYAN ETHNIC GROUP BASED ON MTDNA HAPLOGROUP STUDY

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Besermyan are a small ethnic group living in the Volga-Ural region of Russia. They belong to Finno-Ugric language group, but speak a special dialect. There are some Bulgar-Chuvash borrowings in their adverb vocabulary that are absent in other dialects of the Udmurt language. Besermyan live in the northwestern part of modern Udmurtia in the Cheptsa basin. In 2002 their number was about three thousand. The Besermyan origin is a very interesting issue. There is a view that the endonym Besermyan (beserman) is derived from the Turkic word which means »Muslim« in Arabic. This hypothesis, along with their language, hints at the origin of this ethnic group; however the genetic portrait of Besermyan has not been described yet. In our study we used the data of mitochondrial DNA (mtDNA) HVS-I sequencing from 98 Besermyans representing 10 villages in Udmurtia Republic of Russia. The prevalence of Western Eurasian mtDNA lineages (91.7%) over Eastern Eurasian ones (9.2%) was shown in the studied population which is consistent with the structure of mtDNA pool of Finno-Ugric ethnic groups of the Volga-Ural region. Some Eastern Eurasian lineages in Besermyan are represented by haplogroups D4b, A4b and Z1a which are also common in Udmurts. It is important to note though that the share of Western Eurasian component in Udmurts according to previous study by Bermisheva et al. (2002) is about 74.5% so mtDNA haplogroup distribution in Besermyans is closer to other Finno-Ugric people of the Volga-Ural region: Mordvins and Maris.

Analysis of Ancient DNA

Presentation number: AG15 Abstract number: ABS-204-ISABS-2013

ANTHROPOLOGIC AND MITOCHONDRIAL DNA ANALYSIS OF A MEDIEVAL GRAVEYARD FROM ŠOPOT (CROATIA)

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Anthropologic and DNA analysis of human remains recovered from a graveyard in Šopot near Benkovac (Croatia) dating to the 14th/15th century was conducted in order to reconstruct the origin and life conditions of the people populating the region at that time. The dynamics of the population represented in this graveyard are important for understanding Croatian history because the deceased individuals were buried according to pagan ritual which was uncommon in a post Christianization period. Human remains from a total of 31 graves were analyzed, in which 47 individuals were found (9 female, 23 male and 15 children). Average age at death for adults was lower than expected (for female 28.9, male 32.4 years), suggesting that the living conditions of these individuals were poor. In addition, 10 antemortem traumas were visible on 6 adults, which is a higher rate than expected, and indicates potential violence within the population group. Finally, mitochondrial DNA (mtDNA) analysis was performed on hypervariable regions one and two for 46 of the individuals. Due to the age and condition of the remains, only 19 of the samples yielded full sequence profiles. Haplogroup analysis was performed for these 19 individuals, with the majority of the results falling within the most common groups in present-day Croatia. However, examination of the less-common haplogroups suggested a possible migration of individuals from Asia. Collectively, the physical and molecular results from this study provide evidence to suggest that individuals recovered from this gravesite are not from the current indigenous population.

Presentation number: AG16 Abstract number: ABS-235-ISABS-2013

FORENSIC ANALYSIS OF DEGRADED DNA: EVALUATION OF A PCR AMPLIFICATION ENHANCEMENT REAGENT

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Exposure of biological evidence to high temperatures, ultraviolet (UV) radiation, microbial activity, or oxidative processes, typically results in DNA damage that will affect the overall quality of the DNA typing profiles. The primary focus of this study was to evaluate the PCR amplification enhancement reagent STRboost™ (Biomatrica®) as an aid in the analysis of damaged or degraded DNA. DNA samples were exposed to UV radiation to mimic the damaging effects of sunlight, and to DNase I to mimic microbial enzyme degradation. The experiments were conducted in a controlled manner. Treated and untreated DNA samples were analyzed using commercially available fluorescence-based PCR-STR DNA typing kits. Capillary electrophoresis was used to obtain the DNA profiles. As expected, as the time of exposure to UV or DNase increased, there was a corresponding decrease in the fluorescence intensity of the STR alleles in the DNA profiles. In general, the addition of STRboost™ to the PCR amplification reaction increased the fluorescence intensity of the STR alleles in the DNA profiles. Up to a six-fold enhancement in the fluorescence intensity of the STR alleles was observed with the DNase treated samples. However, the degree of enhancement varied with the extent and type of degradation, and the size of the alleles being amplified. Our results show that the use of STRboost™ can improve the overall quality of the DNA typing profiles from damaged or degraded DNA samples.

Presentation nuber: AG17 Abstract number: ABS-284-ISABS-2013

KINSHIP ANALYSIS OF ANCIENT INDIVIDUALS BY COMBINED USE OF SMALL BIALLELIC INSERTION/DELETION (INDEL) AND SHORT TANDEM REPEAT (STR)

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In bioarchaeological research, the results of DNA typing are often used for sex determination and kinship analysis among ancient individuals. However, the amount of information and the reliability of the results in such analyses are limited by the quality and quantity of isolated ancient DNA (aDNA). The aim of this study was to investigate the possibility of determining putative familial relationships between three ancient individuals using commercially available short tandem repat (STR)-multiplex and insertion/deletion (INDEL)-multiplex kits suitable for degraded DNA samples and those containing PCR inhibitors. Three tooth samples were obtained from human skeletal remains of Vranjevo selo, an archaeological site located in Bosnia and Herzegovina. DNA was extracted using optimized phenol-chloroform protocol with EDTA decalcification pre-extraction step. DNA samples were then quantified using the Quantifiler™ Human DNA Quantification Kit. The Investigator® DIPplex Kit was used for multiplex amplification of 30 small biallelic INDELs, and STR analysis was performed using the Powerplex® ESX17 and ESI17 kits. Both types of kits also include the Amelogenin locus. DNA fragment size analyses were conducted on an ABI PRISM 310 Genetic analyzer. At each stage, requirements for preventing contamination with exogenous DNA were achieved. Kinship analyses were carried out using Familias program. Complete or partial DNA profiles were obtained from all three samples. The results of kinship analyses demonstrated the presence of genetic relationship between three ancient individuals, in accordance with anthropological and archaeological data. The overall results indicate the suitability of improved commercially available multiplex kits for the analysis of aDNA.

Presentation number: AG18 Abstract number: ABS-273-ISABS-2013

DISCONTINUITY SCREENING OF THE EARLY FARMERS' MT-DNA LINEAGES IN THE CARPATHIAN BASIN

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Discontinuous mitochondrial (mt) haplotype data between Central-Europe's first farmers and contemporary Europeans have been described before. Hungary was a key-area of the Neolithisation, in the route of Neolithisation following the River Danube, and that was also the birthplace of the Linear Pottery Culture, which later colonised Western and Northern Europe. Neolithic and post-Neolithic human remains as well as contemporary population of Hungary is involved in our project to gain information on their mt-haplotype pattern and especially on the frequency of Asian haplotypes in the Carpathian Basin. HVS-I sequences from nt15977 to nt16430 of Neolithic specimens with sufficient mtDNA preservation among an extended Neolithic collection were analysed for polymorphisms, identifying 23 different ones. A novel, N9a, N1a, C5, D1/G1a, M/R24 haplogroups were determined among the pre-industrial Hungarians. The presence of Asian haplotypes in the ancient populations must be taken into consideration when reconstructing the population history of Europe and Asia, so a survey of the recent Asian haplotype frequency in Europe is unavoidable. The ancient and recent haplotype pattern of Hungary is definitely worth further investigation to test a theory on the continuous population history of Europe, wheter genetic gaps between ancient and recent human populations of Europe were more likely to be detected. Supported by The Wenner-Gren Foundation for Anthropological Research, Inc., USA (grant No. ICRG 117)

Presentation number: AG19 Abstract number: ABS-234-ISABS-2013

POSSIBLE BIOLOGICAL RELATIONSHIP OF SKELETAL REMAINS FROM MASS GRAVE BASED ON ADNA

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A mass grave containing skeletal remains of 5 individuals was discovered and excavated in 2007 in village Brteč near the city Vysoké Mýto. Initially the official police investigation started because of the violent death of all deceased but artifacts linked the skeletons to the Second Word War. Due to very well preserved remains, anthropologic profiles (sex, age, race, stature) as well as the paleopathological and traumatic changes including the manner of death were established. Confirmation of biological relationships between individuals was essential in further identification: Brteč was located very close to Sudetenland where whole families were massacred during the war; moreover there was the Luftwaffe airport in Vysoké Mýto. DNA was extracted from teeth of the remains and isolated with MinElute PCR Purification kit (Qiagen). Genetic test was made by multiplex amplification of 9 STR polymorphisms (AmpFlSTR Profiler Kit, Applied Biosystems, USA) followed by capillary electrophoresis with fluorescent detection (automatic genetic analyzer ABI PRISM 310). Software GeneScan 3.1.2 (Applied Biosystems, USA) evaluated our results.

Presentation number: AG20 Abstract number: ABS-260-ISABS-2013

MITOCHONDRIAL DNA AND PHYLOGENETIC ANALYSIS OF PREHISTORIC NORTH AFRICAN POPULATIONS

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North Africa is located at a crossroad between Europe, Africa and Asia and has been inhabited since the Prehistoric time. In the Epipaleolithic period (23.000 years to 10.000 years BP), the Western North Africa has been occupied by Mecha-Afalou Men, authors of the Iberomaurusian industry. The origin of the Iberomaurusians is unresolved, several hypotheses have been forwarded. With the aim to contribute to a better knowledge of the Iberomaurusian settlement we analysed the mitochondrial DNA (mtDNA) of skeletons exhumed from the prehistoric site of Taforalt in Morocco (23.000-10.800 years BP) and Afalou in Algeria (11.000 to 15.000 BP -Algeria). Hypervariable segment 1 of mtDNA from 38 individuals were amplified by Real-Time PCR and directly sequenced. Sequences were aligned with the reference sequence to perform the mtDNA classification within haplogroups. Phylogenetic analysis based on mitochondrial sequences from Mediterranean populations was performed using Neighbor-Joining algorithm implemented in MEGA program. mtDNA sequences from Afalou and Taforalt were classified in Eurasiatic and North African haplogroups. We noted the absence of Sub-Saharan haplotypes. Phylogenetic tree clustered Taforalt with European populations. Our results excluded the hypothesis of the sub-Saharan origin of Iberomaurusians populations and highlighted the genetic flow between Northern and Southern cost of Mediterranean since Epipaleolithic period.

Presentation number: AG21 Abstract number: ABS-134-ISABS-2013

DETERMINATION OF Y-HAPLOGROUPS: Y-SNP ANALYSIS VERSUS Y-HAPLOGROUP PREDICTOR

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Determining human Y-haplogroups is the most useful tool in tracing human histories that have arisen a single time in human evolution. The results of Y-haplogroup determination by SNP analysis were compared with the prediction of Y-chromosome haplogroup from a set of Y-STR markers. In study the efficiency of three available softwares for Y-haplogroup prediction was tested on samples with know haplotypes and haplogroups in the Slovak Romany population. The haplogroup with the highest frequency was haplogroup H, particularly H1a-M82, followed by haplogroup E, sublineage E1b1b1a-M78. The proportion of error observed in the allele-frequency haplogroup prediction method was not be so critical and predictors proved to be good estimators for Y-haplogroup prediction. Nevertheless, they are less accurate and Y-SNP analysis is necessary.

Presentation number: AG22 Abstract number: ABS-287-ISABS-2013

ANTHROPOGENETICAL ANALYSIS HELP TO DETERMINE THE IDENTITY OF THE BURIED SKELETONS IN CISTERCIAN CONVENT PORTA COELI

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Molecular anthropology helps to determine the relationship and the geographical origin of individuals. We used the tools of molecular archeology to study the relationship among human remains found in the Cistercian convent Porta Coeli (20 km NW from Brno) in the early nineties, specifically at the now defunct St. Catherine Chapel. The chapel was used during the 13th century construction of the monastery church of the Assumption. Eleven skeletons were found under the floor in the chapel. From the historical record we know that the members of founding families and monastery donors had the privilege of burial in the Cistercian sacred buildings. The excavated bones were in good condition but without objects in the graves that would allow skeleton identification. A tooth of each skeleton was washed, powdered and decalcificated. DNA from obtained material was isolated by Mini ElutePCR purification Kit® (QIAGEN). The STR and YSTR analysis were used for the detection of the relationship between buried individuals (PowerPlex® ESX and ESI Systems, PowerPlex® Y Systems, Promega). The genetic profile was determined for each individual. Amplification of the markers on mtDNA was not successful as humic acids co-isolated with the DNA in the samples inhibited the PCR. Apparently, the individuals buried under the floor in the St. Catherine Chapel were not relatives of the first instance. These results do not correspond with the historical record about buried sponsor family.

DNA Analysis in Victims of Mass Disasters

Presentation number: AG23 Abstract number: ABS-244-ISABS-2013

THE CHALLENGE OF IDENTIFICATION OF BURNED HUMAN REMAINS

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Burned human remains are very common in mass disasters and are always difficult material for forensic identification because often they could not be identified by conventional means. In such cases, when classical methods are not useful, DNA analysis is usually the only suitable method. DNA analysis is very powerful method for individualization and identification of skeletal remains and it is performed for almost 20 years in our laboratory. We will present several cases from our practice involving remains that were badly damaged by fire and made classical identification impossible. We are going to show different examples: war victims' remains, plane and car accident as well as house fire. Although remains were highly carbonized we obtained DNA profiles from all typed samples. The quality of results depended on sample condition, time after death and applied methods and technology. Therefore, DNA analysis is recognized as the precise and straightforward way to answer the question of identity.

Presentation number: AG24 Abstract number: ABS-123-ISABS-2012

MODERN DOPING: THE FUTURE OF SPORTS

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The concept of doping activities goes back to the beginning of sports and recreational activities. There is very little evidence about performance improvement of ancient Greeks, however, during development of sport, doping in sport has become ubiquitous, and foundation of World Anti-doping Agency was necessary in 1999. Modern doping involves gene doping, which as an offshoot of gene therapy progresses in parallel with the progress of medicine. Gene therapy in medicine is considered to be the future treatment of many currently incurable diseases, which is why many studies are conducted, initiated and conceived only in the areas of gene therapy. This review covers methods of gene therapy and gene doping, and a number of candidate genes in gene doping divided by endurance capacity, muscle performance and psychological abilities. Also describes the results of recent investigations of each candidate gene individually, and the opportunities and potential targets for future studies. This work included the area of negative, but also positive side effects of gene doping, detection techniques and their development, and at the end, the bioethical perspective on the whole subject, which is certainly one of the crucial segments in the development of gene doping.

Genetic Basis of Disease

Presentation number: MG1 Abstract number: ABS-221-ISABS-2013

ASSOCIATION OF SOME MORPHOLOGIC AND PHYSIOLOGIC TRAITS IN PATIENTS WITH ALLERGIC AND CHRONIC INFECTIOUS DISEASES IN KOSOVA

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The aim of this study was to investigate the frequency and distribution of some selected phenotypes in patients with allergic and infectious diseases as well as their eventual association with the risk of developing these diseases. For this purpose 466 patients with allergic diseases and 101 patients with hepatitis were examined in the University Clinical Centres and compared with the data of 529 control healthy individuals. The following selected phenotypes were examined: ear lobe (free/ attached), chin (normal/cleft), ability for tongue rolling (roller/non-roller), eye color (dark/light), hair color (dark/light). In addition, the blood groups from ABO and Rh system as phenotypical markers were observed. The results obtained show that tongue rollers are more frequent in patients with allergic rhinitis ($P<0.05$) and allergic asthma ($P><0.005$). Light eye color is more frequent in patients with dermatitis ($P><0.05$), whereas, light hair individuals are more frequent among patients with allergic rhinitis ($P><0.005$) and dermatitis ($P><0.05$). Furthermore, there were specific correlations for allergic rhinitis, dermatitis and allergic asthma observed. The correlations observed were different from those in control individuals. In patients with hepatitis, there was only one specific correlation (statistically significant) observed. Phenotype frequencies and specific associations observed in these preliminary findings could serve as a basis for further in depth investigations involving higher number of patients and parameters, would contribute to an understanding of predispositions and susceptibility to allergic and chronic infectious diseases.

Presentation number: MG2 Abstract number: ABS-227-ISABS-2013

HOMOZYGOSITY MAPPING OF A FAMILY AFFECTED WITH PROGRESSIVE MYOCLONIC EPILEPSY FROM OMAN

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Monogenic disorders are far less common than the genetically complex disorders, which involve more than one gene. Studies of these monogenic disorders have allowed researches to learn more about the molecular mechanisms underlying the phenotypes and to identify new genes involved in cellular pathways. Progressive myoclonic epilpsy (PME) is a group of rare neurological disorders that have been associated with quite a number of genes identified so far. We identified a family with two brothers affected with Unverricht-Lundborg disease (ULD), subtype of PME know to be caused by mutations in CSTB and SCARB2 genes. The two brothers also have a cousin who is affected with the same disease. The patients are in their second decade of life and are on epileptic drugs. The age of onset was at the age of 10. We sequenced the two genes known to cause ULD, which are CSTB and SCARB2 by Sanger sequencing and found no sequence variations from normal. It is worth mentioning here that we identified another two families with ULD in which both had mutations in the CSTB gene promoter region. So we decided to perform homozygosity mapping using 250K SNP chips from Affymetrix to map the disease locus in this family. We are currently looking at candidate genes in the regions of homozygosity and pursuing exome sequencing to identify the genetic cause of the disease in this family.

Presentation number: MG3 Abstract number: ABS-256-ISABS-2013

MITHOCHONDRIAL DNA AND Y-STR ANALYSIS OF TUNISIAN GLYCOGENOSIS TYPE III PATIENTS

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Glycogen storage disease type III is an autosomal recessive inherited metabolic disorder resulting from deficient glycogen debrancher enzyme activity in liver and muscle. In this study, we focused on the p.W1327X AGL gene mutation in 16 Tunisian patients originating from the same region in Tunisia. The study was performed to understand migration flows having contributed to the emergence of this mutation in Tunisia. The patients were investigated for exons 4 and 31 of AGL gene, the hypervariable segment 1 of mitochondrial DNA sequencing and genotyping of 17 Y-STR markers. We found that the p.W1327X mutation was a founder mutation in Tunisia and located on the same haplotype with Turkish and Caucasian patients. Maternal lineages were characterized by an admixture of autochthonous North African, sub-Saharan and a predominance of Eurasian haplogroups. However, paternal lineages were highly homogeneous and originated from the Arabian Peninsula. We hypothesize that the p.W1327X mutation was introduced into Tunisia probably by a recent migration event then the mutation was fixed in a small region due to the high rate of consanguineous marriages and genetic drift. The screening for this mutation should be performed in priority for patients from this region of Tunisia and those from regions sharing the same settlement history.

Presentation number: MG4 Abstract number: ABS-306-ISABS-2013

A PATIENT WITH AUTOSOMAL RECESSIVE CUTIS LAXA TYPE 2B DUE TO PYCR1 GENE MUTATION

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Autosomal recessive cutis laxa (ARCL) is a group rare, inherited disorders named after the characteristic skin phenotype present in patients. Besides wrinkled skin various other clinical manifestations such as intrauterine growth retardation, failure to thrive, developmental delay, progeroid appearance, triangular dysmorphic face, osseous abnormality and CNS manifestations can be found in these patients. Autosomal recessive cutis laxa type 2B (ARCL2B; OMIM #612940) is a subgroup of ARCL caused by mutations in PYCR1 gene encoding mitochondrial pyrroline-5-carboxylate reductase 1, mostly expressed in skin and bones. Diagnosis is often difficult owing to a broad clinical overlap with other cutis laxa or progeroid syndromes. Here we describe an 18-year old male patient with ARCL2B. The patient was initially diagnosed as neonatal progeroid Wiedemann-Rautenstrauch syndrome (WRS; OMIM #264090) at the age of 2 years due to senile facial appearance and dysmorphic craniofacial features. He has been reevaluated at the age of 17 years, when some other salient clinical features including prominent forehead, protruding ears, myopia, wrinkled skin with prominent veins over the dorsum of the hands and feet, hypermobility of small joints, as well as mild intellectual disability and epilepsy were noted. According to these clinical findings diagnosis of ARCL2B was suspected. Sequencing of PYCR1 gene revealed homozygous c.797+2-797+5del splice-site mutation and confirmed the diagnosis ARCL2B. The mutation resides within exon 6, which encodes the most highly conserved part of the PYCR1 protein and is associated with moderate to severe ARCL2B phenotype.

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EFFECTS OF IMMUNE MODULATION THERAPY IN THE FIRST CROATIAN INFANT DIAGNOSED WITH POMPE DISEASE: A 3-YEAR FOLLOW UP STUDY

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The Pompe disease is an autosomal recessive storage disorder characterized by deficient or absent activity of the enzyme acid alpha-glucosidase. Due to ineffective metabolism, glycogen accumulates in muscle tissues. Patients with classic infantile-onset form usually present by the first months of life with hypertrophic cardiomyopathy and muscle weakness. If left untreated, these patients rapidly die of cardiorespiratory failure. A cross-reactive immunological material (CRIM)-negative status is predictive of high anti-alglucosidase alfa antibody titers. CRIM-positive patients also sometimes develop robust antibody titers. High antibody titers complicate therapeutic management, and those patients have worse clinical outcome of enzyme replacement therapy (ERT). Fours years ago, we successfully used an immune modulation therapy (IMT) protocol in a CRIM-positive infantile-onset patient with Pompe disease in whom infusions had to be temporarily discontinued because of severe infusion-associated reactions. She was found to be positive for anti-alglucosidase alfa antibodies (1:6,400). IMT (rituximab, methotrexate and intravenous gamma globulin) was started, and ERT was safely reintroduced during the IMT induction phase without complications. Antibodies disappeared, IMT was tapered and discontinued, and cadiomyopathy steadily improved. During more than three years of follow up, she remained ventilator-dependent and no gains in motor skills were noticed. Antibodies are still undetectable and no adverse reactions associated with IMT had occurred. The cardiomyopathy is gradually increasing, but there is still ~50% reduction as compared to the highest value measured. Although the reversal of clinical decline in our CRIM-positive and antibody-positive infant with Pompe disease cannot be solely attributed to IMT, this protocol proved efficient and safe.

Presentation number: MG6 Abstract number: ABS-288-ISABS-2013

GENETIC TESTING IN MALE INFERTILITY

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Azoospermia is the complete absence of sperm in the both ejaculate and centrifugate. It occurs with a frequency of about 1-3% of the male population and accounts for 10-15% of male infertility. Male infertility as a cause for ART (assisted reproductive technology) is growing rapidly in last 20 years and according to literature, almost half of all infertility problems nowadays are due to male problems. Genetic factors such as chromosomal abnormalities, monogenic disorders, multifactorial genetic disease, etc are discovered to be connected with this issue and more than 2,000 genes have been implicated in male fertility. In our hospital for more than 15 years TESE/ICSI (testicular sperm extraction/intracytoplasmic sperm injection) procedure is used as a method of choice for patients with azoospermia, but after routine history, physical examination and hormonal assessment it is necessary and obligatory to have cytogenetic and Y-chromosome microdeletion analysis as minimum standards for either diagnosis or management of these patients in ART. There is high incidence of chromosomal abnormalities and microdeletions of the long arm of Y chromosome in male infertility. Yq11 region is referred to as azoospermia factor (AZF) region with subregions AZFa, AZFb and AZFc with candidate genes DAZ, RBMY, USP9Y, CDY, PR, CDY, PRY and DBY. In conclusion, infertility at all, but specially male part, has to incorporate genetic testing in everyday routine because all other methods reached the end and efficacy of ART can hardly be more than 30%, as it is now, without novel techniques such as genomics, proteomics, and metabolomics.

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MOLECULAR-GENETIC BASIS OF NON-SYNDROMIC HYPODONTIA: FAMILY REPORTS

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Non-syndromic hypodontia is the most common orofacial deformity in humans. It represents the agenesis of one to six teeth. There is a large amount of molecules involved in the process of dental development. Several candidate genes associated with non-syndromic hypodontia have been described, but molecular-genetic basis of disease is still unknown. The aim of this study was to clarify the role of paired domain box gene 9 (PAX9) in the pathogenesis of non-syndromic hypodontia. We investigated the two families with severe tooth agenesis. Agenesis was present in each quadrant extensively. Tooth agenesis was characterized by panoramic radiographs. The probands and theirs family members were studied. In first family the proband has agenesis of 8 teeth, mother has microdontia of upper second premolars. In second family the proband has affected 18 teeth, his sister 7 teeth and father had absence of 9 teeth. DNA samples of all family members were analyzed. Genomic DNA was isolated from buccal swabs. Mutation analysis was performed by amplifying PAX9 exons and sequencing the products. DNA sequencing analysis was performed by the 24-capillary 3500xL Genetic Analyzer (Life Technologies). Six polymorphisms were found in PAX9 exons 1, 4 and 5. The role of common variants located out of binding domain in pathogenesis of non-syndromic hypodontia is discussed.

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ANALYSIS OF MITOCHONDRIAL DNA POLYMORPHISM IN TUNISIAN TYPE 2 DIABETES

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Genetic variations in the non-coding region of mitochondrial DNA may play an important role in the pathogenesis of type 2 diabetes (T2D) and its complications. Nevertheless, the implication of certain mitochondrial variants in T2D is still controversial. We aimed to explore whether mitochondrial DNA variants contribute to the susceptibility to T2D in Tunisian population. A case control association study was performed on 64 T2D patients and 77 controls. The hypervariable region 1 (HVS1) from np16069 to np16400 of the mitochondrial DNA was amplified and sequenced. Statistical analysis was carried out using STATA program. Analysis of the T16189C variant showed that this SNP is common in Tunisia with a frequency of (~30%) in both T2D patients and controls. Statistical analysis showed that T16189C variant was unlikely to be associated with T2D in Tunisians. In addition, the remaining studied SNPs (87) from the HVS1 region showed that only the distribution of the G16390A variant was significantly different between T2D and controls ($p = 0.04$). Multivariate logistic-regression analysis with adjustment for age, sex and BMI revealed that G16390A variant was not associated with susceptibility to T2D. We found no statistical evidence to support an association between T2D and HVS1 polymorphism in the studied Tunisian population.

Presentation number: MG9 Abstract number: ABS-215-ISABS-2013

ASSOCIATION OF HLA AND ABO BLOOD GROUP WITH KIDNEY GRAFT FUNCTION

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Aim: To determine association of HLA and ABO blood group with kidney graft function 3 years after transplantation (TX) in patients at the Clinical Hospital Center in Osijek (KBCO). **Methods:** Study included 64 patients, median age 50 years, after deceased donor kidney TX in KBCO, transplanted from 2007 to December 2012. Of the total of 64 kidney transplant recipients, we analyzed 62 because two grafts did not survive a week (one for sudden death and another for the renal artery thrombosis, in the first 24 hours, respectively). Data were taken from medical records and statistically analyzed using SPSS. **Results:** Kidney graft function was analyzed at 3, 6, 12, 24 and 36 months posttransplant. 25 of the examinees were A+, while 13 were 0+. 27 donors were A+ and 14 of them were 0+. The majority (n=25) of the recipients had 3 HLA mismatches with their donors, 16 recipients had 4 and 15 had 2 mismatches. No mismatches were found in only 1 of cases. Blood groups were not differently distributed between the different blood groups. The mean creatininemia 3 months after TX (n=58) was 144 μmol/L, 6 months after TX (n=55) was 136 μmol/L, 12 months after TX (n=48) was 147 μmol/L, 24 months after TX (n=29) was 142 μmol/L and 36 months after TX (n=14) was 159 μmol/L. **Conclusion:** The number and type of HLA mismatches, and ABO blood type had no significant effect on graft function.

Presentation number: MG10 Abstract number: ABS-145-ISABS-2013

EGFR/HER2 NEGATIVE ACINIC CELL CARCINOMA ARISING IN NASAL CAVITY: A CASE REPORT

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We present a rare case of acinic cell carcinoma (ACC) arising in nasal cavity. It is a very unusual and atypical location for this tumor. ACC is a rare, slow-growing salivary neoplasm, representing approximately 2.4% of all salivary neoplasm. It occurs in the parotide gland in more then 90% of cases, but it has also been reported in the submandibular gland (0.4%) and in minor salivary glans (0.3-05%). In research, EGFR and HER2-AKT-mTOR signaling pathways are activated in subgroups of salivary gland carcinomas. EGFR overexpression was found in 51% of the tumors, while HER2 overexpression was observed in 21 %. Our patient is a 53-year-old women who reported a nasal obstruction with difficulty in breathing through the left nostril and frequent headaches. The patient had a long history of sarcoidosis, hypothyreosis and had hysterectomy. On physical examination, a roundish mass was found under the left nasal turbinate, spreading to the epipharynx. In this case we wanted to see if there was EGFR/HER2 mutation present, so we did cobas EGFR® mutation test. This is a real-time PCR test for qualitative detection and identification of mutations on exons 18,19,20 and 21 of the epidermal growth factor receptor (EGFR) in DNA derived from formalin-fixed paraffin-embedded acinic cell carcinoma tumor tissue. The test was negative for both genes in our tumor tissue sample. In summary, we have a case of acinic cell carcinoma interesting for its unusual location and negative EGFR/HER2 gene mutations, which are usually positive in this tumor.

Presentation number: MG11 Abstract number: ABS-146-ISABS-2013

P53 AND P16^{INK} IN AN UNUSUAL HISTOPATHOLOGICAL PICTURE OF LOWER LIP LESION: A CASE REPORT

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Actinic cheilitis (AC) is a pre-malignant inflammatory reaction of the lips caused by continuous exposure to solar rays. The reported risk of AC progressing to squamous cell carcinoma (SCC) varies from less than 1% to 20%. Squamous cell carcinoma of the skin is the most common malignant tumor of the lips. SCC is a complex malignancy where environmental factors (sun exposure), virus infections, and genetic alterations interact, and thus give rise to the malignant condition. There are also high-risk factors such as the roles of human papillomaviruses (HPV), the Epstein-Barr virus, and the human herpes simplex virus (HSV). There are also several genes associated with the condition: p53, p16 (INK4) and p21 (WAF1/CIPI), survivin, B-cell lymphoma-2 (BCL-2), keratins, fibroblast growth factor 3 (FGF3), FGF4, FGF19, oral cancer overexpressed gene 1 (ORAOV1), and cyclin D1 (CCND1). We present a rare case of the lower lip lesion in a 71-year-old woman with the microscopic view uncharacteristic for anything so far described. At first she was treated with local application of antibiotic Tyrothricin and lip balm with key ingredients sucralfate, vitamins E and F; as she was getting worse, the pathologically changed tissue was surgically excised. We tested the lesion for HPV, p53 and p16 (INK4). DNA was isolated and tested in TaKaRa Thermal Cycler DiceTM Cat. #6603 but lesion showed negative for HPV. Testing for p53 and p16 showed positive results. We conclude that we have an unusual histopathological picture but with a connection to tumor markers associated with SCC.

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NON-TRAUMATIC MUCOSAL NEUROMA OF GALLBLADDER NON ASSOCIATED WITH MEN 2B: A CASE REPORT

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We are representing a case of non-traumatic mucosal neuroma of the gallbladder that is not associated with MEN 2B. Until now, no similar findings have been reported. There have been many reported cases that associate MEN 2B syndrome with non-traumatic neuroma of the gallbladder. Primary cancer of the gallbladder is very rare and the most common one is adenocarcinoma with various subtypes. Considering tumors with neural crest origin in gallbladder, we count few cases of schwanomas, paragangliomas, traumatic neuromas and neuroendocrine carcinomas. Our patient is 40- year old man who suffers from gallstones for two years. He came to hospital for a removal. His pain was situated in upper abdomen and bellow right costal arch, specially after a meal. Infectologists have been controlling him because of hepatitis C (formal addict) and he wasn't on interferon therapy. His family anamnesis was negative, so were the findings of other tumors associated with MEN 2B syndrome. Histopathological findings after laparoscopic cholecystectomy were cholecystitis chronic and mucosal neuroma. We are proposing an additional differential diagnose for patients who for years suffer of some liver disease or gallstones, indicating that non-traumatic neuroma of gallbladder cannot be excluded. It may be connected to liver transformations and pathological abnormalities.

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MECKEL GRUBER SYNDROME IN EUROPE: A POPULATION BASED STUDY

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Aim: Meckel Gruber syndrome is a rare autosomal recessive lethal disorder caused by mutations in nine genes involved in ciliar protein functioning. We present data based on a large European population regarding prevalence of all birth outcomes, prenatal diagnosis, congenital malformations and other characteristics of this syndrome. **Methods:** Data from 34 birth registries from 16 European countries included in EUROCAT network were analyzed. These registries record congenital malformations in live births, foetal deaths > 20 gestational weeks and terminations of pregnancies. The data for this study were extracted from the central database using ICD/BPA/EUROCAT (Q6190) and OMIM (249 000) codes. **Results:** We identified 190 Meckel Gruber syndrome patients in the population of 13 753 505 births in the 1990-2009 study period. Total prevalence rate was 1.38 per 100 000 births. Most patients were diagnosed prenatally (87.3%). Termination of pregnancy following prenatal detection of severe anomaly/anomalies was performed in 143 patients (75.26%), 38 patients were live births (20%) and 9 (4.7%) stillbirths. The most common congenital anomalies were occipital encephalocele (76.8 %), multicystic dysplasia of kidney (71.09%) and polydactyly in (85%). Other associated anomalies were present in 32 % cases. Post mortem analysis was performed in 60.5 % patients. **Conclusion:** Meckel Gruber syndrome is a rare lethal disease affecting 1 in 72 386 patients across Europe. Prenatal diagnosis led to termination of pregnancy in two thirds of patients. Antenatal ultrasound examination can identify major anomalies, but a post mortem examination is needed to document all anomalies and for confirmation of syndrome diagnosis.

Presentation number: MG14 Abstract number: ABS-223-ISABS-2013

ASSOCIATION BETWEEN HLA AND OCCURRENCE OF NEW ONSET DIABETES AFTER TRANSPLANTATION

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Aim: To assess connection between various human leukocyte antigens (HLA A, HLA B and HLA DR) and occurrence of new onset diabetes after transplantation (NODAT) in patients who have undergone kidney transplantation from deceased donor. **Methods:** Data was collected from patient's medical charts and statistically analyzed using SPSS. There were 64 examinees (39 males, 25 females; median age 50 yrs). **Results:** Prevalence of NODAT in the examined population was 7 out of 58 patients (12.1%). The majority (25 patients; 40.3%) of the recipients had 3 HLA mismatches, 16 (25.8%) had 4 and 15 (24.2%) had 2 mismatches with their kidney donors. We classified kidney graft function into 4 groups, based on the serum creatinine concentration (SCC). In group 1 patients had SCC<130 μmol/L, in group 2 between 130 and 259 μmol/L, in group 3 between 260 and 400 μmol/L and in group 4 SCC was above 400 μmol/L. SCC was observed at release from hospital and then again 3, 6, 12 and 24 months after kidney transplantation. Majority of examinees (n=22; 36.7%) at the release from hospital have belonged to the group 2, after 3 months 36 patients (62.1%) were in the group 1 and the same was after 6 months. After 12 months there were 27 examinees (56.3%) in the group 1 and after 24 months we had 16 examinees (55.2%) in the same group. **Conclusions:** Type of HLA and number of mismatches were not associated with NODAT. NODAT was not associated with kidney graft function.

Presentation number: MG15 Abstract number: ABS-242-ISABS-2013

MOLECULAR-GENETIC STUDY OF CONGENITAL ADRENAL HYPERPLASIA IN BASHKORTOSTAN REPUBLIC (RUSSIA)

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Congenital adrenal hyperplasia is a group of autosomal recessive disorders of adrenal steroidgenesis. 95% of CAH cases are due to 21-hydroxylase deficiency. This defect is the result of gene conversion events between the functional CYP21A2 gene that encodes the 21-hydroxylase and the adjacent inactive pseudogene (CYP21P). We analyzed CYP21A2 gene in 129 unrelated CAH patients from Bashkortostan Republic of Russia. The patients were divided into 2 groups according to clinical findings: classical (88.4%) and nonclassical (11.6%). Our study showed that the gene deletions/large gene conversions were present in 27.3% of unrelated alleles. The most frequent point mutations I2splice and R356W occurred in 13.6% and 10.8%, respectively. Other mutations, I172N, Q318X, V281L and P30L were rare (4.7%, 3.9%, 2.3% and 1.5%, respectively). The clusters of mutations of CYP21A2 gene in one chromosome: Q318X+R356W (1.9%), I172N+Q318X (0.4%), delA2orLGC+V281L (0.4%) and I2splice+P453S (0.4%), were found in 8 CAH patients. Furthermore we identified 3 additional mutations of CYP21A2 gene in CAH patients. In one patient we found previously described missense mutation R426C in exon10. In another patient we found previously unreported deletion of 3 nucleotides in exon9 of CYP21A2 gene that led to the deletion of isoleucine in 384 position of amino acid sequence. And in one patient we identified the insertion of one nucleotide in exon7 of CYP21A2 gene. The frameshift mutation F307+1 nt was found in a heterozygous state with I172N mutation. In conclusion, previously described diagnostically significant CYP21A2 gene mutations were present in about 77% of unrelated CAH alleles.

Presentation number: MG16 Abstract number: ABS-251-ISABS-2013

TOWARD IMPROVED MANAGEMENT OF GENETIC DISEASES IN TUNISIA BY BIOMEDICAL DATA CAPTURING

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Aim: Despite increasing interest for rare genetic diseases they are still neglected in Tunisia due to limited-resources. Investigation of Tunisian families has contributed to the elucidation of disorder genetic basis due to familial structure, consanguinity and founder effects. As no available resource on genetic diseases in Tunisia exists, we aimed to construct a comprehensive database. **Methods:** Data were collected from manual text mining of bibliographic databases. The database construction was performed using the framework "Symphony". **Results:** Mining the database revealed that 425 genetic disorders affect Tunisian patients of which 61% are autosomal recessive and 50% are due to at least one mutation. Over 480 mutations were identified and part of them was the result of a founder effect and the cause of more than 73 genetic disorders. Two classes of founder mutations were identified. The first includes founder mutations reported so far only among Tunisians. The second includes founder mutations shared with other populations. **Conclusion:** Our database will provide a valuable tool for decision making for researchers and clinicians; facilitate molecular diagnosis of Mendelian disorders and help evaluate the burden of genetic diseases on public health and establishment of preventive programs. This comprehensive resource will be useful not only for biomedical community in Tunisia but also to the other North African and Middle Eastern populations.

Presentation number: MG17 Abstract number: ABS-304-ISABS-2013

SPECTRUM OF GJB2 MUTATIONS IN CROATIAN PATIENTS WITH NONSYNDROMIC HEARING LOSS

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Recessive mutations at the DFNB1 locus (13q11-12), where the GJB2 and GJB6 genes are mapped, are the cause of about 50% congenital, severe, nonsyndromic hearing loss (NSHL). Mutations in GJB2 gene represent a major cause of NSHL worldwide. Among them, c.35delG mutation accounts for approximately 70% of all GJB2 mutant alleles in most European Caucasian population. The aim of our study was to specify the prevalence and the spectrum of GJB2 mutations among 120 unrelated subjects with autosomal recessive NSHL from Croatia. The coding region of the GJB2 gene was sequenced. By multiplex ligation dependent probe amplification (MLPA) analysis we have tested splice site mutation c.IVS1+1G>A in noncoding region of GJB2 gene and copy number changes in the GJB2, GJB3, GJB6, WFS1 and POU3F4 genes. About half of our patients presented with mutation in the GJB2 gene. We identified 14 sequence variations. Most of them had previously been reported as disease related. The c.35delG mutation represented 77% (81/105) of GJB2 deafness alleles. Allelic frequencies of other mutations accounted for 2.1-0.4% of analyzed chromosomes. The most frequent mutation found in GJB2-heterozygous patients was c.IVS1+1G>A, similar as in Czech, Hungarian and Turkish population. The c.35delG/c.35delG genotype was associated with severe to profound hearing loss in ~90% of c.35delG homozygotes. In conclusion, the c.35delG, as the most common mutation, has important role in etiology of DFNB1 deafness in Croatian population. Also, we suggest the importance of routine screening for the c.IVS1+1G>A mutation in patients with NSHL.

Presentation number: MG18 Abstract number: ABS-229-ISABS-2013

BRONCHOPULMONARY CANCER IN A BIHOR COUNTY POPULATION

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Bronchopulmonary cancer is one of the most frequent types of cancer in human population. The risk factors that determine this type of cancer are: smoking, work place (mine or chemicals factories) age and inheritance. This study purpose to make evident some aspects as concerns the bronchopulmonary cancer in a population of Bihor county. We studied 200 individuals who had cancer during the 2000 - 2004 period. It were made smears coloured after May-Grunwald-Giemsa method. We measured the size of cells, nuclei and nucleolus, and calculated the variation coefficients for each parameter. We observed an increased incidence of bronchopulmonary cancer in rural because many of these individuals are or were workers in mine or in toxic departments of diverse factories. In 200 cases, 66.5% are smokers with average of smoking of 20,8. The III degree modifications were observed in the smokers who had an age over 40 years (33,2%). The III cytological modifications were directly in accordance with the smoking (26,3% in smokers and 2,5% in non-smokers). Smoking, exposing at harmful factors and age of the patient are very important causes of the bronchopulmonary cancer appearance. There are very important the prophylactic measures to prevent the bronchopulmonary cancer.

Presentation number: MG19 Abstract number: ABS-230-ISABS-2013

DYNAMICS OF SOME FREQUENT TYPES OF CANCER IN BIHOR COUNTY

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Nowadays more and more cases of different cancer types become diagnosed. We can mention many causes responsible for the appearance of cancer: environmental, chemical, physical and biological factors. Of course, in many cases the inheritance has an important role in appearance of different types of cancer. Our study is about the dynamics of cancer in our county during the 1997-2006 period. We analyzed the data of the Bihor Medical Central Board in mentioned period. We realized a statistical study about the incidence of the different types of cancer in our county. There were registered more than 15000 new cases of cancer during the mentioned period. Bronchopulmonary cancer has the most increased frequency (19,23%) and the next are the breast or mammary cancer (12%) and uterine cervix cancer (9,9%). Bronchopulmonary cancer is more increased in the rural area. The frequency of mammary cancer is more increased in the urban area. The abortion, ovarian cysts and fatness are important etiological factors. The frequency of uterine cervix cancer increases year after year. It is increased in the urban area. As etiological factors can be mentioned the many numbers of abortions in the personal antecedents, fatness, infections. We observed that in general, the incidence of cancer in our county depends on patient sort (masculine or feminine), age and proceeding area.

Presentation number: MG20 Abstract number: ABS-160-ISABS-2013

FAMILIAL COMPLEX CHROMOSOME PATHOLOGY

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Aim: To find families in which two chromosome anomalies originated independently one from another. **Method:** Karyotype analysis in family members of chromosome patients performed during the last 40 years. **Results:** Found chromosome pathologies defined as "chromosome interaction" or "interchromosomal effect" could be divided into four types: i. Double chromosome aneuploidy in the same patient in the cases of coincidence, e.g. Down syndrome and Turner syndrome; ii. Chromosome anomaly, different from parental or aroused additionally to that inherited from parents, e.g. trisomy 21 in baby born by mother possessing rearrangement in another chromosome, or reciprocal translocation in daughter born by mother possessing another reciprocal translocation; iii. Chromosome anomalies (the same or different) found in siblings or more distant relatives, e.g. Turner syndrome in two sisters born in interval of five years, or trisomy 21 and monosomy X in first cousins; iv.The "amplification" of the same chromosome aberration in the cells of affected patient. For example, there are found in Down syndrome patient with ring-21 more as one ring-21 chromosomes or dicentric and tetracentric ring chromosomes 21, or up to four Xq izochromosomes in patients suffering from Turner syndrome. **Conclusions:** When the family possesses an affected chromosome individual, the forecast for the birth of chromosome patient is about three times over than the population risk.

Presentation number: MG21 Abstract number: ABS-156-ISABS-2013

ASSOCIATIONS OF OSTEOPOROSIS AND OSTEOPROTEGERIN GENE: A STUDY OF 135 PATIENTS

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Osteoprotegerin gene (OPG) is an important candidate gene of osteoporosis. The aim of this study was to determine if two polymorphisms in the OPG gene influence bone turnover markers and bone mineral density (BMD). A total of 135 patients, aged 41 to 87 years, were included in the study. Lumbar spine, femoral neck, total-hip and distal radius BMD were measured by dual-energy X-ray absorptiometry (DXA) and bone turnover markers were measured by standard biochemical procedures. OPG gene polymorphisms A163>G and T245>G were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The frequencies of A163>G and T245>G polymorphisms in the OPG gene were determined by screening 131 DNA samples. The prevalence of genotypes of the A163G polymorphism was 59,4% for GG, 33,3% for AG and 7,2% for AA genotype in group with osteoporosis, whereas in control group the prevalence was 77,8%, 16,7% and 5,6%, respectively. The prevalence of genotypes of the T245G polymorphism was 88,4% for genotype TT and 11,6% for genotype TG in group with osteoporosis, whereas in control group the prevalence was 94,4% and 5,6%, respectively. Analysis of BMD in the distal radius of postmenopausal women showed a trend to lower levels in the minor allele homozygote group (GG) versus two other groups. Our results suggest that OPG polymorphism influences BMD in postmenopausal women, however further biological and/or functional evidence would be needed to confirm the suggestive influence of OPG polymorphisms on BMD.

Presentation: MG22 Abstract number: ABS-279-ISABS-2013

ADH GENE VARIABILITY IN POPULATIONS OF SIBERIA

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The most important task of genomic studies is to characterize genome variability both at the individual level and at the population level, which comprise the genetic basis of phenotypic variability in humans. Genetic diversity and linkage disequilibrium (LD) characterology is a powerful tool in the genetics of multifactorial diseases directed to identification of functional variants underlying disease susceptibility. Therefore, in the present study LD structure for the four SNPs of two alcohol dehydrogenase genes (ADH1B (rs1229984 (1), rs2066701 (2)) and ADH1C (rs1693427 (3), rs1789920 (4)) was characterized in Altay, Siberian Tatar, Buryat, Ket, Northern and Southern Kyrgyz, Evenk and Tuvinian ethnic groups. LD blocks were identified using «Solid spine» method implemented in the "Haploview" software. Single LD block of 4 SNPs was found in Altay, Ket, Siberian Tatar and Tuvinian populations. Buryats and Northern Kyrgyz single LD blocks included SNPs 2-3-4, in Evenks - SNPs 1-2-3. The shortest LD block in ADH1C gene (SNPs 3-4) was revealed in Southern Kyrgyz. Genetic differentiation index (Fst) was 6.83% among eight populations, indicating the moderate level of genetic subdivision. Almost all studied populations differ significantly in pairwise population comparisons by Fst. Altay was not differentiated significantly from the Southern Kyrgyz and Tatars, and North and South of Kyrgyzstan were also similar. This data indicate that LD architecture in indigenous Siberian populations is highly ethnic specific. Supported by the Russian Foundation for Basic Research (project no. 12-04-00595).

Presentation number: MG23 Abstract number: ABS-209-ISABS-2013

NIEMANN-PICK TYPE C DISEASE IN CZECH AND SLOVAK FAMILIES: EFFECT OF NPC1 MUTATIONS ON TRANSCRIPT AND PROTEIN LEVEL

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Niemann-Pick type C disease (NPC, OMIM #257220, #607625) is a rare autosomal recessive lipid storage disorder characterized by progressive neurodegeneration. It is associated with mutations in one of two genes: NPC1 and NPC2. Approximately 95% of cases are caused by mutations in the NPC1 gene. By Sanger sequencing we have identified 32 different disease causing mutations in 38 NPC1 families. Among Czech and Slovak patients the most common mutations were p.R1186H, p.S954L and p.P1007A. Six genotypes identified in 10 patients (p.R1186H/S954L; p.R1186H/P1007A; p.R1186H/Y276H; p.P1007A/V950G; p. S954L/L176R; p.V664M/Arg404Glyfs*45) were subsequently studied. The allelic expression ratio of the mutated alleles in cultured skin fibroblasts was determined by PCR/RFLP followed by fragmentation analysis. We have used Western blotting for semi-quantitative specification of NPC1 protein levels. RNA transcript ratios ranged from 22/78 to 30/70 and the amount of immunoreactive NPC1 protein was detectable in all samples. The results show that the analyzed missense mutations allow residual protein synthesis and that the fibroblast cultures may be useful for testing of compounds stabilizing mutant NPC1 protein. Support: IGA MZ CR NT12239-5/2011, MH CZ - DRO VFN-64165, PRVOUK-P24/LF1/3

Presentation number: MG24 Abstract number: ABS-197-ISABS-2013

FRAGMENT ANALYSIS OF PHOX2B GENE CAUSING ONDINE'S CURSE: OVERCOMING EXTREME GC CONTENT

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Ondine's curse (also known as congenital central hypoventilation syndrome or primary alveolar hypoventilation) is a rare disease of central nervous system (in 2006 it afflicted about 200 patients worldwide). Afflicted patients suffer from the failure of autonomous control of breathing demonstrated mostly by respiratory arrests during sleep, potentially fatal if untreated. In the Czech Republic, Olomouc region, there is a family with at least two members affected by symptoms of apnea. Ondine's curse is caused in 92 percent of cases by expanded polyalanine repeat in exon 3 of PHOX2B gene at chromosome 4. An extreme GC content (>85 %) makes genotyping very difficult. We present a case report about eight members with unclear clinical status of the Olomouc Ondine´s curse family tested for polyalanine repeat PHOX2B mutation using PCR with one fluorescence tagged primer followed by capillary electrophoresis. While standard PCR and PCR with DMSO added have produced uninterpretable and irreproducible results, new combination of additives and standard Taq in PCR mixture has yielded distinguishable peaks by fragment analysis in two persons with confirmed PHOX2B mutation. Using our PCR cocktail that enables high GC content genotyping, we confirmed results of the international reference laboratory and diagnosis of the physicians for these two patients. However, we did not find mutation in other patients and the length of the mutated fragment differs from result of the index patient from reference laboratory (+ 3 bp). We will follow this discrepancy and patients in future studies. Supported by the ERDF, CZ.1.07/2.3.00/30.0041 and CZ.1.05/2.1.00/01.0030.

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RNA SECONDARY STRUCTURE - GRAPHIC REPRESENTATION AND NUMERIC CHARACTERIZATION

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Graphic approaches have been used in science to visualize complex relationships but also to represent functional relationships, outcomes of complicated processes and interactions, as well as to simplify scientific notation. More recently the methodology of Graph Theory has been extended towards the study of biomolecules, such as characterizations of DNA and proteins, which are of interest for comparative studies of DNA, RNA, and proteins, including also the problem of DNA and protein sequence alignment. We have developed novel graphical schemes for graphical and nongraphic representations of RNA secondary structure and subsequent their numerical characterizations. This topic, even though it has emerged only very recently, appears promising in offering novel quantitative characterizations of similarities and differences among biopolymers. It is important to emphasize that graphic representation of DNA, RNA and proteins are virtual mathematical objects (usually viewed as 2D geometrical structures and occasionally 3D geometrical structures) that are devoid of any relationship with the factual geometries of DNA, RNA or protein structures. Thus, they merely represent alternative fictitious "images" of complex systems but are expected to reflect faithfully on similarities and differences of the underlying sequences. There is an additional distinction between graphical representations of DNA, RNA and proteins on one hand, and quantum chemical models of molecules on the other hand, in that the Kekulé valence structures and various molecular orbitals relate to the actual molecular geometry, which is not the case with graphical representations of DNA, RNA and proteins.

Presentation number: MG26 Abstract number: ABS-208-ISABS-2013

NOVEL LAMP2 EXON COPY-NUMBER VARIATION AND ITS MOSAICISM DEMONSTRATE THE LIMITS OF DANON DISEASE LABORATORY DIAGNOSTICS

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Danon disease is a rare X-chromosome-linked disorder (DD, OMIM 300257) that manifests by variously expressed cognitive deficit, myopathy, cardiomyopathy and hepatopathy. The clinical phenotype in female carriers/patients is generally less severe than in affected males and likely depends on the tissue-specific X-chromosome inactivation ratios. DD is caused by mutations in the lysosomal-associated membrane protein 2 (LAMP2) gene. The majority of mutations completely abolishes the protein expression and leads to late endosomal/lysosomal misprocessing of autophagosomes. LAMP2 protein absence was identified in two brothers by flow cytometry in peripheral blood leukocytes and was subsequently associated in both with Alu-mediated tandem duplication of exons 4 and 5 (g.15815_22218dup6404). The 6.4 kb duplication was detected by a combination of exon dosage qPCR analyses and duplication breakpoint/junction mapping. The clinically asymptomatic mother of probands was tested for the presence of the same mutation and surprisingly, exon dosage analyses of all her LAMP2 exons demonstrated normal values. This result contrasted with the result of the mutation-specific PCR that identified the mutation in a broad range of her tissues (leukocytes and buccal, urinary tract epithelia). As the mutation tissue mosaic was supposed, we adapted the flow cytometry protocol for detection of very rare LAMP2-deficient cells and determined the fraction of granulocytes lacking LAMP2 as 0.06% of the total cell count. Our results point to potentially under-diagnosed DD carriers/patients. LAMP2 flow cytometry, because of its supreme sensitivity, can be an efficient method for pedigree screening. Support: Research Project 0021620806, PRVOUK-P24/LF1/3, MH-CZ-DRO-VFN64165, 00064203, OPPK CZ.2.16/3.1.00/24022, PRVOUK-P35/LF1/5.

Presentation number: MG27 Abstract number: ABS-206-ISABS-2013

EVALUATION OF TSPY AND TSPX GENE COPY NUMBER IN PATIENTS WITH GONADAL TUMOURS, PROSTATIC CELL LINES AND CONTROL GROUPS

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TSPY gene is localized on Y chromosome having a homologue TSPX on X chromosome. TSPY is specifically expressed in testes. TSPX is normally expressed in ovaries and testis. Over expression of TSPY was discovered in tumour tissues. Product of TSPY accelerates a pass trough G2/M phase via cycline D2 and positively affects the cell proliferation. Over-expression of TSPX or SET leads to cell retaining in G2/M. **Aim:** Quantification of TSPX/X gene copy number and study of potential changes in the TSPY/X copies and their mutual ratios. **Method:** There were assessed 10 women and 8 men patients with gonadal tumours, 5 prostatic cell lines (DU-145, LAPC-4, PC-3, RWPE-I, LNCeP), 80 women and 80 men controls in the study. Relative copy number of TSPY/X genes was quantified by capillary electrophoresis in comparison to one-copy genes AMELY/X. **Results:** We observed more TSPY gene copies in patients with seminomas compare to TSPX gene than in control group. Number of TSPX gene copies in men control group is higher than in patients with seminomas. More variability in TSPX gene copies was indicated in women with ovary carcinoma compare to controls. The women control group has more TSPX gene copies than patients with tumours in average. In prostatic cell lines DU-145, LAPC-4 and LNCeP was significantly increased amount of TSPY copies compare to control group. **Conclusion:** Obtained data could contribute to understanding of TSPY/X gene role in tumor-genesis process in gametogenic tissues. Supported by IGA UPOL LF_2011_004.

Presentation number: MG28 Abstract number: ABS-140-ISABS-2013

CLEAVAGE OF THE DROSOPHILA SCREW PROPROTEIN AND BMP MORPHOGEN GRADIENT FORMATION IN EMBRYO

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Bone morphogenetic proteins (BMPs) are involved in development as key regulators by determining cell fate: proliferation, apoptosis and differentiation. BMPs' function relies on their ability to form concentration gradients and this is regulated at post-translational level. One of the first post-translational regulations is proteolytic processing. After processing, the ligands are secreted and in concert with extracellular matrix and binding proteins, they form a gradient to activate target genes in concentration dependent manner. Defective processing of BMP-type proteins is reported in some human developmental disorders, like cleft lip. In Drosophila embryo, decapentaplegic (Dpp) the ortholog of vertebrate BMP2/4, patterns the dorsal surface of the embryo and the proper shaping of gradient requires dimerization with screw (Scw), the ortholog for vertebrate BMP5/6/7/8. This study focuses on the cleavage of Scw, and its effect on gradient formation and signaling. A genetic screen revealed a scw mutant (scw(E1)) that had strong genetic interaction with a recessive lethal allele of dpp (hr4). This mutant turned out to carry a point mutation in its cleavage site of prodomain. Biochemical studies in cell culture and in vivo studies in embryos show that E1 cleavage is crucial for signaling. Failure in processing leads to imbalance in Dpp/Scw heterodimer formation and impaired gradient formation.

Presentation number: MG29 Abstract number: ABS-176-ISABS-2013

NOS3 GENE VARIANTS ARE ASSOCIATED WITH DEVELOPMENT OF HYPOXIC-ISCHEMIC ENCEPHALOPATHY, INTENSITY OF BRAIN DAMAGE AND SEVERITY OF CLINICAL PRESENTATION

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Aim: The aim of this study was to analyze the correlation of different clinical parameters of hypoxic-ischemic encephalopathy with NOS3 gene polymorphisms. **Methods:** A total of 110 children with hypoxic-ischemic encephalopathy and 128 control children were selected for this study. Sex, gestational age, birth weight, Apgar score, cranial ultrasonography and magnetic resonance imaging findings were correlated with genotypic data of six haplotype-tagging single nucleotide polymorphisms and most commonly investigated rs1800779 and rs2070744 SNPs. **Results:** The TGT haplotype of rs1800783, rs1800779 and rs2070744 polymorphisms showed association with hypoxic-ischemic encephalopathy. Children with the TGT haplotype were infants below 32 weeks of gestation and they have the worst degree of brain damage. Higher incidence of TT genotype of NOS3, rs1808593 SNP in the group of hypoxic-ischemic encephalopathy patients with medium and severe brain damage were found. Probability of brain damage was twice higher in children with the TT than TG genotype of the same polymorphism. Furthermore, the T allele of the same polymorphism was twice frequent in children with lower Apgar score. The incidence of hypoxic-ischemic brain damage was up to two times higher in male than in female children. The occurrence of disease was almost two times higher in children with lower Apgar index and around 1.5 times higher in children with lower birth weight and lower gestational age. **Conclusion:** This study strongly suggests associations of NOS3 gene polymorphism with the intensity of brain damage and severe of clinical picture of affected children.

Presentation number: MG30 Abstract number: ABS-224-ISABS-2013

COMORBIDITY AND DIFFERENTIAL-DIAGNOSTIC DILLEMAS IN FORENSIC-PSYCHIATRIC ASSESSMENT AND TREATMENT

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We present a 52-year old patient in therapeutic programme of the Institute for Forensic Psychiatry within Dr. Ivan Barbot Neuropsychiatric Hospital in Popovača, Croatia. The patient was proclaimed mentally incompetent because of the disorder named in ICD-10 as F22 (Persistent Delusional Paranoid Disorder) and is in a forced setting and treatment. Most diagnostic criteria indicate that we are facing primarily a Narcissistic Personality Disorder listed in the spectrum of disorders in ICD-10 as F60-F69. The clinical description is dominated by somatic-neurological disturbances, so an in depth neurological examination was performed (EEG, MSCT, MRI, PET/CT SCAN) that found a extracerebral meningeoma in the right portion of the middle skull cavum, and atrophy of the brain that did not match the chronological age of the patient. Chronological evaluation of the psychic and somatic-neurological status results in the differential-diagnostic dilemma: Does one find the Persistent Delusional Paranoid Disorder in contrast to the Organic Paranoid Disorder, or is the Acute Psycotic Reaction determined by the first episode of Huntington's Disease (as a consequence of a new mutation on the fourth chromosome) that could both explain the psychotic-paranoid clinical picture before and during commitment of the crime and anticipatory brain atrophy and the rapid decline in cognitive functioning? After careful evaluation, it seemed highly likely to emphasize the narcissistic injury in the context of Narcissitic Personality Disorder, and other comorbid diagnostic assumptions to be comprehended as additional triggers, in the realisation of the crime itself.

Presentation number: MG31 Abstract number: ABS-225-ISABS-2013

NARCISSISTIC PERSONALITY DISORDER IN COMORBIDITY WITH ORGANIC CEREBRAL SYNDROMES IN FORENSIC PSYCHIATRY

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People too much preoccupied with themselves and in need of too much adoration from other people for maintaining their self-esteem have Narcissistic Personality Disorder (NPD).The patient suffers from two basic negative feelings, shame and envy. Shame is ontologically older than guilt which people with NPD do not posses. People with NPD are psychologically very vulnerable as they constantly compare themselves with others in their environment. At the moment when they face a psychological loss or disappointment they pass through a so-called narcissistic injury that is followed by narcissistic rage. That is a very dangerous period both for the patient and his environment because the patient enters an affective tunnel in which he can commit a crime. The affective tunnel is the very link between NPD and Organic Cerebral Syndromes (OCS) because people with the latter also fall into affective narrowed consciousness state at the moment of hyperarousal when they either do not understand the nature and consequences of their acts or they cannot control their will. OCS is a pathological state in which one finds a neurobiological substrate in the brain that causes psychopathological phenomena. It can be a benign or malignant tumor, infection of infestation, a degenerative process or some other organic cause of brain pathological functioning. Its localisation is of crucial importance. NPD and OBS in comorbidity act in a synergistic manner. The aim of this presentation is to show phenomenology, but also treatment opportunities by various forensic-psychiatric procedures.

Presentation number: MG32 Abstract number: ABS-226-ISABS-2013

FORENSIC PSYCHIATRY AND SOMATIC-PSYCHIATRIC COMORBIDITY

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Forensic psychiatry is a psychiatric subspeciality closely retaled to criminalistics and, at first, it is a liaison between psychiatry and law. Forensic psychiatrist is an expert witness in criminal and civil judicial proceedings. It is his task to estimate competency to stand trial or, through forensic expertise, to determine mental compentence tempore criminis. He also deals with treatment and rehabilitation of persons without mental competence and with estimation if they would recommit the crime. To work responsibly and professionally, a forensic psychiatrist must have a deep knowledge of general and special psychopathology. Forensic practice has shown that he must equally know somatic medicine and that he should not succumb to duality of cartesianism because most of somatic diseases coexist with psychiatric disorders. There are numerous examples for somatic-psychiatric comorbidity. One could consecutively follow organ systems or somatic medical specialities. We shall indicate only the most known examples: comorbidity of myocardial infarction and depression in cardiovascular system, comorbidity of multiple sclerosis and depression in nervous system, iatrogenic psychosis in Parkinson's disease, comorbidity od renal insufficiency and cognitive deficit in urogenital system, comorbidity of thyroid diseases and panic attacks, in metabolic specialities there is comorbidity of extreme diet fasting and ketonic euphoria, in infectology infestations and psychoses, etc. On this occasion we shall give the appropriate illustration of comorbidity of scleroderma, as a genetic disease, which resulted with a compressive arachnoid cyst, because of the weakness of collagen, and organic paranoid psychosis, which resulted in homicide.

Personalized Genomics

Presentation number: MG33 Abstract number: ABS-269-ISABS-2013

PERSONALIZED MEDICINE - ARE WE READY IN CROATIA?

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Personalized medicine promises to transform the delivery of healthcare to patients. Physicians will require a solid background in genomics and proteomics to make the best use of new data and hospitals will experience the need to adapt. Also, it seems that personalized medicine will result in lower costs because it is cheaper to give the right medicine in the right doses to the right people. With targeted therapies we will not waste our time with drugs that do not work. At least that is the idea. It is obvious that the personalized medicine revolution is almost here. What about healthcare system in Croatia? Do we already have facilities, educational system and management for implementation of personalized medicine? Is it just a classic technology-based transformation? Do we have to enhance awareness about lifestyle and preventive lifestyle changes? Is targeted therapy our future? Will we live longer and better? It is reasonable expecting that personalized medicine will be dominant conversation on a national stage and from topics of recent symposium on personalized medicine in Croatia it is assumable that we already cover all areas. But is it so in a real life?

Presentation number: MG34 Abstract number: ABS-137-ISABS-2013

GENETIC BASIS OF ACUTE PAIN: GENOME-WIDE ASSOCIATION STUDY OF THE POPULATION OF THE ISLAND OF VIS

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Aim: The aim of this study was to investigate the genetic basis of mechanical pressure pain threshold and to contribute to better understanding of pain sensation. **Methods:** This study included 349 subjects of the isolated population of island Vis in Croatia, within 10,001 Dalmatians project. A total of five mechanical pain threshold measurements were performed using manual algometer. All subjects were previously genotyped with the Illumina HumanHap with 317,000 SNP markers. The data was analyzed in genome-wide association study, controlled for familial relatedness, effects of gender and age. **Results:** Several candidate SNPs were identified in the range of $P<10^{-7}$ to $P<10^{-6}$, not reaching formal significance level. However, a total of 11 SNPs clustered within or in close proximity to GOLGA7 gene located on chromosome 8 (p11.21). This gene is involved in protein transport from Golgi apparatus to cell surface, thus possibly suggesting a novel target for mechanism of pain sensation explanation. A few more candidate SNPs were identified, including rs2995026 (gene CAMTA1, $P=5.34x10^{-6}$), rs17100272 (C12orf56, $P=3.22x10^{-6}$) and rs7152869 (gene SYT16, $P=7.1x10^{-7}$), none of them known to be associated with pain sensitivity before. **Conclusion:** Despite the lack of formal statistical significance, the abundance of identified SNPs suggests a possible novel role of GOLGA7 gene in pain sensation. A more detailed analysis is thus merited by investigating sequenced data or by increasing sample size. Either way, further effort is needed for better understanding of the genomic architecture of acute pain.

Presentation number: MG35 Abstract number: ABS-217-ISABS-2013

HIGH-THROUGHPUT ANALYSIS OF HUMAN MICROBIOME FROM FECAL SAMPLES

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The human body consists of 10 times more microorganism cells than human cells: the human microbiota. The microbiota (mostly bacteria, but also fungi and archaea) reside in organs like the skin, gut, genitals and the oral cavity and fulfill important functions contributing to health and well-being in many different ways, such as fermenting unused energy substrates, preventing growth of harmful species and producing vitamins for the host (such as biotin and vitamin K). Changes in the microbiota (bacterial dysbiosis) of the gut have already been associated with diseases like obesity, inflammatory bowel disease, Type 2 diabetes and colon cancer. Bacteria make up most of the microbiota in the colon and 60% of the dry mass of feces. This fact makes feces an ideal source for any gut microbiome related tests and experiments by extracting bacterial DNA from fecal specimens. Here we present our high-throughput procedure for microbiome analysis using automated genomic library preparation on Beckman Coulter Biomek FX Laboratory Automation Workstation and multiplex sequencing on Illumina Hiseq2000 next generation sequencer.

Presentation number: MG36 Abstract number: ABS-198-ISABS-2013

IDH1 MUTATION IN GLIOMA PATIENTS ASSESSED BY CADMA

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Nuclear NADP-dependent isocitrate dehydrogenase 1 (IDH1) or mitochondrial 2 (IDH2) genes code for enzyme catalyzing oxidative carboxylation of isocitrate to a-ketoglutarate in citric acid cycle, resulting in the reduction of NADP to NADPH. IDH1/2 are found mutated in 70 to 80 % of astrocytomas, oligodendrogliomas or oligoastrocytomas of grades II and III, and secondary glioblastomas. Mutated IDH enzyme generates D-2-hydroxyglutarate and several theories have been proposed to explain its role in tumorigenesis, including induction of genome-wide epigenetic changes. Testing for IDH mutations aids the differentiation, diagnosis, and prognosis of various tumors with histologic ambiguity. Therefore, IDH testing method should have robust analytical parameters to be able to detect mutation in heterogenous cancer tissue. To improve detection threshold of minority mutated population in wildtype surplus, several methods were suggested: i.e. COLD PCR (CO-amplification at Lower Denaturation temperature), blocking of wildtype allele, mutation enrichment by restriction of wildtype allele, or CADMA (Competitive Amplification of Differentially Melting Amplicons). In this project, we tested the usefulness of CADMA that combines allele specific PCR with intended mismatch and competition for template between primers to test for the most common mutation in IDH1 (CGT to CAT, Arg to His) and other mutations so far desribed (Ser, Cys, Gly, Val, and Leu). We present comparison of our CADMA results with mutation enrichment protocol. Partially supported by grant CZ.1.07/2.3.00/30.0004 and CZ.1.05/2.1.00/01.0030.

Molecular Diagnostics: Current Technology and Applications

Presentation number: MG37 Abstract number: ABS-193-ISABS-2013

COMPARISON OF METHYLATION STATUS OF IMMUNE RESPONSE GENES IN CELL-FREE DNA IN HEALTHY, HEMODIALYZED NON-DIABETIC AND HEMODIALYZED DIABETIC SUBJECTS

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The process of hemodialysis is regarded as a stimulus activating pathophysiological mechanisms of inflammation and hypersensitivity reaction. Therefore we decided to study the changes in methylation status of promoters of immune response genes at the level of cell-free DNA, which is thought to fulfill regulation function in intercellular communication. We focused on hemodialyzed diabetic subjects in comparison with general hemodialyzed population and healthy subjects. We isolated plasma cfDNA from 18 patients with diabetic nephropathy (DN) and from 17 non diabetic patients before and after a HD session. We examined also cfDNA from 10 healthy volunteers two times per day with 4 h interval. The extent of promoter methylation of 24 genes involved in immune response was examined using the EpiTect Methyl qPCR Array Inflammatory Response and Autoimmunity and cluster analysis (SABiosciences, Qiagen). During a hemodialysis procedure we found significant alterations in methylation patterns of gene IL 13 in diabetic subjects and in genes IL13RA, ATF2, GATA3 and IL6ST in non-diabetic patients. Promoters of genes GATA3, IL15, IL6R, IL6ST, IL7, INHA, TYK2 were significantly more methylated in healthy subjects then in diabetic patients immediately after HD procedure. The patients with higher methylation status of gene sequences IL13RA, IL 15, EDG3 and INHA in interdialytic interval were significantly overrepresented in the group with none or mild anemia therapy what is in agreement with the fact that IL13 and IL 15 are known inhibitor of erythropoiesis. Supported by grant no. PRVOUK-P25/LF1/2 of the Ministry of Education of the Czech Republic.

Presentation number: MG38 Abstract number: ABS-210-ISABS-2013

MOLECULAR DIAGNOSTICS OF SPINAL MUSCULAR ATROPHY BY DETERMINATION OF COPY NUMBERS OF SMN1, SMN2 AND NAIP GENES

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Aim: SMA is autosomal recessive disease with 1/10000 livebirths. 95% SMA patients are homozygous for exon 7 SMN1 deletion, 3.6% are compound heterozygous (with point mutation). SMA carriers are heterozygous with one exon 7 SMN1 copy. **Methods:** We determined SMN1, SMN2 and NAIP copy number using MLPA/capillary electrophoresis. **Results:** Analysis of 222 DNA samples revealed 27 SMA carriers, 21 SMA patients and 174 persons with 2 SMN1copies. Compared to group of 174 individuals: SMA carriers and SMA patients number with 3 SMN2 copies is increased; SMA carriers and SMA patients number with 1 SMN2 copy is decreased; SMA patients number with 2 SMN2 copies is decreased; we detected 4.76% individuals with 4 SMN2 copies; SMA carriers and SMA patients number with 1 NAIP copy is increased; SMA carriers and SMA patients number with 2 NAIP copies is decreased; SMA carriers number with 3 NAIP copies is decreased; we detected absence of SMA carriers with 4 NAIP copies as well as SMA patients with 3 and 4 NAIP copies; we detected 0 NAIP copies in 33.33% SMA patients and in 3.7% SMA carriers. **Conclusion:** Majority of SMA patients are homozygous for deletion of SMN1 and a normal or reduced number of SMN2 copies. Majority of type II and III SMA patients show homozygous absence of SMN1 as a result of gene conversion of SMN1 into SMN2, leading to absence of functional SMN1 and an increase to 3-4 copies of SMN2. NAIP deletions may indicate a severe form of SMA.

Presentation number: MG39 Abstract number: ABS-241-ISABS-2013

ASSESSMENT OF CIRCULATING TUMOR MARKERS IN BREAST CANCER PATIENTS BEFORE AND AFTER CHEMOTHERAPY

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Numerous studies suggest isolation and characterisation of circulating tumor cells as alternative and future approach for early diagnosis of cancers and post-treatment follow-up (monitoring of residual disease). The validation of this strategy that would be applicable to majority of clinical cases would bring a revolution in treatment of cancers. In this work we present preliminary results for evaluated method for detection and expression analysis of experimentally confirmed tumor molecular markers from blood of patients consecutively analysed in an ongoing translational study. Expression level of five markers GA733-2, mammaglobin, MUC1, CK-19 and ERBB2 were assessed using real-time PCR before and after onclogical treatment. All values were normalized against housekeeping gene (GAPDH) expression. Individual and group variation in relative gene expression calculated using Pfapfl method (2001) and comparison against the type of treatment will be presented.

Presentation number: MG40 Abstract number: ABS-211-ISABS-2013

BTK, ELANE AND SBDS GENE SEQUENCING IN CROATIAN PATIENTS WITH PRIMARY IMMUNODEFICIENCIES

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Aim: Primary immunodeficiency diseases (PID) are a heterogenic group of rare inherited conditions, which are result of error in development and/or functioning of the immune system. They are caused by hereditary or genetic defects and can affect anyone, regardless of age or gender. The aim was to confirm clinical diagnosis at molecular genetic level in patients with PID and to define carrier status by analyzing DNA samples of patients' close family members. **Methods:** 22 samples of genomic DNA were analyzed: 9 samples of patients with suspicion on one of the PID and 13 samples of their close family members. For identification of mutations in the coding region of analyzed genes, the sequencing method on Applied Biosystems 3130xl Genetic analyzer and BigDye® Terminator v3.1 Cycle Sequencing Kit were used. **Results:** In 2 patients with X-linked agammaglobulinemia, mutations in the BTK gene were found. In the first patient, mutation was inherited from the mother, and in the other, mutation occurred de novo in mother's egg cells. In 4 patients with suspicion on cyclic or severe congenital neutropenia, 4 mutations occurred de novo in mother's egg cells were found in ELANE gene. In 3 patients with suspicion on Shwachman-Diamond syndrome mutation in SBDS gene were found. **Conclusion:** Molecular genetic analysis is the final diagnostic step in confirmation of the PID diagnosis considering the enormous number of mutations in various genes discovered so far that codes proteins responsible for maturation, functionality and regulation of the immune system.

Presentation number: MG41 Abstract number: ABS-212-ISABS-2013

GENETIC BACKGROUND OF WILSON DISEASE IN CROATIAN POPULATION

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Aim: Wilson disease (WD) is an autosomal recessive disorder of copper metabolism, with an incidence estimated to be one in 30,000 in general population. It is usually characterized by liver, neurological, psychiatric symptoms, but can also present with atypical symptoms. It is characterized by significant genetic heterogeneity, which makes genetic analysis time-consuming and expensive. Because in children copper laboratory test results are often normal, genetic analysis is an important step in establishing diagnosis of disease. **Methods:** Genomic DNA of 76 WD index patients was extracted from peripheral blood by salting-out method. Twenty-one exons of ATP7B gene were analyzed by sequencing method on 3130XL Genetic Analyzer, using BigDye Terminator Cycle Sequencing Kit v3.1 and polymer POP-7 (Applied Biosystems). **Results:** Sequencing analysis of ATP7B coding region confirmed clinical diagnosis in 59 patients by detecting WD mutations in both ATP7B alleles. In 8 patients ATP7B mutations were detected only in one allele, while in 9 patients no mutations were detected. Mutation p.His1069Gln in exon 14 was the most frequent in Croatian WD patients, with allele frequency of 44.1%. 18 different ATP7B mutations in total were detected, of which mutations p.His1069Gln, p.Ala1003Thr, c.2304dupC, p.Arg616Gln and c.3402delC represent 81.7% of mutated ATP7B alleles in this population. Mutation detection rate in this study was 82.9%. **Conclusion:** Analysis of ATP7B gene by sequencing method is recommended in genetic diagnostics of WD because of it's genetic heterogeneity. Diagnosis of WD on molecular genetic level is very important in cases with equivocal copper studies and/or atypical clinical presentation.

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FAMILIAL CASE OF MODY 7 DIABETES

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Maturity-onset diabetes of the young (MODY) is caused by defects in the primary insulin secretion. It is autosomal dominant form of diabetes that is usually manifested before the 25-year of life. Except for early onset of disease, other symptoms are similar to type 2 diabetes, with normal c-peptide levels and insulin resistance. Currently, there are eleven types of MODY, from which the most common types are MODY 2 and MODY 3 (with mutations on GCK and HNF1A genes, respectively). As part of larger study, family with three affected members came to Institute for genetic engineering and biotechnology for MODY diabetes testing. At this time we tested mother, daughter and son, all with same symptoms and diagnosis, but different onset time. Family confirmed also that father has diabetes type 2, but he was unavailable at this time for testing. Material for this testing was 3 ml of whole blood. Isolation of DNA was performed by salting-out method. Identification of mutations was made with MLPA (multiplex ligase-dependent probe amplification) technique, with MLPA kits P241 and P357 (Mrc - Holland). Statistical analyses were performed with Coffalyser software (Mrc-Holland). All samples were typed successfully. After statistical analysis, we found deletions of KLF11 gene in all three samples (mother, daughter and son), which corresponds to MODY 7 type of diabetes.

Presentation number: MG43 Abstract number: ABS-222-ISABS-2013

DEVELOPMENT OF A SERS METHOD FOR ULTRA LOW DETECTION OF DIAGNOSTICALLY IMPORTANT BIOLOGICAL ANALYTES

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Here we report an ultrasensitive method for detecting bioactive compounds in biological samples by means of functionalised nanoparticles interrogated by surface enhanced Raman spectroscopy (SERS). This method is applicable to the recovery and detection of many diagnostically important peptidyl analytes such as insulin, human growth hormone, growth factors (IGFs) and erythropoietin (EPO), as well as many small molecule analytes and metabolites. Our method, developed to detect EPO, demonstrates its utility in a complex yet well defined biological system. Recombinant human EPO (rhEPO) and EPO analogues have successfully been used to treat anaemia in end-stage renal failure, chronic disorders and infections, cancer and AIDS. Current methods for EPO testing are lengthy, laborious and relatively insensitive to low concentrations. In our rapid screening methodology, gold nanoparticles were functionalised with anti-EPO antibodies to provide very high selectivity towards the EPO protein in urine. These "smart sensor" nanoparticles interact with and trap EPO. Subsequent SERS screening allows for the detection and quantisation of ultra trace amounts ($<<10^{-15}$ M) of EPO in urine samples with minimal sample preparation. We present data showing that the SERS spectrum differentiates between human endogenous EPO and rhEPO in unpurified urine, and potentially distinguishes purified EPO isoforms. Elimination of sample preparation and direct screening in biological fluids significantly reduces the time required by current methods. Antibody recognition against a variety of biological targets and the availability of portable commercial SERS analysers for rapid onsite testing suggest broad diagnostic applicability in a flexible analytical platform.

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STRATEGIES FOR FORENSIC DETECTION OF GENE DOPING: ARE THEY POSSIBLE?

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As recognised by the anti-doping community, gene doping, like doping in any form, undermines the principles of fair play in sport and most importantly, involves major health risks to athletes who partake in gene doping. Gene therapy has progressed in leaps and bounds over the years, but the forensic field has proved anything but predictable. As laboratory detection become more sophisticated in doping evaluation genes for erythropoetin (EPO), human growth hormone (hGH), insulin-like growth factor-1 (IGF-1), peroxisome proliferator-activated receptor-delta (PPAR delta), and myostatin inhibitor have been identified as primary targets for doping. The emerging biotechnology industry changed these limitations with the implementation of recombination DNA production of peptide drugs and hormones. Manipulating genes inserted into mammalian cells, the new industry produced recombinant (r) drugs, such as rHGH, rEPO, insulin-like growth factor (r-IGF)-1, and r-insulin in great quantities. With the advent of gene therapy, a more direct way to deliver proteins and hormones to an athlete's tissues and organs became reality. Interesting developments could use patterns of gene activity or gene products to detect abnormal gene activity. The monitoring or visualization of gene activity or gene products through the expression of DNA and RNA by a sophisticated microchip array could monitor thousands of genes, enabling the laboratory to use a sophisticated detective tool for gene doping. Forensic approach would potentially be suitable to detect gene doping through gene transfer by analysis of small volumes of blood using regular out-of-competition testing.

Presentation number: MG45 Abstract number: ABS-157-ISABS-2013

GENETIC VARIATIONS OF D15S541 AND D15S11 LOCI AMONG CROATS AND THEIR APPLICATION IN DETECTION OF UNIPARENTAL DISOMY 15

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Prader Willi Syndrome (PWS) and Angelman Syndrome (AS) are clinically distinct genetic disorders, both mapping to chromosome region 15q11-q13. PWS and AS can be caused by de novo derived deletion of this region, uniparental disomy (UPD) of chromosome 15 or the silencing of alleles. Polymorphism of two STR loci (D15S11 and D15S541) was studied in a sample of 178 healthy unrelated Croatian individuals. The group of 28 patients with the clinical presentation of PWS or AS was also tested for these two loci as well as for D15S642 and D15S659 loci for detection of UPD15. Alleles at tested loci were determined by PCR-STR method. Among healthy individuals 13 and 17 different alleles were identified at D15S11 and D15S541 locus respectively. The most frequent alleles at D15S11 were D15S11-2 allele (50.0%) followed by D15S11-5 allele (12.6%) and D15S11-6 allele (10.4%). Among D15S541 alleles the most frequent was D15S541-5 (36.5%) followed by D15S541-9 (34.8%). Observed heterozygosity for tested STRs were 0.624 for D15S11 and 0.719 for D15S541, while PIC value was as follows: 0.712 (D15S11) and 0.727 (D15S541). No significant deviations from Hardy-Weinberg equilibrium could be observed for these systems. In patients' group the parental origin of chromosome 15 was determined in 26 out of 28 patients, while for two patients only one STR locus was informative and therefore not sufficient for diagnosis of UPD. In conclusion, the combination of four STR loci at chromosome 15 is in most cases sufficient for detection of UPD15.

Presentation number: MG46 Abstract number: ABS-302-ISABS-2013

DETECTION OF SUBTELOMERIC COPY NUMBER ABERRATIONS IN PATIENTS WITH DEVELOPMENTAL DELAY/ INTELLECTUAL DISABILITY BY MLPA

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Developmental delay/intellectual disability (DD/ID) is a clinically and genetically heterogeneous condition. Chromosomal rearrangements involving telomeres have been identified to account for approximately 5-10% causes of DD/ID. Multiplex Ligation-dependent Probe Amplification (MLPA) is a relatively quick method for subtelomeric chromosomal screening. In this study we present the results of MLPA performed in 250 patients with DD/ID with or without dysmorphic features or congenital anomalies using SALSA P036 and SALSA P070 kits, which are specifically designed to detect subtelomeric imbalances. The aim of the study was to determine the ability to detect and confirm subtelomere abnormalities in patients with DD/ID using a combination of MLPA subtelomeric probe mix. The MLPA screening revealed subtelomeric rearrangements in 18 (7.2%) cases: (6 deletions: del4p, del8p, del22q, delXp and two del15q; 5 duplications: two dup9p and three dupX/Yp; 7 deletions/duplications: dup3p/del18q, dup4p/del8p, del8p/dup12p, dup8p/del18q, del12p/dup22q, dupXp/del11q and one del/dup19p). The use of two subtelomeric kits per patient and investigation of all aberrations by follow-up kits has reduced the rate of false positive and negative results and improved diagnostic yield. MLPA specific telomere probe mix has proven to be suitable for confirmation and better characterization of selected aberrations. **Conclusion:** MLPA is a fast, sensitive and cost-effective technique for screening DD/ID patients. The use of combination of appropriate kits improves diagnostic accuracy and is now used in our routine work.

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SELF-SAMPLING OF VAGINAL SWABS: DIAGNOSTIC POTENTIAL AND POSSIBLE APPLICATIONS

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Self-sampling, as a simple, convenient and non-invasive process, is being more and more investigated as an alternative option in collection of biological specimens. Application of self-sampling could be particularly attractive and significant for molecular-genetic human papillomavirus (HPV) detection in self-sampled vaginal swabs, due to the fact that many studies have shown women's preference for self-sampling over gynecological collection of cervical samples. They reported that this procedure was more comfortable, less invasive and less embarrassing. Aim of this small-scale study was to assess vaginal self-sampling as an alternative option to standard gynecological sampling. This was done by comparison of HPV status results obtained from cervical samples and self-sampled vaginal swabs. Thirty-nine examinees provided their vaginal and cervical samples for this purpose. HPV status was analyzed by PCR, using universal HPV primer able to detect a wide spectrum of genital HPV types. The concordance of results was statistically analyzed using Inter-rater agreement kappa test. It was possible to detect HPV in both types of samples. Results of HPV detection showed mismatch in 10 % of the cases. However, the agreement between the results was good (kappa = 0,748), which opens the possibility to apply and incorporate self-sampling in screening strategies of developing countries. Self-sampling could be of a great significance as an alternative sampling approach particularly in regions with certain barriers, such as specific religious and cultural background, lack of adequate and effective health-care system and infrastructure, various socioeconomic factors, sometimes invasive nature of sample collection, national screening strategies STD's and similar.

Presentation number: MG48 Abstract number: ABS-141-ISABS-2013

TRANSFUSION ASSOCIATED GRAFT VS HOST DISEASE ANALYSIS WITH STR MARKERS

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Transfusion associated graft versus host disease (TA-GVHD) is a rare condition. This may occur after blood transfusion in immune-competent or immune-compromised patients and is associated with a mortality rate of 90% or more. The diagnosis of TA-GVHD is often delayed because of its non-specific clinical features. DNA profiling is a technique that provides wide applications in allied basic and clinical sciences apart from its applications in forensic medicine. We are reporting a case of immune-compromised patient who developed TA-GVHD. Short tandem repeat (STR) analysis was performed to detect the presence of donor cells in patient. The results supported the diagnostic potential of using multiple biological specimens for DNA profiling. This is the first case report where donor's DNA fingerprinting pattern in a hair follicle sample (5.74%) of patient was substantiated. Chimerism was also present in blood (45.16%) and buccal swab (10.68%).

Presentation number: MG49 Abstract number: ABS-237-ISABS-2013

OPTIMIZATION OF RNA EXTRACTION FROM URINE SAMPLES

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Extraction of nucleic acids from urine samples may be of great help in forensic cases, as well as molecular genetic analyzes that are used in the diagnostic of disease. In this paper, we focused on total RNA extraction using Tris® Reagent (Sigma-Aldrich Chemie GmbH, Steinhaim, Germany) reagent in order to obtain cDNA by reverse transcriptase, and using of cDNA at the subsequent quantitative analysis, referring primarily to the analysis of gene expression measured by real-time PCR. Thus extracted and processed RNA can be used in the detection of increasing levels of certain tumor markers (PSA - prostate specific antigen) in prostate cancer cases. Followed by specific urological procedures, urine samples contain tumor specific molecules that can be of significance for diagnostic development i.e. for prostate cancers. This would certainly be a relief to patients because urine sampling is less invasive than the initial blood sampling taken for analysis of PSA in serum and final obtaining biopsy samples of prostate tissue for confirmation of the diagnosis which is standard procedure in such cases.

Presentation number: MG50 Abstract number: ABS-300-ISABS-2013

VASCULAR ENDOTHELIAL GROWTH FACTOR IN NEUROBLASTOMA - A NEW PROGNOSTIC FACTOR AND INDICATOR FOR NOVEL TREATMENTS?

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Tumour angiogenesis is an important characteristic of tumour tissues as it considerably differs from healthy tissues. Tumour vascularity influences metabolism, cell division, differentiation and apoptosis, all of which might represent targets for use of novel treatments. In our study we tried to establish whether the expression of vascular endothelial growth factor (VEGF) in relation to tumour vascularity could be found relevant as a prognostic factor for patients suffering from neuroblastoma. We analysed 56 neuroblastoma samples of patients aged from 2 months to 12 years, treated in a ten-year period with the follow-up duration of 60 months, with all relevant clinical criteria included. Samples were analysed semi-quantitatively using anti-VEGF and anti-CD34 antibodies. Tumour vascularity was estimated by calculating the tumour vascular volume fraction (TVVF). We found that patients with high VEGF expression had worse survival, whilst all patients with low VEGF expression survived. Poor tumour vascularity was accompanied by shorter survival. We observed significantly reduced rates of survival (37%, p<0,0001) in patients with high VEGF expression and poor vascularity. Our results indicate a strong influence of tumour angiogenesis on prognosis of neuroblastoma patients. This could contribute to the assessment of high-risk neuroblastoma patients and perhaps additionally stratify them in comparison with the current negative genetic prognostic factors such as n-myc amplification. Furthermore, establishing a correlation of angiogenesis and survival in neuroblastoma patients might indicate that adjacent use of angiogenic or metabolic inhibitors along with the standard therapy, in this group of high-risk patients, could be beneficial.

Presentation number: MG51 Abstract number: ABS-289-ISABS-2013

EPICARDIAL ADIPOSE TISSUE THICKNESS AS PREDICTOR OF ADIPONECTIN SYSTEMIC BLOOD CONCENTRATION

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Aim: Epicardial adipose tissue (EAT) thickness is a risk marker of coronary artery disease (CAD), recently defined as an inflammatory disease. Adiponectin (ADIPOQ) is responsible for the modulation of atherosclerosis through its antiatherogenic effects while interleukin 6 (IL6) and tumor necrosis factor alpha (TNFa) are known proatherogenic cytokines. The aim of this study is to predict possible concentrations of ADIPOQ, IL6 and TNFa in the systemic circulation with EAT thickness. **Methods:** In this study 36 subjects were included, 16 CAD and 20 controls all of whom underwent cardiosurgery. Prior to cardiosurgery, EAT thickness was assessed by echocardiography. During cardiac surgery, blood samples were taken from the aortic root (AR). ADIPOQ, IL6 and TNFa concentrations were measured in the AR with ELISA kits. **Results:** EAT thickness positively correlated with IL6AR (r=0.396, p=0.02) and negatively correlated with ADIPOQAR (r=-0.628, p<0.001) concentration. EAT thickness did not show significant correlation with IL6 and TNFa concentrations in AR. MARS regression analysis showed that ADIPOQAR (R2=0.53) concentration can be predicted with an EAT thickness. EAT thickness was unable to predict IL6 and TNFa concentrations. **Conclusion:** EAT thickness negatively correlated with ADIPOQAR but did not correlate with IL6 and TNFa concentrations. Regression model of EAT thickness can be used, fairly accurately as predictor for adiponectin systemic concentration.

Molecular Therapy

Presentation number: MG52 Abstract number: ABS-294-ISABS-2013

THE EFFECT OF PENTADECAPEPTIDE BPC 157 ON ATROPINE INDUCED MYDRIASIS IN RATS

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Introduction: The aim of this study was to investigate the effect of BPC 157 on atropine induced mydriasis in Whistar albino rats. **Methods:** 30 Female Whistar Albino rats weighing 200-250g were randomly assigned into 6 groups, five per each group. Animals were randomized into pentadecapeptide BPC 157 and control group. Pentadecapeptide BPC 157 group animals were treated by pentadecapeptide BPC 157 intraperitoneally (5.0 ml/kg - µg, ng, pg, fg, ag) and atropine 0.5% administered locally. Control group received saline intraperitoneally (5.0ml/kg) and atropine 0.5% administered locally. Assesment includes change in pupil diameter monitored by "Veho discovery VMS-004 deluxe" camera and radius diameter area software in 4h interval. Calibration by millimeter paper was also performed before every measurement. **Results:** There is a difference in time of disappearance of atropine effect between the control group rats and rats treated with BPC 157. **Conclusion:** Considering the results obtained we suggest that Pentadecapeptide BPC 157 is effective and shortens the time needed for a pupil to return to normal state after initial treatment with atropine. We presume that effect is dependent on the action of the NO system.

Presentation number: MG53 Abstract number: ABS-265-ISABS-2013

ANTICANCER ACTIVITY OF TWO NOVEL PALLADIUM (II) COMPLEXES IN HUMAN LEUKEMIA CELL LINES

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Effective treatments for human leukemias are still being developed, but so far none of them was found to be completely satisfying. It was recently reported that palladium complexes have a significant anti-cancer activity as well as lower toxicity and less side effects compared to some clinically used chemotherapeutics. Anticancer activities of two novel palladium (II) complexes [Pd(sac)(terpy)](sac)·4H2O and [PdCl(terpy)](sac)·2H2O were tested against three human leukemia cell lines: Jurkat, MOLT-4 and THP-1 and in comparison to cisplatin and adriamycin. Cytotoxic effect of two palladium (II) complexes, cisplatin and adriamycin was determined with the MTT viability assay, and IC50 values were calculated. Cell apoptosis was assesed with FITC-Annexin/PI staining for flow cytometry and fluorescent microscopy, and caspase-3 colorimetric assay. Specific apoptotic pathway was elucidated by caspase-8 and -9 colorimetric assays. Furthermore, PARP cleavage and p53 activity were determined by western blot to elucidate whether treatments induce apoptotic or necrotic response. Both complexes exhibited a significant dose-dependent anti-growth effect in vitro, more powerful than cisplatin and less effective than adriamycin, but with less side effects. The complexes predominately induced apoptosis, but necrosis was also observed. In vitro results have shown that palladium (II) complexes may be a potential anti-cancer agents for treating human leukemias, but further analysis to determine putative mechanism of action and in vivo studies on animal models are essential.

Presentation number: MG54 Abstract number: ABS-178-ISABS-2013

ANTIBACTERIAL SCREENING OF EXCRETIONS/SECRETIONS OF THE MAGGOT LUCILIA SERICATA

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Maggots of the green blowfly Lucilia sericata typically live on microbial infested necrotic wounds of animals. For many centuries, they have been used as traditional means of wound healing in many cultures such as American, Maya Indian, Australian Aborigine and Chinese. The medicinal use of maggots in wound management is called Maggot therapy, therapeutic myasis or maggot debridement therapy and dates back to the early centuries. The selectivity of the maggots against pathogenic microorganisms found on infected wounds has ignited interest in the study of antimicrobial effects of maggots. In this study, three different assays were used to study the in vitro antibacterial activity of secretions of the larvae of Lucilia sericata on a selected group of gram positive and gram negative bacteria typically found on infected wounds. Secretions used in this study were obtained from larvae incubated in a sterile environment and also on rotten infected porcine tissue to model an infected wound environment. The results obtained from the zone of inhibition assay and colony forming unit assay indicates that the maggot excretion has antibacterial activity against MRSA, E. coli, S. aureus and B. cereus, but no activity against P. aeruginosa. The turbidometric assay showed negative results for antibacterial activity. Extensive research into the efficacy of maggot therapy will eventually boost the confidence of patients in the use of maggot therapy.

Presentation number: MG55 Abstract number: ABS-318-ISABS-2013

DAMAGE TO ENDOMETRIUM BY CYSTEAMINE AND PROTECTION BY PENTADEKAPEPTIDE BPC 157

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Cysteamine is ulcerogenic to the doudenum and colon, but its effect on the endometrium is unknown. Pentadekapeptide BPC 157 inhibits this effect on the doudenum and colon; hence, we postulated that it might protect the endometrium too. We examined the effect of BPC 157 on cysteamine lesions of the endometrium in Wistar Albino rats. Cysteamine was applied into the horns of the uterus at the dose of 400 mg/kg. Ulcerogenic effects were confirmed by macroscopic and microscopic evaluation after 2h, 3 days and 7 days. Treatment included BPC 157 at the dose 10 ng/kg or 10 µg/kg intraperitoneally or orally while controls received saline (5ml/kg) by the same routes. BPC 157 was applied immediately after cysteamine administration. Preliminary results show that cysteamine caused damage to the endometrium, and that BPC 157 administrated intraperitoneally and orally inhibited the formation of the lesions.

Translational Medicine

Presentation number: MG56 Abstract number: ABS-207-ISABS-2013

TREFOIL FACTORS IN THE EMBRYONIC RESPIRATORY SYSTEM OF MICE

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Aim: To determine the presence of Trefoil factor family (TFF) protein 1, 2 and 3 in the developing respiratory system of mouse embryo. **Methods:** CD1 mouse embryos, at Theiler stage 23, 24 and 25 (15 to 17 days old) were fixed in 4% paraformaldehyde and embedded in paraffin blocks. 6 µm thick sagittal sections were prepared for immunohistochemical staining. Polyclonal, affinity-purified, primary rabbit antibodies were applied, followed by biotinylated anti-rabbit secondary antibody and streptavidin-HRP layers. Complex was visualized using DAB as chromogen. **Results:** TFF1 staining was visible in the epithelial cells of nasal, tracheal and bronchial mucosa. TFF2 was present in nasal mucosa epithelium and in bronchial epithelium, where apical part of cell cytoplasm was stained including the thin mucus layer above bronchial epithelium. Epithelium of nasal cavity and bronchi showed pronounced presence of TFF3. No presence of trefoil peptides was found in the alveolar epithelium or interstitial tissue. **Conclusion:** Distribution of TFF peptides in the embryonic respiratory tract is similar to adult respiratory tract. TFFs have important role in cell migration, differentiation and tissue remodeling in adult tissues. Therefore, TFFs presence in respiratory epithelium during embryonic development points to their involvement and importance during development and maturation of respiratory tract.

Presentation number: MG57 Abstract number: ABS-281-ISABS-2013

AUTOPSY FINDINGS IN ELEVEN PATIENTS WITH FUNGAL MENINGITIS DUE TO CONTAMINATED STEROID INJECTION

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General: As a function of the medical examiner system we investigated the outbreak of fungal infection of the central nervous systems as a result of injections with contaminated steroid injections beginning in September of 2012. The state of Michigan was the epicenter of the epidemic. As of March 2013 a contaminated steroid injections were responsible for 700 infections resulting in 51 deaths nationwide. **Methods:** A total of eleven (11) autopsies on victims of contaminated steroid injections were performed at the University of Michigan. Complete autopsies including fungal cultures and PCR testing were performed. **Results:** The average age of patients was 72.5 years. Laboratory documentation of Exserohilium rostratum was documented in all cases. One case was diagnosed with PCR the remaining with fungal cultures. Three patients developed fatal cerebral hemorrhage or stoke. Seven patients developed fungal meningitis. The median period of incubation from injection to death was 34.7 days (range 5-63 days). The fungus manifested angiophilic invasion as demonstrated by the high number of cerebral infarction with forty percent of patients developed stroke. **Conclusion**: Preliminary autopsy studies from an outbreak of fungal infections from contaminated steroid injections showed substantial mortality. Public health surveillance including medical examiner assisted autopsy studies resulted in prompt recall of the product, notification of exposed persons and medicolegal documentation of cases.

Best Practices in Translational and Personalized Medicine

Presentation number: MG58 Abstract number: ABS-263-ISABS-2013

TLR-4 GENE POLYMORPHISM IN RENAL TRANSPLANT PATIENTS

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The study included 70 patients with a transplanted kidney and 50 healthy volunteers representing a control group. Median age of the transplanted patients was 53 years (48-58 years). Transplanted patients had been receiving an adjuvant kidney therapy for an average of 4 years (2-6 years). Median time lapse from receiving kidney transplant was 3 years (ranging from 2-5years). The most common chronic kidney disease diagnosis was chronic glomerulonephritis (21 patient; 32,8%), followed by a polycystic kidney disease in 7 patients (10,9%). Detection of gene polymorphism for tlr4 rs4986790 (A896G, D299G) i tlr4 rs4986791 (C1196T, T399I) was conducted using multiplex real-time PCR. Polymorphism detection was performed using LightCycler. Detection of the TLR4 rs4986790 polymorphism revealed a great predominance in the AA-wild type (104 patients, 86.7%), 44 (88%) out of which were from the control group and 60 (85.7%) from the transplanted group. When analysing rs4986791 polymorphism, the largest proportion of patients (99; 82.5%) displays a CC-wild type, followed by a CT-heterozygous group (19; 15,8%), while only 2 patients from the tranplanted group (2.9%) showed a TT-mutation. A significant decrease in erythrocytes (Kruskal Wallis test, p=0.037), creatinine clearence (p=0.019) and hemoglobin levels (p=0.009) was observed in the AA-wild type rs4984790 group, while the AG-heterozygous group showed the highest levels of blood glucose. CC-wild type group type rs4986791 shows significantly lower erythrocyte values (p=0.027), creatinine clearance (p=0.030) and hemoglobin levels (p=0.049).

Presentation number: MG59 Abstract number: ABS-252-ISABS-2013

AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS (PAP) IN A PEDIATRIC PATIENT-CASE REPORT

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Pulmonary alveolar proteinosis, (PAP) is a rare disease of unknown etiology, characterized by accumulation of lipoproteinaceous material within alveoli. The prognosis is highly variable, and for over three decades the pathophysiology and treatment of this disease remained a mystery. With recent developments in molecular genetics, our understanding of the pathogenesis of PAP has improved significantly. Four forms of PAP are recognised in children: congenital, acquired, secondary and idiopathic. In adults, and occasionally in older children and adolescents, PAP is usually an autoimmune disease; antibodies to granulocyte macrophage colony-stimulating factor (anti-GM-CSF) are present in 90 percent of cases. Autoimmune PAP with anti-GM-CSF antibodies has been reported in only a few children, who presented in late childhood or adolescence. Thus, unlike adults with PAP, it appears that the majority of infants and children with PAP do not have the autoimmune form of the disease. As an example, in a study of 15 children with PAP, none had anti-GM-CSF antibodies in the serum, and only one had anti-GM-CSF antibodies in bronchoalveolar lavage fluid. We present a rare case of autoimmune PAP in a 10-year old male patient, diagnosed by HRCT, lung biopsy and lab analyses (positive anti-GM-CSF antibodies in serum). Patient was treated with corticosteroids, hidrochloroquine, GM-CSF and supportive therapy. Lung transplantation was performed one and half year after diagnosis.

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MEASUREMENTS OF NITRIC OXIDE IN CHILDREN WITH PRIMARY AND SECONDARY CILIARY DYSKINESIA

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Introduction: Primary ciliary dyskinesia (PCD) is an inherited disease related to ciliary disfunction, with heterogenity in clinical presentation and in ciliary ultrastuctural defect. Up to present day, three types of cilia are known: primary, nodal and motile. Cilia are complex, likely involving more than 1000 gene products. Early diagnosis is important to prevent disease progression. Nasal nitric oxide (nNO) measurement is established first line test in the work-up for PCD. Objective: To examine the usefulness of nasal (nNO) and bronchial exhaled nitric oxide (FeNO) measurements in detecting PCD in children. **Method:** Measurements were done in 5 PCD children and 9 children with secondary ciliary dyskinesia (SCD), aged between 5 and 16 years. They were diagnosed with PCD or SCD syndrome based on clinical presentation and electron microscopy findings of bronchial biopsy sample. **Results:** No significant differences were found on the basis of age or ventilatory function test between the PCD patients and SCD groups. Values of FeNO were significantly lower in PCD group (5-14,9 ppb), compared to group with SCD (7-46 ppb). Values of nNO in PCD group varied from 10 to 2819 ppb, and for SCD group varied from 19,4 to 2136 ppb. Diagnosis of recurrent otitis was established in 4 patients (28,6%), recurrent sinusitis in 9 (64,3%), chronic productive cough in 9 (64,3%), and bronchitis in 8 (57%). **Conclusion:** The measurement of FeNO appears to be a useful tool for screening children with PCD and SCD, so it should be performed before nasal biopsy and electron microscopy studies.

Presentation number: MG61 Abstract number: ABS-151-ISABS-2013

OPTIMIZATION OF IN VITRO TRANSDUCTION OF HUMAN SKELETAL MUSCLE WITH RECOMBINANT ADENOVIRAL VECTOR CARRYING THE LUCIFERASE REPORTER GENE

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Aim: Bone tunnel widening is frequent complication after anterior cruciate ligament (ACL) reconstruction. Induction of bone formation using autologous skeletal muscle genetically modified to overexpress BMP-2 represents a promising strategy to solve this problem. The conventional, two step approach, ex vivo gene therapy is an expensive and time consuming procedure. Successful clinical applications needs ex vivo gene therapy protocol in which all procedure would be performed in operating theatre during surgical procedure. The purpose of this study is to optimize ex vivo transduction of autologous skeletal muscle with respect to the duration of contact and the concentration of recombinant adenoviral vector. **Methods:** This experimental study consists of two phases. Human muscles from 20 volunteer donors harvested during ACL reconstruction were used. The aim of the first phase was to optimize in vitro transduction of the human muscle by changing the concentration of the adenoviral vector carrying the luciferase reporter gene (AdLuc). During the second phase we explored various time points necessary for successful transduction using optimized concentration of AdLuc. **Results:** It is possible to transduce human muscle in vitro by AdLuc within 30 minutes. **Conclusions:** Preliminary results of this study suggest the possibility of application of human skeletal muscle in vitro gene therapy with recombinant adenoviral vector carrying the human BMP-2 gene during reconstruction of the anterior cruciate ligament.

Presentation number: MG62 Abstract number: ABS-272-ISABS-2013

ASSOCIATION BETWEEN CYCLOSPORINE CONCENTRATION IN BLOOD AND SIDE EFFECTS IN RENAL TRANSPLANT PATIENTS

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The primary object of this research is to test the suitability of a set therapeutic ranges and the necessity of cyclosporine therapeutic monitoring in renal transplant patients. The secondary object was to define whether or not there was an interaction between cyclosporine and other drugs used simultaneously during treatment. Research was conducted as a retrospective analysis of medical documentation. In the process of statistical analysis Receiver operating characteristic was used. We analyzed if there was a connection between cyclosporine blood concentration and the onset of the following side effects: tremor, thrombocytopenia, nephrotoxicity, hepatotoxicity, mycosis, hyperglycemia, hyperkalemia, gum hyperplasia, hyperlypidemia, hirsuitism and CMV infection. Research results indicate that TDM is a justifiable method for a clinical risk evaluation of certain dose dependent side effects. Tremor appears at concentrations above 123.6 µg/L and the risk of CMV infection is high at concentrations 202,6 µg/L or higher. Considering the fact that the observed sample is small, some of the named side effects occurred rarely, therefore such analysis cannot be considered relevant. Thrombocythopenia and fungal infections are dose independent side effects of cyclosporine. Also, our results suggest that the interaction between cyclosporine and prednisone occurs and results in additive effect, which is the occurrence of hyperlipidemia and hyperglycemia at subtherapeutic cyclosporine concentrations. We conclude that TDM is a relevant tool for kidney transplant patient follow up, in purpose of avoiding some of the undesirable effects. Also, genotyping of genes included in pharmacokinetics and pharmacodynamics would be necessary for more detailed cyclosporine dose presumption.

Presentation number: MG63 Abstract number: ABS-250-ISABS-2013

SLEEP-DISORDERED BREATHING IN CHILDREN WITH PRADER-WILLI SYNDROME

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Introduction: Prader-Willi syndrome (PWS) is a genetic disorder caused by the absence of expression of the paternal copy of genes in chromosome region 15q11-13. The genetic subtypes of PWS are deletion (~70%) and maternal uniparental disomy (mUPD; 25-30%). Diagnosis was confirmed by methylation test, and genetic subtypes were established using FISH or multiplex ligation-dependent probe amplification and microsatellite analyses. **Methods:** The sleep unit database was used to analyse all identified cases with PWS (14 patients). Standard overnight polysomnography (PSG) was performed in sleep laboratory. Obstructive sleep apnoea (OSA) was defined by an obstructive apnoea-hypopnoea index, AHI >1/h. Age, symptoms of OSA, tonsillar size and BMI-Z-score were obtained in all cases. **Results:** Subjects included were 9 months to 11 yrs old. OSA was diagnosed in 11 of 14 (78%) cases, with both obstructive and central apneic events, with mean AHI=7,0. Those with OSA were significantly older ($P < 0.01$) and more likely to have enlarged tonsils ($P< 0.01$) than those without OSA. There was difference in BMI Z-score or the presence of symptoms of OSA, p=0.02. GH was deferred in 5 (36%) pending treatment for OSA. **Conclusions:** OSA was frequently present in children with PWS referred simply to meet the requirement for PSG before starting GH, and indication for operation of enlarged tonsils. We recommend routinely performance of PSG prior to GH therapy,and upper airway surgical intervention. Further studies are necessary to determine optimal treatment for some children with PWS and sleep-disordered breathing.

Prenatal Diagnostics

Presentation number: MG64 Abstract number: ABS-192-ISABS-2013

RECURRENT PREGNANCY LOSS DATA FROM GENETIC COUNSELLING UNIT, UNIVERSITY HOSPITAL CENTRE SPLIT

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Spontaneous abortion is one of the most common complications of pregnancy, caused by exogenous factors, infections and chromosomal abnormalities. In the Genetic Counseling Unit (GCU), the relevant information can be given by taking careful and detailed anamnestic data and drawing family trees. We present results obtained from data obtained from the GCU at the University Hospital Split from1985 to 2010 for couples with recurrent pregnancy loss. We found a higher number of de novo chromosomal changes in aborted material from those who had normal constitutional karyotype and urogenital infections than among those who had balanced chromosomal translocations. We included 451 couples with repeated or habitual abortions in the absence of »maternal age effect«, but with male partners of women who have experienced three miscarriages slightly older. Siblings in the second generation of women and men had higher number of spontaneous abortions than the general population. Both men and women had significant statistical correlation with previously urinary and/or genital infections. We found carriers of balanced translocations in only 1,8%. Samples from aborted material had somatic chromosome trisomy and triploidy. For couples who have balanced chromosomal translocation we have cytogenetic proof that the translocation causes recurrent miscarriages and could recommend the preimplantation diagnosis before next pregnancy. This constitutes an important framework for further progress in explanation of the causes of complex multifactorial process such as recurrent miscarriages.

Presentation number: MG65 Abstract number: ABS-214-ISABS-2013

FETAL ALCOHOL SYNDROME PREVALENCE IN RURAL COUNTY OF KRAPINA AND ZAGORJE

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Maternal alcohol consumption during pregnancy can cause Fetal alcohol spectrum disorders (FASD) in their children. The prevalence of FASD in Western countries is not known, as there are few epidemiologic studies performed so far. We present the results of study performed in rural County of Krapina and Zagorje with the objective to determine Fetal alcohol syndrome (FAS) and Partial fetal alcohol syndrome (PFAS) prevalence in a sample of schoolchildren and alcohol drinking habits during pregnancy among their mothers. The study involved 1110 schoolchildren attending 1st to 4th grade and their mothers. We used an active case ascertainment method with passive parental consent and Clarified IOM criteria. The study involved clinical examination of schoolchildren and data collection from their mothers. Out of 1110 mothers, 917 (82.6%) answered the questionnaire. Pregnancy alcohol consumption was admitted by 11.5% of interviewed mothers and 1.4% admitted binge drinking during pregnancy. Clinical examination involved 824 (74.2%) out of 1110 enrolled children. The examination revealed 14 children (1.7%) with clinical signs of FAS and 41 (5.0%) of PFAS. Based on 74.2% participation rate in the clinical examination, the observed FAS prevalence was 16.9, PFAS 49.7 and combined prevalence was 66.7/1000 schoolchildren. This is the first FAS and PFAS prevalence study performed in rural community of Croatia and Europe. The study revealed a high prevalence of FAS/PFAS and pregnancy alcohol consumption in this rural winegrowing region, which constitutes a serious health problem with the need for future studies and development of preventive programmes.

Presentation number: MG66 Abstract number: ABS-205-ISABS-2013

RHD AND KELL GENOTYPING FOR NONINVASIVE PRENATAL DIAGNOSTICS PURPOSES

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There are two reasons for establishing a methodology for non-invasive determination of RHD and KELL genotypes in early pregnancy: 1) to identify fetuses which are at risk of hemolytic disease of fetus and newborn by alloimmunized pregnant women, and 2) to prevent alloimmunization during pregnancy. There is no method validation on a representative number of samples in the Czech Republic, which would allow introducing the methodology into clinical practice. **Aim:** Evaluate two different cell free fetal (cff) DNA separation procedures based on adsorption on the surface of silica gel and on the separation on magnetic particles. Optimize and evaluate RHD and KELL genotyping. **Material and methods:** We tested both isolation procedures in 76 cffDNA samples. Together 200 control samples were used for genotype assessment. Optimization and calibration of RHD and KELL genotyping was done using Real-Time PCR and by capillary electrophoresiss minisequencing. **Results and Conclusion:** There were significant differences in the yield of cell free fetal DNA between the tested cffDNA isolation methods. Silicagel membrane based method for isolation of cffDNA shorter molecules is more suitable than the magnetic particle one. To determine the sensitivity threshold there were performed RHD and KELL calibrations by Real time PCR and capillary electrophoresis with a dilution series RHD and KELL genotypes. Both methods are able to clearly recognize the fetal genotype. The optimization was further examined to detect RHD and KELL genotypes simultaneously and together with multiplex SNP assay as an internal cffDNA control. Supported by IGA MZ CR: NT12225.

Protein Glycosylation in Diagnostics and Therapy

Presentation number: MG67 Abstract number: ABS-148-ISABS-2013

EFFECTS OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS ON ACUTE-PHASE ALTERATIONS OF CEREBRAL MONOAMINES

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The neuroprotective potential of the nonsteroidal antiinflammatory drug "indomethacin" was tested against lipopolysaccharide-produced biosynthesis of monoamines in six brain regions of male rat. Observations were based on a single intraperitoneal injection of each of lipopolysaccharide (250 μg Kg-1 body wt) and indomethacin (20 mg Kg-1 body wt) followed by sampling and assaying of brain specimens after 2, 8, 12 and 24 hrs. In virtually all brain regions tested, lipopolysaccharide stimulated the biosynthesis of cerebral monoamine. Yet, pretreatment with indomethacin provoked substantial mitigation predominately after 24 hrs. A time-based manner attended by a regionally nonselective manner characterized lipopolysaccharide-induced monoamine biosynthesis; whereas, indomethacin alleviation seemed to proceed in a time-dependent and regionally selective pathway. The response towards lipopolysaccharide implied a role of prostaglandin synthesis given that it was abolished by the cyclooxygenase-inhibitor indomethacin. Data verified the potent therapy potential of indomethacin in protecting cerebral catecholamine systems against lipopolysaccharide-induced acute phase reactions.

Presentation number: MG68 Abstract number: ABS-275-ISABS-2013

ALTERATION IN NUMBER OF INTERNEURONS IN CORTICAL REGIONS OF ST3GAL2, ST3GAL3 AND DOUBLE KNOCKOUT MICE

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Besides the afferent and efferent neurons, interneurons are necessary for normal function of nervous system since they possess modulator role in information flow. Over time many studies confirmed that they are critical for cortical plasticity and are implicated in the etiology of neuropsychiatric diseases like schizophrenia, autism, anxiety disorder and seizures. Using the primary antibodies against calcium binding proteins calbindin (CB) and parvalbumin (PV) we investigated density and distribution of interneurons in brains of wild type (WT), St3Gal2 (beta-galactoside alpha-2,3-sialyltransferase 2) null, St3Gal3 (ST3 beta-galactoside alpha-2,3-sialyltransferase 3) null and double null (DKO) mice. PV and CB neurons were quantified relative to all neurons in: striatum (St), motor (M1), visual (V1), somatosensory (S1) and auditory (Au1) cortex. ST3Gal2 mice had a significantly lower proportion of CB positive neurons in all regions except Au1 cortex. At the same time the proportion of PV positive neurons was lower in all sensory regions and St, but not in M1. ST3Gal3 mice had a significantly lower proportion of CB neurons in M1 and St, but the proportion of PV neurons was similar to WT. DKO mice had more CB and PV positive neurons in M1 than WT. These findings are the first report of changes in the ratio of neuronal subtypes in sialyltransferase null mice investigated up to now indicating potential role of sialic acid epitopes in the proliferation, migration or maintenance of interneurons. Further studies should determine functional changes in interneuron deficient microcolumns and its behavioral consequences.

Presentation number: MG69 Abstract number: ABS-285-ISABS-2013

OPTIMAL SAMPLE CHOICE FOR ANALYSIS OF PROTEIN ANTENNARY FUCOSYLATION AS SCREENING TOOL FOR HNF1A-MODY

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Maturity-onset diabetes of the young (MODY) is a dominantly inherited form of non-insulin dependent diabetes caused by mutations in several genes. A subtype of MODY is caused by mutations in HNF1A, a nuclear transcription factor, which appears to be one of the key regulators of metabolic genes. Recently we showed that deleterious coding mutations in HNF1A have profound effects and that antennary fucosylation of plasma proteins is significantly decreased in HNF1A-MODY patients. The proportion of HPLC peak DG9 in the sum of DG8 and DG9 (DG9 index) roughly indicates the level of antennary fucosylation of triantennary glycans in plasma. Low values of this index appeared to be very indicative of HNF1A-MODY. HNF1A-MODY patients could be nearly completely separated from Type 1 diabetes, Type 2 diabetes, GCK-MODY and general population on the basis of the HAFU index with Receiver-Operator Characteristic (ROC) curves approaching 90% specificity at 90% sensitivity. In order to optimize and simplify collecting and shipping procedure for samples, we compared the analysis of standard plasma samples, dried blood spots that were generated from whole blood (venous blood) and dried blood spots generated from capillary blood by finger prick test. All analyzed sample types displayed only small differences in HPLC peaks DG8 and DG9 and preserved the information value of HAFU index.

Presentation number: MG70 Abstract number: ABS-184-ISABS-2013

IMMUNOGLOBULIN G GLYCOSYLATION IN ATOPIC CHILDREN

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Immunoglobulin G (IgG) is considered one of modulators of immune responses in atopic diseases. Affinity of IgG binding to Fcγ receptors can be influenced by differential N-glycosylation of IgG Fc regions. This study aimed at examining serum IgG glycoprofile in children suffering from atopic diseases (allergic asthma, allergic rhinitis, atopic dermatitis and food allergy). Peripheral blood was collected from 61 patients and 74 healthy controls of both sexes aged 5-18 years. Patients' atopic status was confirmed by positive skin prick tests and elevated total serum IgE. Plasma was separated by centrifugation and IgG isolated by affinity chromatography on CIMProtein G Monolithic Plate. Total plasma N-glycans (TPNG) and IgG N-glycans were released and processed by in-gel-block method, which included protein denaturation and N-glycan release by N-glycosydase F. After labeling with 2 aminobenzamide TPNG were examined by hydrophilic interaction high performance liquid chromatography, and IgG N-glycans by hydrophilic interaction ultra performance liquid chromatography. TPNG differed between two groups in surface area of only three out of sixteen glycan peaks containing mixed glycan populations. In contrast, IgG N-glycans different markedly between the two groups, concerning both carbohydrate unit number and composition. The atopic population showed a tendency towards bigger glycan structures (containing more than 10 monomer sugar units), as well as an increase in percentage of fully galactosylated and terminally sialylated structures. Thus, there is a markedly different and protein specific IgG N-glycoprofile in atopic vs. healthy children. This feature may be developed into a predisposition, prognostic or diagnostic biomarker of atopic diseases.

Presentation number: MG71 Abstract number: ABS-257-ISABS-2013

LOCI ASSOCIATED WITH N-GLYCOSYLATION OF HUMAN IMMUNOGLOBULIN G SHOW PLEIOTROPY WITH AUTOIMMUNE DISEASES AND HAEMATOLOGICAL CANCERS

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Glycosylation of IgG influences IgG effector function by modulating binding to Fc receptors. To identify genetic loci associated with IgG glycosylation, we quantitated N-linked IgG glycans using two approaches. After isolating IgG from human plasma, we performed 77 quantitative measurements of N-glycosylation using ultra performance liquid chromatography (UPLC) in 2247 individuals from four European discovery populations. In parallel, we measured IgG N-glycans using MALDI-TOF mass spectrometry (MS) in a replication cohort of 1848 Europeans. Meta-analysis of genome-wide association study (GWAS) results identified 9 genome-wide significant loci ($P<2.27x10^{-9}$) in the discovery analysis, and two of the same loci (B4GALT1 and MGAT3) in the replication cohort. Four loci contained genes en coding glycosyltransferases (ST6GAL1, B4GALT1, FUT8 and MGAT3), while the remaining 5 contained genes that have not been previously implicated in protein glycosylation (IKZF1, IL6ST-ANKRD55, ABCF2-SMARCD3, SUV420H1, and SMARCB1-DERL3). However, most of them have been strongly associated with autoimmune and inflammatory conditions (e.g., systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, diabetes type 1, multiple sclerosis, Graves' disease, celiac disease, nodular sclerosis) and/or haematological cancers (acute lymphoblastic leukaemia, Hodgkin lymphoma, and multiple myeloma. Our study shows that it is possible to identify new loci that control glycosylation of a single plasma protein using GWAS.

Presentation number: MG72 Abstract number: ABS-277-ISABS-2013

CHANGES IN IGG COMPOSITION AND TOTAL PLASMA GLYCOME DURING SYSTEMIC INFLAMMATORY RESPONSE INDUCED BY CARDIAC SURGERY

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Controlling the inflammatory cascade still represents a great challenge, due to its complexity and individual physiological differences. Although known to have a great impact on protein functions, changes of glycosylation in acute inflammation have not been extensively studied due to absence of a good human model. The aim of this study was to follow intra-individual changes of total plasma and IgG glycans during the early course of systemic inflammation caused by cardiac surgery. The levels of N-linked glycans have been analysed in 109 patients prior to surgery and on the first and third day following the surgery. Both total plasma protein and IgG linked glycans showed prominent, dynamic changes even during the first 24 hours of inflammatory response. Contrary to the plasma glycome, which changes in the same way in nearly all individuals, pattern of IgG glycosylation changes was rather different between individuals. Correlation was observed between basal levels of IgG fucosylation and the rate of its change with the severity of the inflammatory response, suggesting prognostic potential of glycosylation. These results imply new potential targets for controlling the inflammatory response and must be considered when immunomodulating therapies are subjected.

Presentation number: MG73 Abstract number: ABS-258-ISABS-2013

EPIGENETIC SILENCING OF HNF1A ASSOCIATES WITH CHANGES IN THE COMPOSITION OF THE HUMAN PLASMA N-GLYCOME

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Protein glycosylation is a ubiquitous modification, which affects protein structure and function. Recent genome wide association study identified transcription factor HNF1A as an important regulator of plasma protein N-glycosylation. To evaluate the potential impact of epigenetic regulation of HNF1A on protein glycosylation we quantified its CpG methylation in 810 individuals. Correlations between methylation of four CpG sites and the composition of plasma and IgG glycomes were analyzed. Several significant associations were observed between the level of HNF1A methylation and plasma N-glycans, the most significant one at the level of branched N-glycan structures, while there were no significant associations with IgG glycans. The hypothesis that inactivation of HNF1A promotes glycan branching was supported by the analysis of plasma N-glycomes in 61 patients with inactivating mutations in HNF1A, where the increase in plasma glycan branching was observed as well. This study represents the first demonstration of epigenetic regulation of plasma N-glycome composition, suggesting potential mechanism by which epigenetic deregulation of the glycome may contribute to disease development.

Presentation number: MG74 Abstract number: ABS-259-ISABS-2013

EPIGENETIC MODULATION OF HELA CELL MEMBRANE N-GLYCOME BY EPIGENETIC INHIBITORS AND REVERSIBILITY OF INHIBITION EFFECTS IN A DRUG-FREE ENVIRONMENT

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Glycans are essential regulators of protein function and are now in the focus of research in many physiological and pathophysiological processes. For instance, changes in glycan structures are a hallmark of virtually every cancer, and the majority of cancer markers are glycoproteins. There are numerous modes of regulating glycan biosynthesis, including epigenetic mechanisms implicated in the expression of glyco-genes. Since N-glycans located at the cell membrane define intercellular communication as well as a cellular response to a given environment, we developed a method to preferentially analyze this fraction of glycans. The method is based on incorporation of living cells into polyacrylamide gels, partial denaturation of membrane proteins with 3M urea and subsequent release of N-glycans with PNGase F followed by HPLC analysis. Using this newly developed method, we revealed multiple effects of epigenetic inhibitors Trichostatin A, sodium butyrate and zebularine on the composition of N-glycans in human cells. The induced changes were found to be reversible after inhibitor removal. Given that many epigenetic inhibitors are currently explored as a therapeutic strategy in treatment of cancer, wherein surface glycans play an important role, the presented work contributes to our understanding of their efficiency in altering the N-glycan profile of cancer cells in culture.

Legislation Pertinent to DNA Databases

Presentation number: FG41 Abstract number: ABS-305-ISABS-2013

DEATH OF IVICA KUGLI: ETHICAL, MEDICAL AND LEGAL REASONS AND THE NEED FOR FORENSIC ANALYSIS OF THE REMAINS OF A HIGH SCHOOL STUDENT BURIED IN 1945

Primorac D[1,2,3,4,5], Mance M[6], Anđelinović Š[5], Primorac D[5], Letica S[6]

[1]Medical School, University of Split, Split, [2]Medical School, Josip Juraj Strossmayer University, Osijek, Croatia; [3]Eberly College of Science, Penn State University, University Park, Pennsylvania, [4]University of New Haven, New Haven, Connecticut, USA; [5]Department of Forensic Sciences, University of Split, [6]Medical School, University of Zagreb, Zagreb, Croatia

According to medical documentation, the Zagreb high school student, Ivan ''Ivica'' Kugli died of natural causes, on Christmas Eve, 1945 at 22:00 as a result of the lasting consequences of poliomyelitis, a disease which he contracted as a child. At the time, he was not yet 17, being born February 14, 1929. Of the few living relatives of the once very prestigious and respected Zagreb Kugli family (none of whom live in Croatia today), nobody was convinced that Ivan died because of his childhood illness. Dr. Košcica conducted the post-mortem examination; he handwrote the cause of death on the death certificate: Poliomyelitis. Sixty-six years later and 21 years after the fall of Communism, three witnesses who were Ivan's classmates told a very different story of how he died. One witness is a retired physician (I.D.), another a university professor of architecture (B.M), and the third was Ivan's best friend (B.J). All three independent witnesses stated to an author of this abstract (S.L.) that Ivan died as a result of physical trauma sustained from the actions of fellow students politically motivated by Communistic intolerance. There are many legal, ethical and scientific reasons for exhumation and forensic investigation of Ivan Kugli's remains after almost seven decades. The authors will deliver all available documentation to the Ministry in charge and Office of the Croatian Prosecutor in order to open the criminal investigation.

DNA Autopsy in Unexplained Deaths

Presentation number: FG42 Abstract number: ABS-171-ISABS-2013

DECEPTION AND VAGINAL SLIDES: DO WE NEED TO PRESERVE A CONTROL SAMPLE OF VICTIMS IN EVERY CASE OF RAPE?

Raina A[1], Dogra T[1], Leenaars A[2], Mahapatra B[3], Leenaars L[2]

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Two women were found dead on the bank of a river in India. It was determined that the women had been raped and on postmortem examination, the cause of death was determined to be drowning. The mechanisms of the deaths let to probability that the women were murdered. The doctors, who conducted the examinations, submitted vaginal slides of the deceased for forensic examination. DNA analysis was conducted and male and female fractions of DNA were isolated. The viscera of the deceased were also preserved for toxicological analysis at the time of post mortem examination and subjected to DNA analysis. The female DNA profiles obtained from the vaginal slides and viscera did not match. Blood samples of four suspects were also collected, subjected to DNA analysis, and the profiles did not match with the male DNA profiles from the vaginal slides. This resulted in questionable samples, and therefore, the exhumation of the bodies was recommended and undertaken. DNA profiles were generated from the long bones, teeth, and viscera of the exhumed bodies, which matched only with the DNA profiles generated from the viscera, not with the vaginal slides. From a forensic investigation, it was determined that the vaginal slides were falsified by one of the autopsy doctors. There was intentional deception. Implications of this case are made for future investigations of rape victims.

LATE SUBMITTED ABSTRACT:

New technologies, information security and legislation pertinent to DNA databases in Croatia

Boban M

Faculty of Law, University of Split, Split, Croatia

The use of advanced information technologies requires particular attention to the legislation pertinent to DNA databases, information security and data protection. The global trends in the development of methods to mitigate these problems cannot bypas Croatia. The goal of this study was to determine the impact of new technologies and the rising trend of global threats to security of DNA databases and to establish the key for determinig and building legislation pertinent to DNA databases. From these facts arises the need of thorough research and analysis of protection of DNA databeses on security, in regarding disaster management control, and building the legal protection model to a higher level. Narrower field of work relates to the analysis of existing legislation on protection of personal data and security settings od DNA databeses in order to define the fundamental quality of proposed model. The study also includes an overview of related international standards, guidelines and recommendations of the countries of the European Union to ensure a higher level of personal data protection in the field of information security.

ABOUT INVITED SPEAKERS

Zwi Berneman (University of Antwerp, Antwerp, Belgium) Zwi N. Berneman M.D., Ph.D., is Professor of Hematology at the University of Antwerp and Head of the Division of Hematology of the Antwerp University Hospital, Edegem, Belgium. He is the Medical Director of the Center for Cell Therapy and Regenerative Medicine, the GMP facility of the Antwerp University Hospital for hematopoietic stem cell processing and cell therapy with eye limbus stem cell cultures and dendritic cells. His laboratory pioneered the method of mRNA electroporation and has applied it to the fields of dendritic cells and stem cells. His main clinical research focus is on dendritic cell vaccination in malignant conditions and in chronic viral infections such as AIDS. Basic research ongoing in his laboratory include the interactions between dendritic cells and the innate immune system, the use of dendritic cells for induction of tolerance and the fate of (stem) cells implanted in the central nervous system.

Frederick Bieber (Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA) Dr. Bieber serves as a Medical Geneticist at Brigham and Women's Hospital and as Associate Professor of Pathology at Harvard Medical School in Boston, MA, USA. His work involves clinical laboratory genetic diagnostics and forensic medicine. He has a special interest in forensic DNA data banks and in genetic kinship analysis and participated in the DNA-based identification of victims of the twin tower attacks on September 11, 2001 and of Hurricanes Katrina and Rita. Dr. Bieber serves on Advisory Boards of the Royal Canadian Mounted Police, the Virginia Department of Forensic Science, and the U.S. Department of Defense.

Malcolm Brenner (Baylor College of Medicine, Houston, TX, USA) Malcolm Brenner was educated at Forest School London and Emmanuel College, Cambridge England. He received his medical degree and subsequent Ph.D. from Cambridge University, England. He conducted one of the first human gene therapy studies when he transduced bone marrow stem cells with a retroviral vector with the intention of marking them to study their survival and fate. This seminal study demonstrated that engrafted bone marrow stem cells contribute to long-term hematopoiesis and also that contaminating tumor cells in autografts can cause relapse. More recently, his group has become interested in the genetic-modification of T-cells for cancer therapy, cancer vaccines and monoclonal antibodies. Appointed Editor in Chief of Molecuar Therapy Nature in 2009. He is currently Director of the Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas and serves as a faculty member for the Texas Children's Cancer Center at Texas Children's Hospital.

Zoran Budimlija (Office of Chief Medical Examiner, New York, NY, USA) Dr. Zoran M. Budimlija works at the NYC Office of Chief Medical Examiner, Department of Forensic Biology. His field of interest is the application of the techhniques of Forensic Biology in the field of Pathology and vice versa. He teeaches at three private universities in New York City.

Robert J. Deans (Athersys, Inc., Cleveland, Ohio, USA) Dr. Deans graduated from the Massachusetts Institute of Technology with the B.S. degree and obtained the Ph.D. degree from the University of Michigan. He spent his postdoctoral training in molecular immunology at the University of California in Los Angeles. He was on the faculty of the University of Southern California Medical School for eight years before commencing his more than twenty-year career in stem cell research and therapeutics. Dr. Deans was Director, Research and Development at Baxter Healthcare, where he developed the biological components of the Isolex300i hematopoietic stem cell purification platform. Subsequently, he served as Vice-President, Research at Osiris Therapeutics before moving to Athersys where he is Executive Vice-President, Regenerative Medicine and in charge of the Athersys' European subsidiary ReGenesys. Dr. Deans authored numerous highly cited papers in cell therapy, is an active member of cell therapy professional societies and an acknowledged leader in the field of cellular and regenerative medicine. Under Dr. Deans' leadership Athersys is developing therapeutics based on adherent stem cells (MultiStem) isolated from adult bone marrow. Athersys has completed two Phase I safety studies of MultiStem in the treatment of acute myocardial infarction and in prophylaxis of graft v. host disease in allogeneic hematopoietic stem cell transplantation. MultiStem is currently in Phase II clinical studies for treatment of ischemic stroke and ulcerative colitis.

Katja Drobnič (Faculty of Criminal Justice and Security, UM and National forensic laboratory, MNZ Slovenia) I'm professor of forensic science UM and forensic genetics UL. I'm a guest lecturer at many others faculties, institutions and societies in Slovenia and abroad. I have been training at many well known institutions (FBI Academy, Institute of legal medicine, University of Bern, Forensic Science Service (UK), Institute of toxicology, Madrid, Section of Forensic Genetics, University of Copenhagen). My current job position is a quality manager at National forensic laboratory, and I'm also DNA court expert. My first research were focused on methods for isolation of DNA from crime evidence, identification of victims from mass graves, SRY markers, STR and SNP typing in forensic and population genetic, the latest are oriented on SNP-based prediction of human visible characteristics, species identification using cytB and body fluids identification by mRNA. I have more than 20 articles in journal with science citation index in the forensic and population genetic field.

Moran Elishmereni (Institute for Medical BioMathematics, Bene Ataroth, Israel) Moran holds a BSc in Computational Biology from Bar-Ilan University, and an MSc at Pharmacology Department in the Faculty of Medicine at the Hebrew University of Jerusalem. This summer she is completing her PhD at the Institute for Drug Research at the Hebrew University of Jerusalem. In the Institute for Medical BioMathematics (IMBM) in Israel, Moran takes part in Cancer Immunotherapy projects: she has applied advanced methods of mathematical modelling and computational analysis in order to invstigae and enhance cytokine and cell-based vaccination treatments for melanoma, renal cell carcinoma, and prostate cancer.

Henry Erlich (Roche Molecular Systems, Pleasanton, CA, USA) Dr. Erlich is the Vice President of Discovery Research and Director of the Human Genetics Department at Roche Molecular Systems, Inc. He is a molecular biologist, geneticist, and immunologist, and has been engaged in the development and application of PCR in basic research, medical diagnostics, evolution and anthropology, and forensics. One of his major interests is the analysis of polymorphism in the HLA genes and the development of HLA typing tests for tissue typing, disease susceptibility, and individual identification. He has authored over 250 articles and is the recipient of various scientific awards. He received his B.A. from Harvard University and his Ph.D. in Genetics from the University of Washington, Seattle and has been a postdoctoral fellow in the Department of Biology at Princeton and the Department of Medicine at Stanford.

Arezou Ghazani (Harvard Medical School and Massachussetts General Hospital, Boston, MA, USA) Dr. Ghazani is a medical geneticist and a nanoengineer. She earned graduate degrees in genomics and nanoengineering from the University of Toronto, after which she completed the American Board of Medical Genetics in clinical cytogenetics at Harvard Medical School. She conducts research in the field of nanodiagnostics at the Massachusetts General Hospital in Boston. Her work focuses on the application of nanotechnology instrumentation for detection of rare malignant cells in patients with cancer and her published work has appeared in numerous journals. Dr. Ghazani has lectured internationally and is Course Director of Principles of Genomics, a popular graduate course at Harvard University.

Mitchell Holland (Eberly College of Science, Penn State University, PA, USA) Bachelor of Science in Chemistry from Hobart Collage, Ph.D. in Biochemistry from the University of Maryland College Park, and Postdoctoral Fellowship at the Johns Hopkins University School of Medicine in Human Genetics. Fellow in the American Academy of Forensic Sciences, served as an associate professorial lecturer at George Washington University, and has been adjunct faculty at other colleges and universities. He has been on the Editorial Board of the Journal of Forensic Sciences and the International Journal of Legal Medicine. He was the Senior Vice President and Laboratory Director of The Bode Technology Group from 2000-2005. While there, he participated in the identification efforts of victims from the World Trade Center disasters. He was with the Armed Forces DNA Identification Laboratory (AFDIL) from 1991-2000, the Scientific Laboratory Director from 1993-2000. While there, he participated in the identification of the remains of the Vietnam Unknown Soldier and Tsar Nicholas Romanov.

Edwin Huffine (Bode Technology Group, Springfield, VA, USA) As vice president for international development and humanitarian services at Bode Technology Group, Ed Huffine advises and assists nations in developing or upgrading their forensic DNA systems as well as has overall responsibility for providing identification assistance and mass disaster response for regions that have experienced conflicts or natural disasters. Providing these types of services requires frequent inter-

action with both domestic and foreign political, scientific, and diplomatic representatives and various non-governmental organizations and potential donors.

Manfred Kayser (Erasmus MC - University Medical Center Rotterdam, Rotterdam, Netherlands) Prof. Kayser is a world-leading expert in anthropological genetics and forensic molecular biology. He was instrumental in introducing Y-chromosome analysis to forensic and evolutionary genetics where it now is widely employed. He is well- known for his ground-braking work on human genetic history of Oceania. Recently his research focus widened-up further now additionally including topics like the genetic basis and DNA prediction of human appearance traits, DNA-based inferences of bio-geographic ancestry, and various other aspects of human molecular biology and genetics with putative applications to forensics, such as forensic time estimations and forensic tissue identification. He authored over 100 articles in peer-reviewed scientific journals, is the co-Editor-in-Chief of Investigative Genetics and serves as Academic Editor and Editorial Board Member of several other scientific journals. Currently he is Professor of Forensic Molecular Biology and chairs the Department of Forensic Molecular Biology at the Erasmus University Medical Center Rotterdam, The Netherlands.

Yuri Kogan (Institute for Medical BioMathematics, Bene Ataroth, Israel) Over many years of scientific research, I have been involved in biomathematical modeling of several cancer diseases, as well as in cancer stem cell research, intracellular signal transduction and clinical immunotherapy. My current research at IMBM is focused on modeling cancer growth and treatment, in particular immunotherapy of prostate cancer and glioblastomas, modeling cancer stem cells, and modeling intracellular signal transduction.

Doron Lancet (The Weizmann Institute of Science, Rehovot, Israel) Genome analysis and variation.

Gordan Lauc (Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia) Dr. Gordan Lauc is professor of biochemistry and molecular biology at the University of Zagreb and CEO of Genos Ltd. He graduated molecular biology at the University of Zagreb in 1992 and obtained PhD in Biochemistry from the same univeristy in 1995. He got his postdoctoral training at the Institute for Medical Physics and Biophysics in Münster and the Johns Hopkins University in Baltimore. Gordan Lauc is author of over 70 research papers published in international journals and six international patents. He was invited to lecture at numerous international conferences, and was also elected for visiting professor at the Johns Hopkins University. For his work he was awarded Hans Seyle award for young scientists, Croatian national award for science for young researchers and Award for young researchers from the International Glycoconjugate Organization. He is a member of the Croatian National Science Council and the Johns Hopkins Society of Scholars.

Henry Lee (The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA) Dr. Lee is one of the world's foremost forensic scientists. He has investigated 8000 cases. His work figured prominently in reinvestigation of JFK, RFK assassination, Vincent Foster death and O. J. Simpson trial, the murder of Jon Benet Ramsey & Chandra Levy, suicide of White House Counsel Vincent Foster, kidnapping of Elizabeth Smart. Dr. Lee was the Commissioner of Public Safety and served as Chief Forensic Lab. Currently, he is Distinguish Professor at University New Haven and Director Forensic Research Training Center. He has co-authored hundreds articles and thirty books. He is the principle investigator for many research projects. He is a recipient of twenty honorary Doctorate Degrees. He is the recipient of Medal of Justice from Justice Foundation, Lifetime Achievement Award from Science and Engineer Association, Distinguished Criminalist Award from AAFS, the J. Donero Award from IAI, Alice Island Medal form US, Gusi Award from Philippines.

Timothy Palmbach (The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA) I am an Associate Professor and Chair of the Forensic Science Department at University of New Haven, West Haven, CT. I am also Executive Director of the Henry Lee Institute of Forensic Science. In 2004, I retired from the Connecticut State Police as a Major and Director in charge of the Ct State Police Division of Scientific Services, including the Forensic Science Laboratory, Controlled Substance and Toxicology Laboratory and Digital Forensics Lab. I am an expert witness in Crime Scene Reconstruction and testify throughout the United States in Criminal and civil matters.

Dragan Primorac (University of Split, Split, University of Osijek, Osijek, Croatia, The Pennsylvania State University, USA, University of New Haven, USA) Dragan Primorac is a pediatrician, forensic expert and geneticist. Currently he serves as professor at medical schools in Split and Osijek in Croatia and adjunct professor at Penn State University and University of New Haven in the United States. Prof. Primorac is one of the founders of forensic DNA analysis in Croatia and a pioneer in the application of DNA analysis for identification of bodies in mass graves. He authored hundred and sixtyscientific papers and abstracts in forensic science, population genetics, genetic legacy of Homo sapiens sapiens, clinical medicine and molecular genetics. His papers have been cited close to 1600 times in the scientific literature, and his research program has received continuous funding from the Seventh Framework Programmee (FP7). Together with Professors Moses Shanfield and Stanimir Vuk-Pavlović, Prof. Primorac founded ISASB and has contributed to the academic part of organizing its meetings. From 2003 to 2009 he served as Minister of Science, Education and Sports, Republic of Croatia.

Antti Sajantila (University of Helsinki, Helsinki, Finland) Profeesor, MD, PhD, currently vice-director of Hjelt Insitute, and head of Department of Forensic Medicine, University of Helsinki, Finland. Research interests focus on forensic genetics and pathology. Paarticularly forensic identification, population genetics and ancient

DNA. Recently, research focus also in the post mortem pharmacogenetics. Published over 150 scientific and popular articles.

Moses Schanfield (George Washington University, Washington, DC, USA) Professor Schanfield is one of the original organizers of the ISABS conferences. He has been working in forensic since before DNA technology was initiated and has contributed to the use of DNA in the field. Professor Schanfield's group at Analytical Genetic Testing Center in Denver, Colorado were the discoverers of the in-lane sizeladder that is used in all size based modern DNA technology, he has been involved in testing evidence and testifying on DNA cases since 1989, and though no longer running a DNA Laboratory still consults on DNA cases. Professor Schanfield's research includes developing DNA technology for forensic and anthropological applications as well as forensic statistics.

David I. Smith (College of Medicine, Mayo Clinic, Rochester, MN, USA) David Smith is a Professor in the Department of Laboratory Medicine and Pathology at the Mayo Clinic. He is also he Chairman of the Technolog Assessent Group which is responsible for evaluating new technologies for their potential impact on research and its clinical translation at the Mayo Clinic. Dr. Smith has also been reponsible for developing the neessary infrastruture for Next Generation sequencing at the Mayo Clinic. In his own work he has been using the power of Next Generation sequencing to bette understand the molecular events that underlie the development of cancers of the head and neck.

Andre Terzic (College of Medicine, Mayo Clinic, Rochester, MN, USA) By integrating advanced technology with a focus on clinical problems addressed at a fundamental level, Dr. Terzic has pioneered at Mayo Clinic pathogenomic research of maladaptation in heart disease, and the application of cardioprotective and cardioregenerative therapeutic modalities. He has authored over 300 scientific manuscripts, advancing the development of diagnostic and management strategies for heart failure and ischemic heart disease. His works include reports on the discovery of genes for dilated cardiomyopathy and atrial fibrillation. More recently, he has led the effort in the discovery and development of advanced stem cell-based regenerative therapies applied to cardiovascular medicine. He serves as Co-Principal Investigator of the C-Cure international clinical trial, the first-in-man study using lineage specified stem cells for heart repair. His papers have been cited over 7,000 times in the scientific literature, and his research program has received continuous funding from the National Institutes of Health.

Carmen Perez-Terzic (College of Medicine, Mayo Clinic, Rochester, MN, USA) My research program focuses on cardiomyocyte and regenerative biology and includes basic science and clinical research in regenerative cell therapy as well as research addressing musculoskeletal issues in patients participating in a cardiac rehabilitation program.

Peter Underhill (Stanford University Medical Center, Stanford, CA, USA) Dr Underhill has worked at Stanford University as a member of the research staff for more then 20 years investigating human genetic variation. His main research expertise involves tracing human migrations and deciphering population affinity and substructure in contemporary populations using haploid Y Chromosome compound SNP and STR haplotypes. He has co-authored numerous peer-reviewed publications, most relating to molecular anthropology oriented Y chromosome studies of human populations.

Richard Villems (University of Tartu and Estonian Biocentre, Tartu, Estonia) I am population geneticist interested in the history of genetic structuring of the present-day human populations.

Stanimir Vuk-Pavlović (College of Medicine, Mayo Clinic, Rochester, MN, USA) Stanimir Vuk-Pavlović is a professor of biochemistry and molecular biology at the College of Medicine, Mayo Clinic in Rochester, Minnesota, and Director Emeritus of the Stem Cell Laboratory, Mayo Clinic Cancer Center. His recent research interests include cancer immunotherapy, cell graft engineering, regenerative medicine and practical application of systems biology. Together with Professors Moses Shanfield and Dragan Primorac, Prof. Vuk-Pavlović founded ISABS and has contributed to the academic part of organizing its meetings.

SPONSOR INFORMATION

PLIVA

Organization Information	
Organization:	Pliva Hrvatska d.o.o.
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E-Mail:	info@pliva.hr
URL:	www.pliva.hr
key words (up to 10):	A Teva Group member, the largest pharmaceutical company in Croatia
Product(s) (up to 50 words):	We at Pliva are dedicated to providing our customers with high quality, affordable medicines for a better quality of life. Thanks to our state-of-the-art development andpro-duction capacities, Pliva's production portfolio consists of high quality generic products offering superior therapeutic solutions in almost all therapheutic areas.

BELUPO

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Fax:	385 1 2371 411
E-Mail:	belupo@belupo.hr
URL:	www.belupo.hr, www.belupo.com
key words (up to 10):	Let's be healthy together
Product(s) (up to 50 words):	Belupo is the market leader for the group of medicine with the impact to the skin, heart and blood vessels, central nervous system, muscular skeletal and gastrointestinal system as well as systematic infections drugs. Belupo's programme of over-the-counter products comprises of non-prescription drugs as well as herbal and dietary drugs.

BIOSISTEMI

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E-Mail:	biosistemi@biosistemi.hr
URL:	www.biosistemi.hr
key words (up to 10):	Biosistemi is part of Biosistemi grupa which is Life technologies distributor for 7 Countries in SEE region.
Product(s) (up to 50 words):	Instruments, software and reagents for: research in molecular biology, molecular diagnostic, food safety, animal health, bioproduction, human identification…

CROATIA zdravstveno osiguranje

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Fax:	385 1 6332 777
E-Mail:	croatiazdravstvenoosiguranje@crosig.hr
URL:	www.croatia-zdravstveno.hr
key words (up to 10):	CZO is a member of the Croatia Insurance Group, company with a long tradition in the insurance CZO conducts exclusively voluntary health insurance
Product(s) (up to 50 words):	Voluntary health insurance, additional health insurance - covering larger scope of insurance rights and higher standard of medical services, supplemental health insurance - covering the costs provided by the Regular Health Insurance Act (extra payments)

GENOS

Organization Information	
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URL:	www.genos.hr
key words (up to 10):	DNA laboratory, SME, molecular genetics, glycomics, forensic, expertise, research & development, EU projects
Product(s) (up to 50 words):	Human DNA Tests: Paternity Test, Coagulation Factors DNA Test, Chromosomal Abnormality Test, Non-invasive Prenatal Sex Determination, Lactose Intolerance DNA Test, Secretor Status DNA Test, Animal DNA Tests: Dogs DNA Tests, Cattle DNA Test, Birds DNA Sex Determination

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URL:	www.inel-mt.hr
key words (up to 10):	Distribution, exclusive distibutorship, representative for Croatia, devices, consumables
Product(s) (up to 50 words):	Forensic devices, kits, HID kits, genetics, anthropology, scientific devices, laboratory equipment, life science equipment and consumables

NOVARTIS

Organization Information	
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Country:	Croatia
Phone:	385 1 6274 220
Fax:	385 1 6274 255
E-Mail:	
URL:	
key words (up to 10):	Right treatment to the right patient at the right time!
Product(s) (up to 50 words):	Aclasta, Afinitor, Aredia, Certican, Co-Diovan, Comtan, Cubicin, Diovan, Emselex, Estradot, Eucreas, Exelon, Exforge, Exforge HCT, Exjade, Femara, Galvus, Gilenya, Glivec, Jakavi, Jalra, Lamisil, Leponex, Lescol XL, Lioresal, Lucentis, Myfortic, Navoban, Onbrez Breezhaler, Rasilez, Sandimmun, Sandimmun Neoral, Sandostatin, Sandostatin Lar, Sebivo, Signifor, Simulect, Stalevo, Syntocinon, Tasigna, Trileptal, Votubia, Xolair, Zomarist, Zometa

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8th ISABS Conference in Forensic, Anthropologic and Medical Genetics
and Mayo Clinic Lectures in Translational Medicine
June 24—28, 2013, Split, Croatia

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and Mayo Clinic Lectures in Translational Medicine
June 24—28, 2013, Split, Croatia

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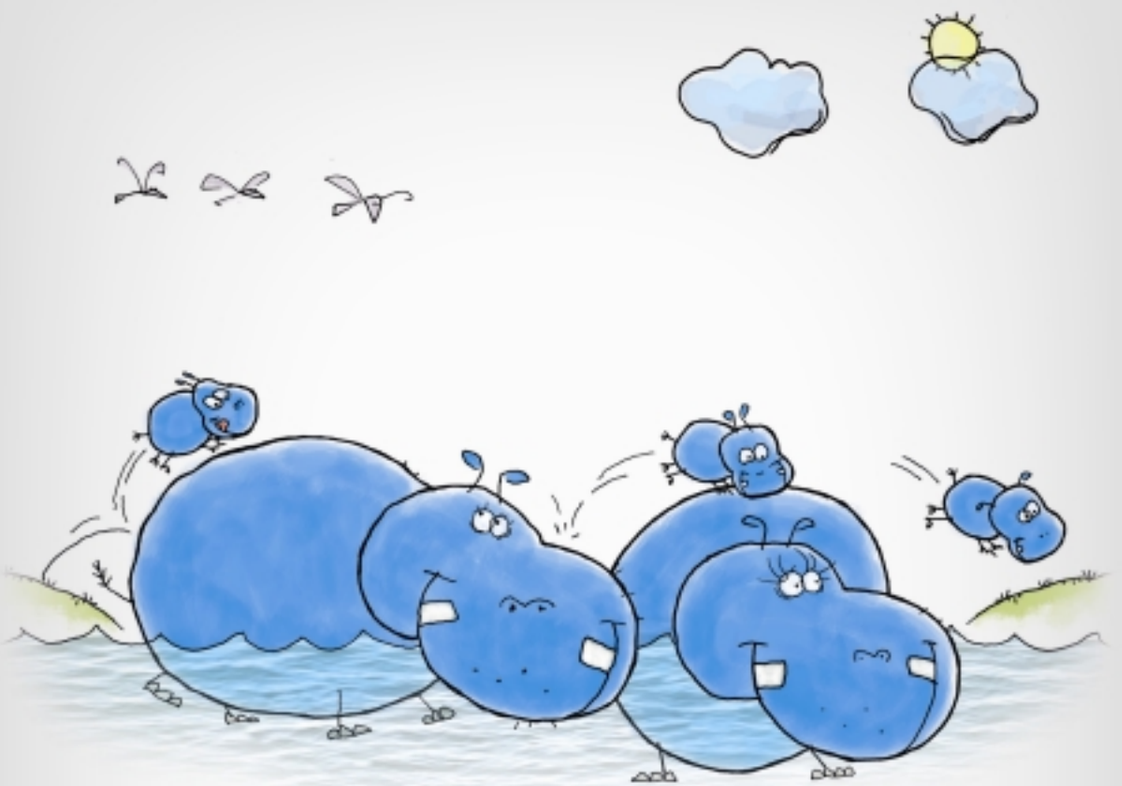

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