ISABS

7th

MAYO CLINIC

FINAL PROGRAM AND ABSTRACTS

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

JUNE 20-24, 2011
Bol, island of Brač
CROATIA

www.isabs.hr info@isabs.hr

CONFERENCE ORGANIZED BY:

ISABS

member of FUTURE NETWORK IN COMMUNE BONUM

MAYO CLINIC

ORGANIZED UNDER THE AUSPICES OF:

Croatian Academy of Science and Arts

ACADEMIA SCIENTIARUM ET ARTIUM CROATICA MDCCCLXI

GENERAL SPONSOR:

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OFFICIAL JOURNAL:

CMJ CROATIAN MEDICAL JOURNAL

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CROATIAN SOCIETY OF HUMAN GENETICS

St. Catherine HOSPITAL

EUROPEAN SCIENCE FOUNDATION

Genos DNA in your hands

svjetlost klinika

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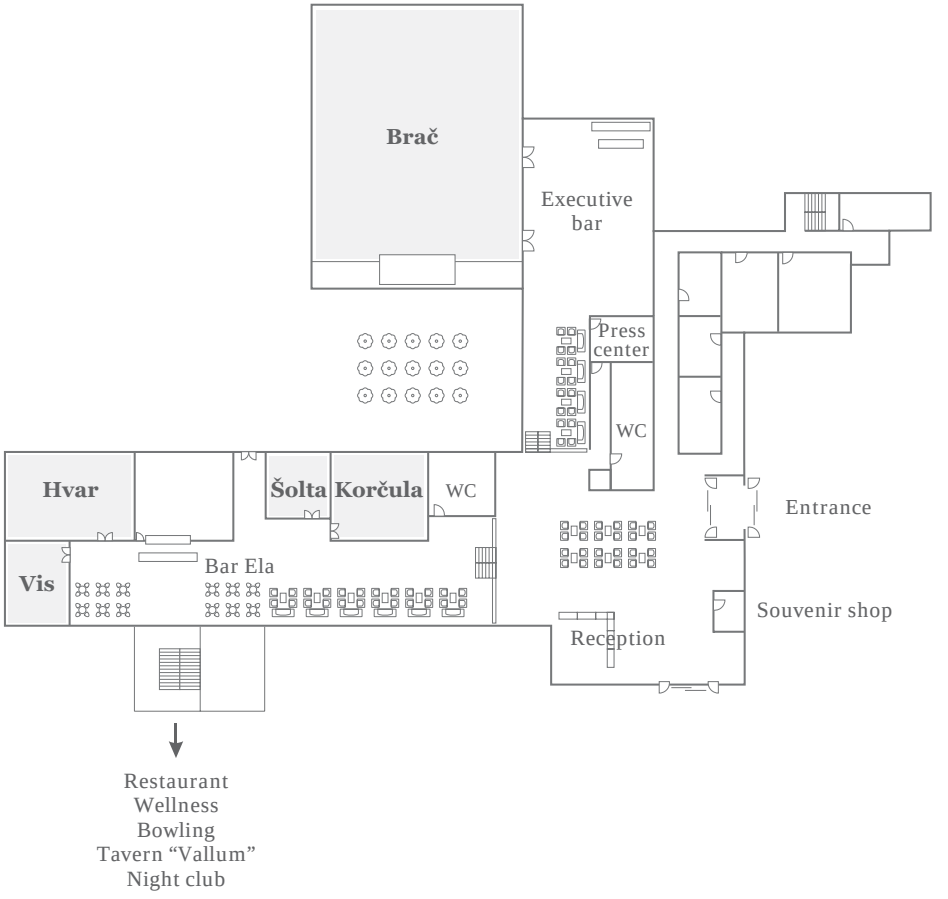
Video and/or audio-taping of any session is not permitted without prior approval from the speakers and from the scientific committee of the 7th ISABS Conference in Forensic, Anthropologic and Medical Genetics and Mayo Clinic Lectures in Translational Medicine


Brač
Executive bar
Press center
WC
Hvar
Šolta
Korčula
WC
Vis
Bar Ela
Entrance
Souvenir shop
Reception
Restaurant
Wellness
Bowling
Tavern "Vallum"
Night club

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Welcome note

Dear Colleagues,

Welcome to the 7th 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 the promotion of applied molecular biology (www.isabs.hr).

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. However, 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 we decided to include the newest and most interesting topics related to the molecular medicine.

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, a day trip and other social occasions that are meant to enhance opportunities for scientific intercourse, but also to introduce the participants to the town of Bol, one of the best known tourist destinations in Croatia due to its unspoiled nature, beautiful beaches, cultural and historical attractions and well developed tourist and sport offers.

Enjoy!

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

CONFERENCE ORGANIZER

Organizer:

International Society for Applied Biological Sciences
e-mail: info@isabs.hr
http://www.isabs.hr

Conference is organized under the auspices of the Croatian Academy of Science and Arts

Program/Conference Directors:

Moses Schanfield, George Washington University, Washington, DC, USA
Dragan Primorac, University of Split and University of Osijek, Croatia; The Pennsylvania State University and University of New Haven, USA
Stanimir Vuk-Pavlović, Mayo Clinic College of Medicine, Rochester, MN, USA

Program Committee:

Gordan Lauc (Genos, Ltd, DNA Laboratory, Zagreb, Croatia)
Damir Marjanović (Institute for Genetic Engineering and Biotechnology (INGEB), Sarajevo, Bosnia and Herzegovina and Genos and Forensic DNA Laboratory, Zagreb, Croatia)
Pavao Rudan (University of Zagreb, Zagreb, Croatia)
Stanimir Vuk-Pavlović (Mayo Clinic College of Medicine, Rochester, MN, USA)

Scientific Committee:

Forensic Genetics:
Antonio Alonso (National Institute of Toxicology and Forensic Sciences, Madrid, Spain)
Zoran Budimlija (Office of Chief Medical Examiner, New York, NY, USA)
Cecelia Crouse (Palm Beach Sheriff's Office, West Palm Beach, FL, USA)
Jürgen Henke (Institut für Blutgruppenforschung, Köln, Germany)
Mitchell Holland (The Pennsylvania State University, USA)
Henry Lee (University of New Haven, West Haven and Connecticut Forensic Science Laboratory, Meriden, CT, USA)
José Antonio Lorente (Pfizer - University of Granada & Andalusian Government Centre for Genomics & Oncology, Granada, Spain)
Marilyn Menotti-Raymond (National Cancer Institute, Frederick, MD, USA)
Antti Sajantila (Department of Forensic Medicine, University of Helsinki, Helsinki, Finland)

Molecular and Cellular Medicine:
Henry Erlich (Roche Molecular Systems, Inc., Alameda, CA, USA)

Francis Glorieux (Genetics Unit, Shriners Hospital for Children and McGill University, Montreal, QC, Canada)
Robert Huber - Nobel Laureate 1988 (Max Planck Institute for Biochemistry, Martinsried, Germany)
Doron Lancet (Weizmann Institute of Science, Rehovot, Israel)
Gordan Lauc (Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia)
Pier Franco Pignatti (Institute of Biology and Genetics and Faculty of Medicine and Surgery, University of Verona, Italy)
Richard J. Roberts - Nobel Prize 1993 (New England Biolabs, Ipswich, MA, USA)
Igor Rudan (University of Edinburgh Medical School, Scotland, UK)
David I. Smith (Mayo Clinic College of Medicine, Rochester, MN, USA)

Molecular Anthropology:
Pavao Rudan (Institute for Anthropological Research, Zagreb, Croatia)
Peter Underhill (Stanford University Medical Center, Stanford, CA, USA)

Local Organizing Committee:

Šimun Anđelinović
Ivana Erceg Ivkošić, Chair
Ante Ivkošić
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Inga Marijanović
Damir Marjanović
Petar Projić
Duje Rako - webmaster
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Josip Madunić
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Igor Matić
Marija Mazor
Zrinka Romić
Jerko Štambuk
Ante Vulić

Official service agency of the conference:

VenEvent
Gundulićeva 3, 10000 Zagreb, Croatia
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fax ++385 (1)48 200 49
isabs2011@venevent.com
www.venevent.com
ID Code: HR-A-01-080443208

ISABS Committees

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

President: **Ivana Erceg-Ivkošić** (Clinical Hospital "Sveti Duh", Zagreb, Croatia)
Vice President: **Inga Marijanović** (University of Zagreb, Faculty of Science, Zagreb, Croatia)
General Secretary: **Vedrana Škaro** (Genos, Forensic DNA Laboratory, Zagreb, Croatia)

ISABS Conferences Founding Members:

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

Scientific Committee:

Forensic Genetics:

Antonio Alonso (National Institute of Toxicology and Forensic Sciences, Madrid, Spain)
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Zoran Budimlija (Office of Chief Medical Examiner, New York, NY, USA)
Cecelia Crouse (Palm Beach Sheriff's Office, West Palm Beach, FL, USA)
Jürgen Henke (Institut für Blutgruppenforschung, Köln, Germany)
Mitchell Holland (The Pennsylvania State University, USA)
Henry Lee (University of New Haven, West Haven and Connecticut Forensic Science Laboratory, Meriden, CT, USA)
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David I. Smith (Mayo Clinic College of Medicine, Rochester, MN, USA)

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Pavao Rudan (Institute for Anthropological Research, Zagreb, Croatia)
Peter Underhill (Stanford University Medical Center, Stanford, CA, USA)

Course Committee:

Frederick Bieber (Harvard Medical School and Birgham and Women's Hospital, Boston, MA, USA)
Damir Marjanović (Institute for Genetic Engineering and Biotechnology Sarajevo, Sarajevo, B&H and Forensic DNA Laboratory, Zagreb, Croatia)
Timothy Palmbach (The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA)
Thomas Parsons (International Commission on Missing Persons, Sarajevo, B&H)

ISABS Young Investigator Programme Committee:

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Edwin Huffine (Bode Technology Group, Lorton, VA, USA)
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Daniel Vanek (Forensic DNA Service, Prague, Czech Republic)
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Ivana Šamija Projić (Clinical Hospital Center Zagreb, Zagreb, Croatia)
Vedrana Škaro (Genos, Forensic DNA Laboratory, Zagreb, Croatia)

Students Committee:

Ante Vulić (Medical School, University of Zagreb, Zagreb, Croatia)
Ante Mihovilović (Medical School, University of Split, Split, Croatia)

The Young Investigator Awards

Recipients of the 2011 Young Investigator Awards

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

Recipients of the 2009 Young Investigator Awards

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

Recipients of the 2007 Young Investigator Awards

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

Recipients of the 2005 Young Investigator Awards

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

Recipients of the 2003 Young Investigator Awards

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

Recipients of the 2001 Young Investigator Awards

Forensic IdentityTesting: Frontiers in Molecular and Cellular Medicine:

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

Scientific Program Information

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

The Young Investigator Awards
The members of the scientific board reviewed all submitted abstracts and four selected authors will receive Young Investigator Award. Recipients of the award will receive 500 € as well as a special certificate.

Credits
The 7th ISABS Conference in Forensic, Anthropologic and Medical Genetics has been approved for 20 (participants) or 25 (lecturers) points by the Croatian Medical Chamber. CMC Credits are intended for medical doctors, members of Croatian medical Chamber, in order to extend their medical doctor's license. CMC credits are valid for all other MD's as well according to their national medical chamber's policy.

Sponsor Exhibition
Setup: Sunday, June 19, 2011, 18:00 – 20:00
Monday, June 20, 2011, 08:00 – 11:00
Tuesday, June 21, 2011, 08:30 to 20:00
Thursday, June 23, 2011, 08:30 to 20:00
Dismantling: Friday, June 24, 2011, 12:30 to 14:00

Poster Setup
Sunday, June 19, 2011, 18:00 – 20:00
Monday, June 20, 2011, 08:00 – 11:00
Poster board numbers can be found in the author's index. The staff at the registration and info desk will help you in finding both the number and location of the board.

Poster Discussion
Monday, June 20, 2011, 18:20 – 19:30 Posters discussion (even numbers)
Thursday, June 23, 2011, 18:10 – 19:30 Posters discussion (odd numbers)

If you or a co-author will not be able to be at your board at this time, please leave a note on your poster stating date and time when you will be present.

Wine and cheese meeting with speakers
Monday, June 20, 2011, 19:30 – 21:30
Thursday, June 23, 2011, 19:30 – 21:30

In order to make speakers more available to the participants we invite you all to participate in speaker/participant program. A speaker will be assigned a table (tables will be scattered around the various venues) so that the participants can come and talk and move around to various speakers.

Poster Removal
Friday, June 24, 2011, 12:30 to 14:00

Program Changes
The organizers cannot assume liability for any changes in the program due to external or unforeseen circumstances.

Registration Desk Opening Hours
Sunday, June 19, 2011 18:00 – 20:00
Monday, June 20, 2011 08:00 – 20:00
Tuesday, June 21, 2011 08:00 – 16:00
Wednesday, June 22, 2011 08:00 – 16:00
Thursday, June 23, 2011 08:00 – 16:00
Friday, June 24, 2011 08:00 – 16:00

Language
The official language of the conference is English (no simultaneous translation).

Slide and PowerPoint Preview Room
A slide and PowerPoint preview room will be available to all presenters.

Message Center
A Message Center will be available at registration desk.

Service Center
The following services will be available at cost of the Service Center: photocopying, typing, and computer printouts.

Smoking Policy
The 7th ISABS Conference in Forensic, Anthropologic and Medical Genetics and Mayo Clinic Lectures in Translational Medicine is officially declared as a "Non-smoking-Conference".

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

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

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

General Information

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

Bank Services
The official currency in Croatia is the Croatian kuna (HRK). To see current exchange rates provided by Croatian National Bank. The official exchange rates:
1 EUR = 7, 44 HRK* (June 8, 2011)
1 USD = 5, 07 HRK* (June 8, 2011)
* Please note that exchange rates are variable

Opening hours of official Banks
Banks and post offices are usually opened from 8:00 a.m. to 7:00 p.m., Monday through Friday and from 8:00 a.m. to 12:00 p.m. on Saturdays.

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

Electricity Supply
220-240 V, 50 Hz.

Insurance
Participants need to make their own arrangements pertinent to health and travel. By registering for the *7th ISABS Conference in Forensic, Anthropologic and Medical Genetics and Mayo Clinic Lectures in Translational Medicine*, participants agree that neither the organizers and its agents nor the sponsors and exhibitors nor the Bluesun hotel ELAPHUSA, Bol assume any liability whatsoever.

Restaurants
Most restaurants in Bol are opened from 8:00 a.m. – 11:00 p.m. 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
Shops in Bol are usually opened from 8:00 a.m. – 9:00 p.m., Monday to Friday, and from 8:00 a.m. – 3:00 p.m. on Saturdays. Some are opened on Saturdays afternoon. Most shops accept major credit cards.

Taxi
Numerous Taxi stands are located throughout Bol city centre and in front of the hotels. All receptionists will be glad to help you.

Hotel Information
Mornings bathed in sunlight, the scent of Mediterranean herbs and the shade of hundred-year-old pine trees – this is a dream that comes true in Bluesun Hotel Elaphusa, 4-star hotel, the leading wellness & spa hotel in Croatia.
Grand Class Centre at the Bluesun Elaphusa Hotel covers more than 4, 000 m2 of indoor and outdoor space and belongs to one of the largest wellness centers on the Adriatic.
Luxurious and spacious rooms, the Hotel's many amenities and superior service are the main characteristics of this supreme hotel.
The Hotel is located approximately 50 meters from the sea.

INVITED SPEAKERS

Christopher Asplen (Gordon Thomas Honeywell Governmental Affairs, Washington, DC and DNA 4 Africa, Chalfont, PA, USA)

Dušica Babović-Vuksanović (College of Medicine, Mayo, Clinic, Rochester, MN, USA)

Zwi Berneman (University of Antwerp, Antwerp, Belgium)

Frederick Bieber (Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA)

Nicolai Bovin (Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation)

Malcolm Brenner (Baylor College of Medicine, Houston, TX, USA)

Zoran Budimlija (Office of Chief Medical Examiner, New York, NY, USA)

Theresa Caragine (Office of Chief Medical Examiner, New York, NY, USA)

Filippo Castiglione (NRC Institute for Computing Applications, Rome, Italy)

Katja Drobnič (Faculty of Criminal Justice and Security, UM and National forensic laboratory, MNZ Slovenia)

Jakov Dulčić (Institute of Oceanography and Fisheries, Split, Croatia)

Moran Elishmereni (Institute for Medical BioMathematics, Bene Ataroth, Israel)

Henry Erlich (Roche Molecular Systems, Pleasanton, CA, USA)

Christopher Evans (Center for Advanced Orthopaedic Studies, Harvard Medical School, Boston, MA, USA)

Matthew Ferber (College of Medicine, Mayo Clinic, Rochester, MN, USA)

Dennis Gastineau (College of Medicine, Mayo Clinic, Rochester, MN, USA)

Arezou Ghazani (Harvard Medical School and Massachussetts General Hospital, Boston, MA, USA)

Esther Guetta (Danek Gertner Institute of Human Genetics, Sheba Medical Center, Tel-Hashomer, Israel)

Karin HaLevi (Institute for Medical BioMathematics, Bene Ataroth, Israel)

Carol Henderson (Stetson University College of Law, Gulfport, FL, USA)

Thierry Hennet (Institute of Physiology, University of Zürich, Zürich, Switzerland)

Mitchell Holland (Eberly College of Science, Penn State University, PA, USA)

Edwin Huffine (Bode Technology Group, Springfield, VA, USA)

Stipan Jonjic (Faculty of Medicine, University of Rijeka, Rijeka, Croatia)

Sree Kanthaswamy (California National Primate Research Center, University of California, Davis, CA, USA)

David Kaye (Eberly College of Science, Penn State University, PA, USA)

Manfred Kayser (Erasmus MC - University Medical Center Rotterdam, Rotterdam, Netherlands)

Ofer Klein (Rebecca Sieff Medical Center and BioCep, Ltd., Safed, Israel)

Jean-Pierre Kocher (College of Medicine, Mayo Clinic, Rochester, MN, USA)

Yuri Kogan (Institute for Medical BioMathematics, Bene Ataroth, Israel)

Gunnar Kvalheim (Norwegian Radium Hospital, University of Oslo, Oslo, Norway)

Doron Lancet (The Weizmann Institute of Science, Rehovot, Israel)

Gordan Lauc (Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia)

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

Hakon Leffler (Lund University Hopsital Sölvegatan, Lund, Sweden)

Henning Madry (Saarland University Hospital and Saarland University Faculty of Medicine, Homburg, Germany)

Damir Marjanović (Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina and Genos, Zagreb, Croatia)

Boris Martinac (Victor Chang Cardiac Research Institute, Sydney, NSW, Australia)

Marilyn Menotti-Raymond (National Cancer Institute, Frederick, MD, USA)

Timothy Palmbach (The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA)

Giorgio Palù (University of Padova, Padova, Italy)

Jef Pinxteren (ReGenesys, Heverlee, Belgium**)**

Giulio Pompilio (University of Milan, Milan, Italy)

Dragan Primorac (University of Split and University of Osijek, Croatia; The Pennsylvania State University and University of New Haven, USA)

Erdmann Rapp (Max Planck Institute for Dynamic of Complex Technical Systems in Magdeburg, Germany)

Pauline Rudd (The National Institute for Bioprocessing Research and Training, Dublin, Ireland)

Antti Sajantila (University of Helsinki, Helsinki, Finland)

Moses Schanfield (George Washington University, Washington, DC, USA)

Eliezer Shalev (Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel)

Stavit A. Shalev (Human Genetic Center, Ha'emek Medical Center, Afula and Technion-Israel Institute of Technology, Haifa, Israel)

Artemis Simopoulos (The Center for Genetics, Nutrition and Health, Washington, DC, USA)

David I. Smith (College of Medicine, Mayo Clinic, Rochester, MN, USA)

Daniela Steinberger (Institute of human genetics, Justus Liebig University, Giessen and biologis, Frankfurt am Main, Germany)

Mark Stoneking (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany)

Ron Tepper (Sapir Medical Center, Kfar Saba, Israel)

Andre Terzic (College of Medicine, Mayo Clinic, Rochester, MN, USA)

Carmen Terzic (College of Medicine, Mayo Clinic, Rochester, MN, USA)

Peter Underhill (Stanford University, Stanford, CA, USA)

Daniel Vanek (Forensic DNA Service, Prague, Czech Republic)

George Vasmatzis (College of Medicine, Mayo Clinic, Rochester, MN, USA)

Richard Villems (University of Tartu and Estonian Biocentre, Tartu, Estonia)

Samuel Volchenboum (University of Chicago, Chicago, IL, USA)

Stanimir Vuk-Pavlović (College of Medicine, Mayo Clinic, Rochester, MN, USA)

Eske Willerslev (University of Copenhagen, Copenhagen, Denmark)

George Wu (University of Connecticut Health Center, Farmington, CT, USA)

Manfred Wuhrer (Leiden University Medical Center, Leiden, The Netherlands)

Simcha Yagel (Hadassah Medical Center, Hebrew University of Jerusalem, Jerusalem, Israel)

Kristin Young (University of Kansas Medical Center, Kansas City, KS, USA)

7TH ISABS CONFERENCE IN FORENSIC, ANTHROPOLOGIC AND MEDICAL GENETICS AND MAYO CLINIC LECTURES IN TRANSLATIONAL MEDICINE

Bluesun hotel ELAPHUSA,
Bol, Island of Brač, Croatia,
June 20-24, 2011

SCIENTIFIC PROGRAM

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

Sunday, June 19, 2011

18:00 - 20:00 Registration and Poster and Sponsors' booth Setup
(in front of the „Brač" conference room)

Monday, June 20, 2011

08:00 – 18:00 Registration
08:00 – 11:00 Poster Setup (Conference floor)

Introductory Plenary Session („Brač" conference room)

08:30 Directors' introductory remarks
08:35 **Peter Underhill** (Stanford University Medical Center, Stanford, CA, USA)
A Y chromosome perspective of post-glacial human migrations in Southeast Europe
09:05 **Doron Lancet** (The Weizmann Institute of Science, Rehovot, Israel)
Variogenomics–human individual variation as the cornerstone of next-generation genomics
09:35 **Giulio Pompilio** (University of Milan, Milan, Italy)
Cell therapy for myocardial refractory ischemia with bone-marrow derived CD133+ cells: translational path and preliminary results
10:05 Coffee Break

10:15 **Inauguration of the conference**

10:30 **Keynote Address - Manfred Kayser** (Erasmus MC - University Medical Center Rotterdam, Rotterdam, Netherlands)
Human appearance: genetic basis, DNA prediction, and forensic application
11:10 **Malcolm Brenner** (Baylor College of Medicine, Houston, TX, USA)
Implementing complex biological therapies in academic centers
11:40 **Henry C. Lee** (The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA)
DNA evidence in high profile cases
12:10 Adjourn

12:30-14:30 **Sponsor Exhibit Opening and Poster Presentation**
(Conference floor)

Parallel Session: Forensic Science 1 („Brač 2“ conference room)

15:00 Introduction from the chair (Katja Drobnič and Timothy Palmbach)
15:05 **Timothy Palmbach** (The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA)
A retrospective analysis of cold-case investigations
15:40 **Moses Schanfield** (George Washington University, Washington, DC, USA)
A review and update of forensic statistics
16:15 **Zoran Budimlija** (Office of Chief Medical Examiner, New York, NY, USA)
Chimerism and forensics–Examples and importance
16:50 **Vedrana Škaro (Selected Oral Presentation)** (Genos Ltd, Forensic DNA Laboratory, Zagreb, Croatia)
Mitochondrial DNA polymorphisms in 312 individuals of Croatian population determined by 105 probe panel targeting 61 hypervariable and coding region sites
17:05 **Katja Drobnič** (Forensic Science Centre, Ministry of Interior, Ljubljana, Slovenia)
Perspectives and pitfalls of mRNA profiling for identification of menstrual blood and vaginal secretion
17:40 **Theresa Caragine** (Office of Chief Medical Examiner, New York, NY, USA)
Estimating the number of contributors to two-, three-, and four-person mixtures containing DNA in high template and low template amounts
18:15 Discussion

Parallel Session: Workshop on Biomathematical Modeling and Cancer Immunotherapy 1 („Brač 1“ conference room)

15:00 Chairs' overview of Workshop goals (Gunnar Kvalheim and Yuri Kogan)
15:15 **Stanimir Vuk-Pavlović** (College of Medicine, Mayo Clinic, Rochester, MN, USA)
The fall and rise of immunity
15:45 **Gunnar Kvalheim** (Norwegian Radium Hospital, University of Oslo, Oslo, Norway)
Dendritic cell based cancer vaccines targeting tumor stem cells
16:15 **Yuri Kogan** (Institute for Medical BioMathematics, Bene Ataroth, Israel)
Improving alloreactive CTL immunotherapy for malignant gliomas using a mathematical model
16:45 **Malcolm Brenner** (Baylor College of Medicine, Houston, TX, USA)
Increasing safety and practicality of cell-based immunotherapy
17:15 **Filippo Castiglione** (NRC Institute for Computing Applications, Rome, Italy)
Mathematical modeling and optimal control of dendritic cell transfection cancer immunotherapy
17:45 Discussion

18:20-19:30 **Poster presentation I** (Conference floor)

19:30-21:30 **Meet the Professor I, wine and cheese** (swimming pool)

Tuesday, June 21, 2011

Parallel Session: Molecular Medicine 1 („Brač 1“ conference room)

08:30 Introduction from the chair (Dušica Babović-Vuksanović and Henry Erlich)
08:35 **Dušica Babović-Vuksanović** (College of Medicine, Mayo, Clinic, Rochester, MN, USA)
Clinical genetics in individualized medicine
09:10 **Mark Stoneking** (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany)
High-throughput sequencing of complete human mtDNA genomes: insights into population history and heteroplasmy
09:45 **Henry Erlich** (Roche Molecular Systems, Pleasanton, CA, USA)
HLA polymorphism, disease susceptibility, and pharmacogenetics
10:20 **Renato Polimanti** (YIA)(University of Rome "Tor Vergata", Rome, Italy)
Role of the glutathione S-transferase gene polymorphisms as markers in complex diseases
10:35 **Pauline Rudd** (The National Institute for Bioprocessing Research and Training, Dublin, Ireland)
Systems glycobiology: From genome to glycome an integrated strategy for identifying and screening potential clinical markers
11:10 **Christopher Evans** (Center for Advanced Orthopaedic Studies, Harvard Medical School, Boston, MA, USA)
Gene therapy for bone and cartilage healing
11:45 Discussion

Parallel Session: Workshop on Legal and Ethical Aspects of DNA Forensic Analysis („Brač 2“ conference room)

08:30 Introduction from the chair (Edwin Huffine and Frederick Bieber)
08:35 **Frederick Bieber** (Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA)
Forensic analysis of DNA data banks: process, progress, and prospects
09:10 **David Kaye** (Eberly College of Science, Penn State University, PA, USA)
Kinship Matching with DNA Databases (aka “Familial Searching”): Legal and Ethical Arguments
09:45 **Christopher Asplen** (Gordon Thomas Honeywell Governmental Affairs, Washington, DC and DNA 4 Africa, Chalfont, PA, USA)
International perspectives on forensic DNA databases
10:20 **Edwin Huffine** (Bode Technology Group, Springfield, VA, USA)
Increasing role of forensic DNA testing in legal/judicial process
10:55 **Carol Henderson** (Stetson University College of Law, Gulfport, FL, USA)
Legal and ethical aspects of bloodstain pattern evidence
11:30 Discussion

Parallel Session: Anthropological Genetics („Brač 2“ conference room)

15:00 Introduction from the chair (Daniel Vanek and Richard Villems)
15:05 **Eske Willerslev** (University of Copenhagen, Copenhagen, Denmark)
What is to be learned from ancient DNA?
15:35 **Daniel Vanek** (Forensic DNA Service, Prague, Czech Republic)
Famous DNA lineages from the perspective of archaeogenetics and genetic genealogy
16:05 **Šimun Anđelinović (Selected Oral Presentation)** (University Hospital Split, Split, Croatia)
Anthropological, genetic and chemical analysis of ancient skeletons from southern Croatia
16:20 **Richard Villems** (University of Tartu and Estonian Biocentre, Tartu, Estonia)
An attempt to place the Balkans on the canvas of genetic variation of West Eurasia and North Africa
16:50 **Brendan Keating** (Selected Oral Presentation) (University of Pennsylvania, Philadelphia, PA, USA)
Genome-wide 200,000 SNP array for profiling of external visible traits, kinship and ancestry
17:05 **Kristin Young** (University of Kansas Medical Center, Kansas City, KS, USA)
Basques in the genetic landscape of Europe
17:35 **Damir Marjanović** (Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina and Genos, Zagreb, Croatia)
DNA analysis of ancient skeletal remains from old Bosnian graves
17:50 Discussion

Parallel Session: Workshop on Biomathematical Modeling and Cancer Immunotherapy 2 („Brač 1“ conference room)

15:00 Introduction from the chair (Filippo Castiglione and Zwi Berneman)
15:05 **Moran Elishmereni** (Institute for Medical BioMathematics, Bene Ataroth, Israel)
A validated mathematical model of systemic IL-21 immunotherapy in solid cancers for the design of beneficial treatment regimens
15:40 **Zwi Berneman** (University of Antwerp, Antwerp, Belgium)
Dendritic cell vaccination in leukemia and AIDS
16:15 **Karin HaLevi** (Institute for Medical BioMathematics, Bene Ataroth, Israel)
A method for in-treatment personalization of cancer immunotherapy
16:50 **Samuel Volchenboum** (University of Chicago, Chicago, IL, USA)
New systems biology tools for cancer diagnostics
17:25 **Stipan Jonjić** (Faculty of Medicine, University of Rijeka, Rijeka, Croatia)
How viruses can teach us to make better vaccines and vaccine vectors
17:35 Discussion and drafting conclusions

20:00 **Welcome Reception**
(Borak Beach Restaurant, located by the sea, right below the promenade, approximately 50 meters from hotel „Elaphusa“)

20:20 **Conferring Young Investigator Awards**

Wednesday, June 22, 2011

9:00 - 16:30 Half-day excursion

18:00 **Special ISABS lecture**
Jakov Dulčić (Institute of Oceanography and Fisheries, Split)
The open sea (a movie) followed by lecture Marine Science and Croatian Naturalists: 430 Years of Adriatic Marine Science

18:30 **ISABS Annual Meeting** („Brač 2“ conference room)

Thursday, June 23, 2011

Parallel Session: Forensic Science 2 („Brač 2“ conference room)
Workshop on Legislation Pertinent to DNA Databases

08:30 Introduction from the chair (Antti Sajantila and Mitchell Holland)
08:35 **Dragan Primorac** (University of Split and University of Osijek, Croatia; The Pennsylvania State University and University of New Haven, USA)
From dust to dust: Forensic DNA analysis
09:10 **Mitchell Holland** (Eberly College of Science, Penn State University, PA, USA)
Second generation sequencing allows for mtDNA mixture deconvolution and high resolution detection of heteroplasmy
09:45 **Sree Kanthaswamy** (California National Primate Research Center, University of California, Davis, CA, USA)
Non-human forensic genetics
10:20 **Mark Barash (YIA)** (Bond University, Gold Coast, Australia)
Identification of SNPs involved in determination of facial morphology
10:35 **Marilyn Menotti-Raymond** (National Cancer Institute, Frederick, MD, USA)
Genetics of coat patterns in the domestic cat
11:10 **Antti Sajantila** (University of Helsinki, Helsinki, Finland)
Post mortem pharmacogenomics - concepts, cases and research
11:45 Discussion

Parallel Session: Advances in Noninvasive Prenatal Diagnosis in First Trimester („Brač 1“ conference room)

08:30 Introduction from the chair (Eliezer Shalev)
08:35 **Eliezer Shalev** (Human Genetic Center, Ha'emek Medical Center, Afula and Technion-Israel Institute of Technology, Haifa, Israel)
Early prenatal diagnosis–an overview
09:10 **Ron Tepper** (Sapir Medical Center, Kfar Saba, Israel)
Early fetal 3D ultrasound examination
09:45 **Stavit Shalev** (Human Genetic Center, Ha'emek Medical Center, Afula and Technion-Israel Institute of Technology, Haifa, Israel)
Genetic assessment of pregnancies with increased nuchal translucency and normal karyotype
10:20 **Esther Guetta** (Danek Gertner Institute of Human Genetics, Sheba Medical Center, Tel-Hashomer, Israel)
Application of Free Nucleic acids in Maternal Blood for Non-Invasive Prenatal Diagnosis: Techniques, Advantages and Limitations
10:55 **Simcha Yagel** (Hadassah Medical Center, Hebrew University of Jerusalem, Jerusalem, Israel)
First trimester screening for trisomy 21 with ultrasound and biochemical markers vs. fetal cell free nucleic acids in maternal serum
11:30 **Ofer Klein** (Rebecca Sieff Medical Center and BioCep, Ltd., Safed, Israel)
CEP as innovative method for isolation of fetal nucleated red blood cells for noninvasive prenatal diagnosis
12:05 Discussion

Technical Workshop („Brač 2“ conference room)

12:00-12:20 **Stephen Lupton** (Promega GmbH)
Optimization of the forensic workflow with innovations from Promega: automated sample prep and new kits for STR-Analysis
12:20-12:40 **Hellwig Daniel** (Sorenson Forensics, Salt Lake City, UT, USA)
Sorenson forensics investigative leadsm: determination of suspect genetic ancestry from crime scene evidence

Parallel Session: ESF Workshop on protein glycosylation in diagnostics and therapy („Brač 1“ conference room)

15:00 Introduction from the chair (Nicolai Bovin and Gordan Lauc)
15:05 **Nicolai Bovin** (Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation)
Diagnostic potential of antiglycan antibodies
15:35 **Thierry Hennet** (Institute of Physiology, University of Zürich, Zürich, Switzerland)
Congenital disorders of glycosylation, from genotypes to therapies

16:05 **Gordan Lauc** (Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia)
Antennary fucosylation of plasma proteins is a reliable diagnostic marker for HNF1A-MODY: Translation of a glycome-GWAS hit into a clinically useful screening tool
16:35 **Erdmann Rapp** (Max Planck Institute for Dynamic of Complex Technical Systems in Magdeburg, Germany)
Tools for the glycome analysis in the post-genomic era
17:05 **Manfred Wuhrer** (Leiden University Medical Center, Leiden, The Netherlands)
Glycosylation profiling for biomarker discovery of autoimmune and alloimune diseases
17:35 **Hakon Leffler** (Lund University Hospital Sölvegatan, Lund, Sweden)
Galectin binding glycoforms of serum proteins, a functional biomarker in cancer and inflammation
18:05 Discussion

Parallel Session: Cell Therapy („Brač 2“ conference room)

15:00 Introduction from the chair (Andre Terzic and Hennig Madry)
15:05 **Andre Terzic** (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Stem cell platforms for heart repair
15:40 **Jef Pinxteren** (ReGenesys, Heverlee, Belgium)
Adult stem cells as biologics delivery platform for ischemic injury
16:15 **Martina Smolić (YIA)** (University of Connecticut Health Center, Farmington, CT, USA; University of Osijek, Osijek, Croatia)
Low CYP2E1 gene expression permits pharmacological selection of transplanted human hepatocytes in vivo
16:30 **Pavel Čapek** (Selected Oral Presentation) (Charles University, Prague, Czech Republic)
Hypertrophic cardiomyopathy: from mutation to functional analysis of defected protein
16:45 **Dennis Gastineau** (College of Medicine, Mayo Clinic, Rochester, MN, USA)
A day at the Human Cell Therapy Laboratory, Mayo Clinic
17:20 **Henning Madry** (Saarland University Hospital and Saarland University Faculty of Medicine, Homburg, Germany)
Regeneration of cartilage defects using gene-based approaches and tissue engineering of cartilage based on genetically modified cells
17:55 Discussion

18:10-19:30 **Poster presentation II** (Conference floor)

19:30-21:30 **Meet the Professor II, wine and cheese** (Conference floor)

Friday, June 24, 2011

Parallel Session: Molecular Medicine 2 („Brač 1“ conference room)

08:30 Introduction from the chair (Christopher Evans and Giorgio Palù)
08:35 **Arezou Ghazani** (Harvard Medical School and Massachussetts General Hospital, Boston, MA, USA)
Advances in Molecular Diagnostics: Role of Nanotechnology and Future Perspectives
09:05 **Artemis Simopoulos** (The Center for Genetics, Nutrition and Health, Washington, DC, USA)
Nutrigenetics and nutrigenomics in personalized nutrition and medicine
09:35 **Carmen Terzic** (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Prediction of pathophenotype through transcriptome deconvolution
10:05 **George Wu** (University of Connecticut Health Center, Farmington, CT, USA)
A practical immunocompetent animal model of hepatitis C viral infection
10:35 **Christopher Evans** (Center for Advanced Orthopaedic Studies, Harvard Medical School, Boston, MA, USA)
Gene therapy for arthritis
11:05 **Giorgio Palù** (University of Padova, Padova, Italy)
Virus-host interactions: The role of microRNA in tumorigenesis and immune evasion
11:35 **Boris Martinac** (Victor Chang Cardiac Research Institute, Sydney, NSW, Australia)
In search of novel types of antibiotics by targeting bacterial mechanosensitive channels
12:20 Discussion

Parallel Session: NextGen Sequencing („Brač 2“ conference room)

08:30 Introduction from the chair (George Vasmatzis)
08:35 **David I. Smith** (College of Medicine, Mayo Clinic, Rochester, MN, USA)
The Next Generation Sequencing Revolution
09:00 **David I. Smith** (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Next Generation Sequencing of oropharingeal cancers
09:25 **Henry Erlich** (Roche Molecular Systems, Pleasanton, CA, USA)
Resolution of DNA mixtures and degraded DNA by Next-Generation Sequencing
09:55 **Jean-Pierre Kocher** (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Integration of multiple data types obtained from genomics experiments: challenges and opportunities
10:25 **Rebecca Just (YIA)** (Armed Forces DNA Identification Laboratory, Rockville, MD, USA; University of Maryland, College Park, MD, USA)
Investigation of next generation sequencing for mitochondrial DNA applications in forensics

10:40 **Daniela Steinberger** (Institute of human genetics, Justus Liebig University, Giessen and biologis, Frankfurt am Main, Germany)
Personal genomics services (PGS): Digitalization of expert genetic knowledge and possible consequences
11:10 **George Vasmatzis** (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Molecularly-defined cancer subtypes using NextGen Sequencing
11:40 **Matthew Ferber** (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Clinical NextGen Sequencing of hereditary colon cancer genes
12:10 Discussion

Conclusion of the Conference

Announcement of the next ISABS Conference

12:30-14:00 Poster removal

ABSTRACTS – ORAL PRESENTATIONS

INVITED LECTURES

Abstract number: ABS-349-ISABS-2011

INTERNATIONAL PERSPECTIVES ON FORENSIC DNA DATABASES

Asplen C

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Maximizing the crime fighting potential of forenisc DNA technology depends not only on the technical expertise of the forensic laboratory. What the law authorizes police to do with that evidence is critical. Legislatively authorized forensic DNA databases continue to be implemented and expanded around the world. This presentation will examine the various legislative trends occurring in different countries. Included in the analysis will be lessons learned from contrasting approaches to database establishment, international connectivity issues and recommendations for future legislative initiatives. Approaches to profile inclusion, profile retention and sample retention will be examined. Examples from six continents will be provided.

Keywords: legislation, databases, DNA, forensic, international

Suggested Reading:

1. www.dnaresource.com
2. www.DNA4Africa.org
3. www.DNASaves.org
4. www.dna.gov
5. www.forensicmag.com

Abstract number: ABS-366-ISABS-2011

CLINICAL GENETICS IN INDIVIDUALIZED MEDICINE

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The molecular genetic cause of over 3.000 monogenic disorders is currently unknown. Novel genomic techniques opened new avenues in elucidation of genetic defects causing monogenic disorders and exome sequencing already led to identification of new genes. Rapid sequencing technology has a potential to advance personalized medicine, as illustrated by recent reports of successful application of human sequence data to predict response to drugs and allow for pre-symptomatic diagnosis of treatable conditions. However, these few optimistic reports are far overweight by number of concerns raised by physicians, researchers, ethicists, and patients regarding consequences of inappropriate use of information about content of human genome that could result in distress and adverse effect to patient wellbeing. Clinical genetics has a critical role in translation of genomic information into the clinical practice. Examples will be discussed.

Keywords: human genome, whole genome sequencing, personalized medicine, medical genetics, genetic testing

Suggested Reading:

1. Nature 2008 Apr 17, 452(7189):872-6.

Abstract number: ABS-342-ISABS-2011

DENDRITIC CELL VACCINATION IN LEUKEMIA AND AIDS

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Immunization using antigen-loaded dendritic cells (DC) holds promise for the adjuvant treatment of cancer and chronic infections to control residual disease. In a phase I/II trial, we investigated the effect of WT1 mRNA-electroporated autologous DC vaccination in 17 patients with acute myeloid leukemia (AML) in remission but at high risk of full relapse. There was a return to normal of the AML-associated tumor marker following DC vaccination, compatible with the induction of molecular remission in 8/17 patients, including 2 patients in partial remission who were brought into complete remission following intradermal administration of DC. Survival in responders was significantly longer than in non-responders. Clinical responses were correlated with elevated levels of activated natural killer cells post-vaccination and long-term responses with an increase in WT1 tetramer+ CD8+ T-cell frequencies. In analogy to AML, we have performed a phase I/II vaccination trial in 6 patients with AIDS under antiretroviral therapy (HAART), using autologous monocyte-derived DC electroporated with mRNA encoding HxB2 Gag and TatRevNef fusion protein DC were injected subcutaneously and intradermally 4 times at 4 weeks interval. Immunogenicity, evaluated before, during and after immunization showed that pre-existing responses to HxB2 peptides were gradually expanded upon vaccination. Importantly, the CD8+ T-cell mediated capacity to inhibit superinfection of autologous CD4+ T cells by HIV-1 IIIB strain improved significantly after DC immunization. In conclusion, DC-based immunotherapy emerges as a feasible and effective strategy to control residual disease in AML and potentially in HIV disease.

Keywords: dendritic cell vaccination, leukemia, AIDS, immunotherapy, gene therapy

Suggested Reading:

1. Van Driessche A et al. Leukemia 2005, 19:1863-1871
2. Van Gulck ER et al. Blood 2006, 107: 1818-1827
3. Van Gulck ER et al. J Virol 2008, 82:3561-3573
4. Van Driessche A et al. Cytotherapy 2009, 11:653-668
5. Van Tendeloo VF et al. Proc Natl Acad Sci USA 2010, 107:13824-13829

Abstract number: ABS-337-ISABS-2011

FORENSIC ANALYSIS OF DNA DATA BANKS: PROCESS, PROGRESS, AND PROSPECTS

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Familial searching refers to the deliberate analysis of forensic DNA databases to indirectly identify individuals in the database who may be closely related to unidentified perpetrators who left DNA at unsolved crimes. A number of methods, including formal genetic kinship analysis, have potential for success in familial searching, both theoretically and in practice. In the U.S. the California Attorney General became the first to approve familial searching in his state. Remarkably, one of the very first applications of familial searching, using kinship analysis of the California DNA offender database, quickly led, in 2010, to the successful identification and arrest of a suspect in a major serial rape/murder investigation which had failed for over 20 years using traditional investigative methods. A second California success quickly followed in another abduction/rape investigation. The realization that another suspect charged with a long series of rapes in the East coast of the US has a brother in prison in another state after his homicide conviction has now prompted legislators in many U.S. states to study and implement new policies for familial searching. Despite it potential and demonstrated successes, familial searching of DNA data banks has stirred considerable legal and policy debate about the effects of such data mining techniques and their use in the criminal justice system. This presentation will review the current state of familial searching methods worldwide, with special emphasis on recent developments in the U.S., where policy statements and kinship searching protocols are now being developed for implementation on a broader scale.

Keywords: kinship analysis, familial searching, DNA database, DNA data bank, convicted offender

Suggested Reading:

1. Bieber FR. Brenner CH, Lazer D. Finding criminals through the DNA of their relatives. Science, 2006, 312: 1315-1316
2. Bieber FR. Turning base hits into earned runs: Improving the effectiveness of forensic DNA data bank programs, J Law Med Ethics 2006, 134: 222-233
3. Cantrell FL. Familial DNA Database Searching. Journal of Public Inquiry Spring/Summer 2010
4. Curran JM, Buckleton JS. Effectiveness of familial searches. Science &Justice 2008, 48(4):164-167
5. Weir BS, Anderson AD, Hepler AB. Genetic relatedness analysis: Modern data and new challenges. Nature Reviews Genetics 2006, 7:771–780

Abstract number: ABS-355-ISABS-2011

DIAGNOSTIC POTENTIAL OF ANTIGLYCAN ANTIBODIES

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Anti-glycan antibodies (Abs) appear in number of pathologies, the group of particular attention includes Abs to tumor-associated glycans (TAGs) such as Tn, Thomsen-Friedenreich antigens, and related ones. Anti-glycan Abs are identified in number of other pathologies, including: cancer, diabetes, neuropathies, multiple sclerosis, peptic ulcers, cold agglutination disease, paroxysmal cold hemoglobinuria, hemolytic anemia, rheumatoid arthritis, Crohn's disease, etc. Allo-antibodies against blood group antigens of AB0, P and Lewis systems are also well known. However, question about existence of auto-antibodies in healthy donors remains to be open; Development of multi-hundred glycan arrays printed on chip platform offers finally a possibility of systematic, large-population based research. The present study, in which profiles of glycan-binding Abs in a cohort of >200 healthy donors were evaluated using glycochip, reveals completely new Abs and unexpected facts of existence of formally autologic Abs to numerous antigens of RBC and EC. Particularly, we observe in all healthy donors, independently on blood group, Abs against Pk, P1, A, B and "core" motifs. Interestingly, very limited number of TAGs demonstrated binding both in cohort of healthy donors and cancer patients. However, we identified glycans with high potency of differentiation the antibodies of cancer patients vs. healthy individuals, many of them directed to core motifs of glycans. Here, we explain reasons of appearing of anti-core Abs, and report first results on cancer diagnostics based on "signatures" of glycans, i.e. combinations of 8-10 glycans specific for this type of disease.

Keywords: glycoarray, anti-glycan antibodies, glycoligands, tumor-associated glycans, glycobiology

Suggested Reading:

1. Blixt et al. Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. Proc Natl Acad Sci USA, 2004, 101, 17033-17038
2. Dyukovaet et al. Hydrogel glycan microarrays. Anal. Biochem, 2005, 347, 94-105
3. Huflejt et al. Anti-carbohydrate antibodies of normal sera: findings, surprises and challenges. Molecular Immunolology, 2009, 46, 3037-3049
4. P.S.Obukhova, N.V.Bovin. Normal human serum contains anti-alpha-Gal1-4GlcNAc antibodies. Autoimmunity Rev, 2004, 3, suppl.2, 102
5. P.Obukhova, R.Rieben, N.Bovin. Normal human serum contains high levels of anti-Gal alpha 1-4GlcNAc antibodies. Xenotransplantation, 2007 14, 627-635

Abstract number: ABS-341-ISABS-2011

IMPLEMENTING COMPLEX BIOLOGICAL THERAPIES IN ACADEMIC CENTERS

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Development of complex biological therapies in a Academic Centers

Genetically modified cells (e.g. cell vaccines, antigen presenting cells, effector T lymphocytes) may be an effective tool for treating cancer and other serious disorders, but cannot readily be fitted into the conventional drug development path. These complex biotherapies are best viewed as analogous to pre-existing cellular therapies, such as stem cell transplantation, or even to complex surgeries: in all these examples, the skills and experience of a team in an academic center are required to develop a therapy to effective fruition, one patient at a time. This presentation will use cellular biotherapies of lymphoma and childhood malignancy to illustrate how this model is developing in practice, and will focus on the problems that need to be considered and overcome in academic centers, before their undoubted potential can become therapeutic reality. These issues include the need for: 1) Extensive and prolonged funding to support the manufacturing and regulatory infrastructure during the inevitably prolonged period of therapeutic development: 2) Training of physician scientists in bench to bedside research in the available 3-4 year period they have available when real bench to bedside research usually takes 6-10 years; 3) Incorporation of iterative pre-clinical and clinical studies into the development program: 4) Developing reimbursement for experimental therapies prior to final approval. No "one size fits all" approach can address all these difficulties, but there are examples of success and failure that allow us to identify some crucial basic requirements.

Keywords: biological , therapies, implementing, academic, complex

Abstract number: ABS-347-ISABS-2011

CHIMERISM AND FORENSICS – EXAMPLES AND IMPORTANCE

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AIM: Mosaics and chimeras are organisms with genetically distinct populations of the cells, depending on the number of zygotes that they originate from. Artificial mosaics could occur during the transplantation of solid and non-solid tissues. Its importance from forensic point of view is that a chimera/mosaic could present itself as a mixture and can complicate the interpretation of genetic profiles. The goal of this project was to examine if recipients of solid tissue allograft exhibit evidence of chimerism/mosaicism in organs unrelated to their transplants and compare real casework examples related to the issue. METHODS: Complex organ transplants were examined and compared with cases from routine forensic casework. Different archival tissues were analyzed by current methods of forensic STR analysis: Phenol: Chloroform: Isoamyl Alcohol extraction, real-time PCR (Rotorgene™) for DNA quantity estimation, amplification using Identifiler ™ amplification kit (28 cycles), product separation by 3130xl genetic analyzer, followed by Genescan™ and Genotyper™ analysis. RESULTS: In all analyzed cases, there was evidence of chimerism/mosaicism in the transplanted tissues, with the presence of recipient's alleles in them, but no evidence of alleles from the transplants in the donor's tissues. CONCLUSION: The study confirmed that in the cases of post-allogenic transplantation of solid tissue organs, the mosaicism was found only in the tissues related to the transplant, while other tissues showed clean profiles of the recipient. Results were discussed and compared in the milieu of the interpretation of real life forensic cases presenting different types of natural and/or artificial mosaics/chimeras.

Keywords: chimerism, mosaicism, transplantation, mixture, forensic

Suggested Reading:

1. Castella V, Lesta Mdel M, and Mangin P One person with two DNA profiles: a(nother) case of mosaicism or chimerism, Int J Legal Med 2009, 123(5): 427-430.
2. Laura K Conlin et al. Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. Hum. Mol. Genet., Vol. 19, No. 7. (1 April 2010), pp. 1263-1275.
3. Ashira Zamir, Moshe Shpitzen, Carla Oz, Uzi Motro, Vardiela Meiner, and Ron Gafny, Presentation of a Three-Banded Allele Pattern—Analysis and Interpretation J Forensic Sci, July 2002, Vol. 47, No. 4.
4. Lion T. Summary: reports on quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection. Leukemia 2003, 17: 252–254.
5. Starzl TE, Demetris AJ. Transplantation tolerance, microchimerism, and the two-way paradigm. Theor Med Bioeth 1998, 19:441-55.

Abstract number: ABS-367-ISABS-2011

ESTIMATING THE NUMBER OF CONTRIBUTORS TO TWO-, THREE-, AND FOUR-PERSON MIXTURES CONTAINING DNA IN HIGH TEMPLATE AND LOW TEMPLATE AMOUNTS

Perez J, Mitchell AA, Nubia D, Tamariz J, **Caragine TA**

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To develop guidelines to estimate the number of contributors to two-, three-, and four-person mixtures containing either High Template DNA (HT-DNA) or Low Template DNA (LT-DNA) amounts. 728 purposeful two-, three-, and four-person mixtures composed of 85 individuals of various ethnicities with template amounts ranging from 10 to 500 pg were examined. The number of alleles labeled at each locus, and the number of labeled different and repeating alleles at each locus as well over all loci for two (HT-DNA) or three (LT-DNA) replicates were determined. Guidelines based on these data were then evaluated with 117 mixtures generated from items handled by known individuals. The number of different alleles over all loci and replicates was used to initially categorize mixtures. Ranges were established based on the averages plus and minus two standard deviations, and to encompass all observations, the maximum and the minimum values. To differentiate samples that could be classified in more than one grouping, the number of loci with four or more repeating or different alleles, which were specific to three- and four-person mixtures, were verified. Misclassified samples showed an extraordinary amount of allele sharing or stutter. These guidelines proved to be useful tools to distinguish low template and high template two-, three-, and four-person mixtures. Due to the inherent higher probability of allele sharing, four-person mixtures were more challenging. Because of allelic drop-out, this was also the case for samples with very low amounts of template DNA or extreme mixture ratios.

Keywords: DNA, mixtures, high template DNA, low template DNA, estimation

Suggested Reading:

1. Paoletti et al. Empirical analysis of the STR profiles resulting from conceptual mixtures. J Forensic Sci 2005, 50(6):1361-1366.
2. Buckleton JS, Curran JM, Gill P. Towards understanding the effect of uncertainty in the number of contributors to DNA stains. Forensic Sci Int 2007, 1(1):20-28.
3. Egeland T, Dalen I, Mostad PF. Estimating the number of contributors to a DNA profile. Int J Legal Med 2003, 117(5):271-275.
4. Haned H, Pene L, Lobry JR, Dufour AB, Pontier D. Estimating the number of contributors to forensic DNA mixtures: does maximum likelihood perform better than maximum allele count? J Forensic Sci 2011, 56(1):23-28.
5. Caragine et al. Validation of testing and interpretation protocols for Low Template DNA analysis using AmplFister Identifiler. Croat Med J 2009:50(3):250-267.

Abstract number: ABS-325-ISABS-2011

MATHEMATICAL MODELING AND OPTIMAL CONTROL OF DENDRITIC CELL TRANSFECTION CANCER IMMUNOTHERAPY

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A crucial question in clinical immunology is the following: what is the best time/dose for a certain therapeutic agent to be administered to the patient in order to decrease/eradicate the pathological condition? In cancer immunotherapies the therapeutic agent is something able to elicit an immune response against cancer. The immune response has its own dynamics that depends on the immunogenicity of the therapeutic agent and on the duration of the immune response. The question then is "how can we decide when and how much of the drug to inject so to have a prolonged and effective immune response to the cancer?". This question can be addressed in mathematical terms in two stages: first one construct a mathematical model describing the cancer-immune interaction and secondly one applies the theory of optimal control to determine when and to which extent to stimulate the immune system by means of an immunotherapeutic agent administered in discrete variable doses within the therapeutic period. The solution of this mathematical problem is described and discussed. We show that the method employed can be applied to find the optimal protocol in a variety of clinical problems where the kinetics of the drug or treatment and its influence on the physiologic/pathologic functions have been described by a system of ordinary differential equations.

Keywords: cancer, optimal control, drug scheduling, immunotherapy, autologous cells transfection

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Abstract number: ABS-321-ISABS-2011

PERSPECTIVES AND PITFALLS OF MRNA PROFILING FOR IDENTIFICATION OF MENSTRUAL BLOOD AND VAGINAL SECRETION

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Besides determination of donor of the biological material, the identification of the biological source of a stain can be of crucial importance in much forensic casework. Identification of menstrual blood and vaginal secretions can play an important role in forensic investigations of alleged sexual assaults. Since there is no conventional protein-based test available for confirming the presence of these two body fluids, we have investigated perspectives and pitfalls of a promising new method for identifying body fluids from biological stains, namely mRNA profiling. The aim of this study was to evaluate the sensitivity of menstrual blood specific mRNA - markers MMP7 and MMP11 and vaginal secretion specific mRNA-marker MUC4. Since the minimum sample size needed for unambiguous identification of those body fluids with this method had not been determined yet we showed that at least a half of a swab is needed to claim with certainty that menstrual blood is present in the biological sample and that one third is enough to confirm vaginal secretions. We will discuss the optimization of the method for avoiding misinterpretation of cross-reactivity with some other biological fluids. In addition, DNA profiling method was tested for sensitivity with the rest of the samples used for body fluid identification. The results were promising as DNA concentration was high enough in as many as a third of the samples to obtain the full profile of the donor in both types of samples.

Keywords: forensic science, mRNA profiling, menstrual blood, vaginal secretion, sensitivity

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Abstract number: ABS-350-ISABS-2011

A VALIDATED MATHEMATICAL MODEL OF SYSTEMIC IL-21 IMMUNOTHERAPY IN SOLID CANCERS FOR THE DESIGN OF BENEFICIAL TREATMENT REGIMENS

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Interleukin (IL)-21 is an attractive antitumor agent with potent immune functions. Yet thus far, the cytokine has yielded only partial responses in solid cancer patients, and settings for beneficial IL-21 immunotherapy remain unclear. We aimed to identify clinically-relevant IL-21 regimens with enhanced efficacy, using a generalized mathematical model. For this purpose, pharmacokinetic (PK) and pharmacodynamic (PD) data were acquired from a preclinical study applying systemic IL-21 therapy in murine solid cancers. We developed an integrated disease/PK/PD model for the IL-21 anticancer response, and calibrated it using selected "training" data from the experiments. The accuracy of the model was retrospectively verified under different IL-21 treatment settings, by comparing its predictions to independent "validation" data in melanoma and renal cell carcinoma-challenged mice ($R^2>0.90$). Simulations of the verified model provided important therapeutic conclusions: (1) Fractionating the standard daily regimen (50 μg/dose) into a twice daily schedule (25 μg/dose) is advantageous, yielding a significantly reduced tumor mass (a 45% decrease); (2) A low-dose (12 μg/day) regimen exerts an effect similar to that obtained under the 50 μg/day treatment, indicative of an equally efficacious dose with potentially reduced toxicity. Further experiments in melanoma-bearing mice confirmed both of these predictions with high precision ($R^2>0.89$), thereby validating the model also prospectively in vivo. Our PK/PD model can therefore rationalize IL-21 therapy, and pinpoint improved feasible treatment schedules. Collectively, this work highlights the value of employing mathematical modeling and in silico-guided design of tumor immunotherapy in the clinic.

Keywords: cancer immunotherapy, mathematical modeling, interleukin, melanoma, renal cell carcinoma

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Abstract number: ABS-338-ISABS-2011

ADVANCES IN MOLECULAR DIAGNOSTICS: ROLE OF NANOTECHNOLOGY AND FUTURE PERSPECTIVES

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Nanotechnology refers to the controlled creation of ultra-small devices and systems with a dimension of 1-100 nm, that exhibit novel functions associated with their size. The size-dependent functionality of nanoparticles is a key defining factor that separates them from other particles or molecules, such as DNA or monoclonal antibodies that exist in a nanometer scale yet lack the “tunable” functionality. In the recent past, nanotechnology has offered remarkable applications, ones that transcend disciplines in science and embrace many fields, including medicine and molecular diagnostics. Depending on the application, nanoparticles include semiconductor quantum dots, iron oxide and nanocrystals that have unique optical, magnetic or structural properties. When linked with biological compounds, nanoparticles can be used to target diseased cells and organs with high affinity and specificity. Owing to their unique properties, one of the prospects of nanoengineering is the development of smart diagnostic sensors and novel techniques to bring testing from the laboratory bench directly to the patient bedside. The key to this “point of care” diagnostic approach is to provide rapid, highly sensitive, portable, and low cost diagnosis and early detection to allow therapeutic and preventive regimens to be initiated. This presentation will review the recent development of nanodiagnostic sensors and portable biocompatible devices with emphasis on applications to diagnostic clinical pathology. It also will describe state-of-the-art screening nanodevices for the magnetic-based detection and characterization of scarce and minute quantities of biomarkers in cancer. New approaches and avenues that will directly impact patient care will be discussed.

Keywords: nanotechnology, nanoparticles, molecular diagnostic, point of care diagnostic, cancer

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Abstract number: ABS-326-ISABS-2011

APPLICATION OF FREE NUCLEIC ACIDS IN MATERNAL BLOOD FOR NON-INVASIVE PRENATAL DIAGNOSIS: TECHNIQUES, ADVANTAGES AND LIMITATIONS

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The undisputed presence of fetal cells and cell-free fetal DNA molecules in maternal peripheral blood and their application in non-invasive prenatal diagnosis have been the source of anticipation and enthusiasm over the last few decades for researchers and clinicians alike. However, the technical challenges are immense: mainly the rarity of fetal material in maternal blood and difficulty in definite fetal attribution. Despite these obstacles, certain aspects of noninvasive prenatal diagnosis have shifted from the research laboratory to clinical application. Most of the progress has been achieved with cell-free fetal DNA, which is less problematic than targeting whole cells. The diagnostic potential of cell-free DNA was initially limited to paternal-specific sequences, for example: Y-chromosome, paternal RhD allele in cases of maternal RhD-, as well as paternal dominant mutations. For example, our efforts led to a test for fetal sex detection based on Y-chromosome sequences starting at week 7 of pregnancy. Fetal gender is routinely detected through invasive procedures and ultrasound scanning from 14 weeks. Earlier, risk-free knowledge of fetal sex is beneficial in pregnancies at risk for X-linked disorders and aids in planning prenatal testing. This type of diagnosis is based on real-time PCR. Recently, next-generation sequencing and analysis of differential fetal-maternal methylation patterns have been incorporated into gene-dosage studies for aneuploidy detection in cell-free plasma nucleic acids. This progress will probably lead to clinical implementation of noninvasive aneuploidy testing. Conceivably, future developments enabling the differentiation between fetal and maternal sequences might enable full replacement of invasive procedures.

Keywords: noninvasive prenatal diagnosis, matermal blood, nucleic acids, real time PCR, gene-dosage

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Abstract number: ABS-356-ISABS-2011

A METHOD FOR IN–TREATMENT PERSONALIZATION OF CANCER IMMUNOTHERAPY

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Immunotherapy is a promising option in cancer treatment, yet its progress is limited by the complexity of the tumor-immune system relationships, and by the lack of predictive measurables for clinical response. Most clinical trials testing immunotherapy modalities have failed to show statistically significant benefit, but success in some individual patients was remarkable. Thus, a method by which therapeutic outcomes can be predicted on a patient-specific basis, and treatment can be adjusted to improve response of individual patients, would be highly valuable. We present a general method for personalizing immunotherapy treatment. The method uses pre-treatment and early-treatment clinical data for constructing personalized mathematical models, which describe the essential dynamic interactions in the system, namely, those between cancer progression, the relevant immune arms and the specific immunotherapy modality. Subsequently, these models are simulated to predict improved individual treatments while still in-treatment. We demonstrate the benefit of the method by its retrospective application to data of a clinical trial in which prostate cancer patients underwent cellular vaccination. Using the method we generated and validated individual models, relatively early in-treatment. These models were, then, employed for identifying improved treatment protocols, which were predicted to yield stable disease in most patients. This study demonstrated that our method can be employed for adjusting personalized protocols early in-treatment, with the potential to enhance the clinical outcomes of immunotherapy. The work proposes that a collaborative interaction between modelers and clinicians may be significant for improving the efficacy of immunotherapy. Prospective validation of the method is under way.

Keywords: immunotherapy, in-treatment personalization , personalized mathematical models, patient-specific treatment , prostate cancer cellular vaccination

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Abstract number: ABS-229-ISABS-2011

CONGENITAL DISORDERS OF GLYCOSYLATION, FROM GENOTYPES TO THERAPIES

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N-linked glycosylation is the most frequent modification of secretory proteins in eukaryotic cells. This highly conserved process is initiated in the endoplasmic reticulum, where the Glc3Man9GlcNAc2 oligosaccharide is first assembled on the lipid carrier dolichylpyrophosphate and then transferred to selected asparagine residues of nascent polypeptides. In the last 20 years, several inherited human diseases called congenital disorders of glycosylation (CDG), have been associated with deficiencies in this pathway in about 1000 patients. The detection of underglycosylated glycoproteins, like serum transferrin, by isoelectric focusing represents a simple diagnostic tool, although this test does not discriminate between the different causes of CDG. The analysis of lipid-linked oligosaccharides (LLO) in CDG cells provides a way to identify alterations of the oligomannose core biosynthesis, because a defective assembly results in the accumulation of intermediate oligosaccharide structures. Using this approach, multiple forms of CDG have been identified and characterized at the molecular level in the last ten years. This achievement has only been made possible by the application of the yeast Saccharomyces cerevisiae as a model to establish the relationship between the genotypes and phenotypes detected in human patients. To date, only two types of CDG can be treated by dietary supplementation with the carbohydrates mannose and fucose. Recently, the squalene synthase inhibitor zaragozic acid has been shown to increase dolichylpyrophosphate levels and to normalize truncated LLO in CDG cells, suggesting that manipulation of the dolichol biosynthesis pathway may represent a valuable therapeutic approach.

Keywords: glycosylation, disease, yeast, dolichol, endoplasmic reticulum

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Abstract number: ABS-320-ISABS-2011

SECOND GENERATION SEQUENCING ALLOWS FOR MTDNA MIXTURE DECONVOLUTION AND HIGH RESOLUTION DETECTION OF HETEROPLASMY

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Sanger dideoxy-terminator-based sequencing has been the technology of choice since the 1980's, and while the instruments used to separate the products of a sequencing reaction have improved over time, the chemistry and resolving power have remained relatively unchanged. However, the scientific community has now embarked on the journey of cyclic-array, hybridization-based, nanopore and single molecule sequencing, all commonly referred to as Next Generation Sequencing (NGS). So, how will NGS be applied in forensics? The answer to this question is complex, and will require careful thought and planning. For example, if we assume that STR analysis will remain the core forensic approach in the foreseeable future, how does NGS fit into this model? The development of an amplicon sequencing method using the 454 Life Sciences NGS technology may be the answer. Experiments were run in our laboratory with the 454 FLX and GS Junior instruments to evaluate the systems' ability to generate reliable sequence data from forensic STR and Y STR loci, as well as mtDNA control region sequences, all from the same sequencing reaction. The presentation we plan to give will focus on our assessment of the 454 GS Junior system when evaluating the mtDNA control region: 1) generating reliable sequence data from mtDNA amplicons, while identifying the artifacts normally encountered, 2) resolving conventional mixtures down to levels below 1:250 (0.4%), and 3) identifying low-level heteroplasmic variants in single source samples that can be used to enhance the discrimination potential of mtDNA testing.

Keywords: forensic science, 454 Life Sciences, human mitochondrial DNA, STR, second generation sequencing

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Abstract number: ABS-377-ISABS-2011

THE INCREASING ROLE OF FORENSIC DNA TESTING IN LEGAL/JUDICIAL PROCESS

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Forensic DNA testing has increased assisted nations in helping to convict the guilty as well as to exonerate the innocent. Many lessons regarding the use of DNA results have been learned and the impact of forensic DNA testing has not only altered legislation by always increasing the scope of DNA sample collection and testing, but also has impacted and modified a variety of laws. The introduction of forensic DNA technology not only holds individuals accountable to the government, but also the government more accountable to the people. In doing this, forensic DNA testing technology assists nations in creating a more open and fair legal-judicial system, results in better governance, and helps to foster increased societal confidence in the government.

Keywords: DNA, legal, judicial, governance, rule of law

Abstract number: ABS-375-ISABS-2011

HOW CAN VIRUSES TEACH US TO MAKE BETTER VACCINES AND VACCINE VECTORS

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To avoid control by various immune control mechanisms, many viruses encode immunoevasin proteins. NKG2D is a potent activating receptor expressed by cells of innate and adaptive immunity. It is well established that signaling via NKG2D receptor plays an important role in control of cytomegalovirus (CMV) infection. Its importance in CMV immunesurveillance is best illustrated by numerous viral evasion mechanisms evolved to avoid NKG2D. Deletion of viral genes involved in downregulation of cellular ligands for this receptor resulted in virus attenuation in vivo. In order to circumvent CMV immunoevasion of NKG2D, we generated recombinant mouse CMV expressing cellular ligand for NKG2D receptor. This resulted in profound virus attenuation in vivo and lower latent virus DNA load. Surprisingly, despite tight innate immune control, virus expressing NKG2D ligand elicited strong, long-lasting protective immunity, suggesting that similar approaches can be used for development of a safe and immunogenic CMV vaccine. The potential of this and similar types of recombinant viruses as vaccines, or vaccine vectors, will be discussed in my talk.

Keywords: viral immunosurveillance, immunoevasion, vaccines, vaccine vectors, cytomegalovirus

Abstract number: ABS-180-ISABS-2011

DEVELOPMENT OF A REAL TIME QUANTITATIVE PCR (QPCR)-BASED HUMAN-DOG-CAT SPECIES IDENTIFICATION AND DNA QUANTIFICATION ASSAY

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In a typical crime scene, human forensic biomaterial is mixed with those from dogs and cats. Knowledge of the concentration of nuclear DNA extracted from a crime scene biological sample and the species from which the sample originated is essential for DNA profiling. Accurate detection and quantification of template DNA in mixed-species samples is important when target DNA may be overwhelmed by non-target DNA. A species-specific qPCR assay for human, dog and cat DNA has been designed and validated. Human primers and probes were taken from previously published data. Dog and cat primers and probes were designed with information obtained after sequencing the MC1R region of nuclear DNA from each species. Each primer and probe was tested for species-specificity and none of them produced any ambiguity, cross-specific reactions or off-target issues. This triplex assay will enable the robust and reliable identification and quantification of minute amounts of DNA from all three species simultaneously in mixed template or homogenous samples.

Keywords: speciation, DNA quantification, qPCR, human, non-human

Abstract number: ABS-336-ISABS-2011

KINSHIP MATCHING WITH DNA DATABASES (AKA "FAMILIAL SEARCHING"): LEGAL AND ETHICAL ARGUMENTS

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Despite warnings of "a political firestorm" in the U.S., several states have adopted regulations or legislation permitting the "familial searching" of law enforcement DNA databases (generally under limited circumstances). Although kinship analysis of these DNA profiles is still uncommon, it has produced some well publicized successes and no widespread backlash. However, it has provoked a debate in the press and the legal literature over its constitutionality and fairness. Although some proponents portray it as a high-tech kind of sleuthing that will solve many cases, critics characterize it as of "lifelong genetic surveillance" of "entire families" that offends "the presumption of innocence." This talk briefly describes the logic and effectiveness of kinship matching and analyzes the major arguments advanced against the practice. It describes a system of database trawling that would make kinship matching routine rather than exceptional and defends this system from the constitutional objections.

Keywords: kinship analysis, familial searching, DNA databases, search and seizure law, racial discrimination

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Abstract number: ABS-353-ISABS-2011

HUMAN APPEARANCE: GENETIC BASIS, DNA PREDICTION, AND FORENSIC APPLICATION

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Although scientific interest in natural determination of how we look goes back a longer time, we currently know much more about genes involved in numerous diseases than we know about the genetic basis of human appearance. Finding out which genes determine human externally visible characteristics is of general anthropological and genetic interest, can provide evolutionary hints on human adaptation, may allow understanding diseases with appearance manifestation, but is also expected to improve forensic investigations. Because current forensic DNA profiling is completely comparative, it only allows the identification of persons already know to the investigating authorities while unknown people cannot be identified with this approach. If the genetic knowledge of human appearance traits indeed allows their prediction from DNA, with reasonable accuracy and using technologies suitable for analysing forensic materials, this information is expected to help police investigations to reduce the number of potential suspects in cases where the evidential DNA profile does not match that of any known suspects. This lecture will provide an overview on our current knowledge about the genetic determination and DNA-based prediction of human appearance traits including forensic applications.

Keywords: appearance, forensic DNA phenotyping, genetic anthropology, DNA prediction, physical traits

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Abstract number: ABS-376-ISABS-2011

CEP – AN INNOVATIVE NEW APPROACH FOR IMMUNOMAGNETIC CELL SEPARATION OF RARE CELLS

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Immunomagnetic based cell separation techniques have been shown to be a promising process for rare cell isolation in general and FNRBC, specifically. The major drawbacks which prevent this method to become, an efficient, low cost automated procedure are: relatively small volumes of specimen, altered cell morphology and contamination of the final specimen. Current technologies magnetically attract the marked cells to the column surface. The cells that come in contact with the surfaces sustain abrasion and damage resulting in non-viable cells and cell aggregates. In this work, we introduce the CEP (Cell Enrichment Process). The CEP represents a novel design in biomagnetic isolation systems. The CEP combines continuous flow technology with “virtual mesh” design producing unrivaled separation of specimens. As the sample flows through silicon tubing, the marked cells are held in stasis by the magnetic flux lines while the non-marked cells pass through. The significance of the closed circuit, generated "virtual mesh", is the total elimination of any surface contact with the targeted cells. Another advantage is the continuous, linear separation areas created by use of tubing as both the transport and separation vessels. The results demonstrated by NRBC isolation from 10ml maternal PBMC mixed with a different ratio of CB-RBC tagged with CD71 mAb are significantly higher yields of targeted cells after one passage. Comparison of CEP method vs. Dynal, yield the following results: The ratio of CB-NRBC to total cell was 1:1.6 compare to 1:27 (Dynal). The recovery # of cells was over 87% compare to 19% (Dynal). The CEP results in prompt new possibilities in the field of rare cell isolation.

Keywords: FNRBC, prenatal, CEP, BioCEP, isolation

Abstract number: ABS-267-ISABS-2011

INTEGRATION OF MULTIPLE DATA TYPES OBTAINED FROM GENOMICS EXPERIMENTS: CHALLENGES AND OPPORTUNITIES

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The Mayo Clinic in collaboration with IBM is developing an informatics system named BORA (Biologically Oriented Repository Architecture) to integrate clinical information with genomics data and biological relationship such as gene set and pathway information. BORA is interfaced with our Mayo's repository of clinical data that provides rich phenotypic annotations on patient's sample. The system can integrate data from public sources such as TCGA datasets. BORA is designed to support advanced genomics research activities and clinical decision support. It will enable Integrative Molecular Analysis that combines multiple molecular features to discover relationships and correlations that will lead to the development of predictive or interpretative models of disease. I will discuss the architectural design and current area of application of the BORA system at the Mayo Clinic.

Keywords: genomics, data integration, individualized medicine, biomarker discovery, functional analysis

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IMPROVING ALLOREACTIVE CTL IMMUNOTHERAPY FOR MALIGNANT GLIOMAS USING A MATHEMATICAL MODEL

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High grade gliomas (HGG), a central nervous system cancer, poses a firm challenge to oncotherapists, as no efficacious strategy to defeat this pathology has been found as yet. T cell transfusion has been suggested as a promising immunotherapeutic treatment in these tumours, but this approach still requires further clinical evidence. I will present implications of a mathematical model we developed for interactions between tumour and T cells, in order to study the CTL immunotherapy option. The tumour–immunity interactive dynamics were modelled by six ordinary differential equations describing tumour cells, T cells, secreted cytokines and immune-mediating receptors. We analyzed the model under T cell immunotherapy, exploring its general behaviour, that is, under no constraints of specific parameters. Analysis suggests that in untreated patients, the patient's own immune system is never sufficient for eliminating HGG. In contrast, infusion of T cells above a calculable level makes tumour reduction possible. The efficacy of specific regimens depends on individual parameters, such as tumour load and tumour aggressiveness (i.e., growth rate). Results further indicate the existence of a patient-specific minimum cell infusion rate (cells/h) required for tumour elimination. We suggest individual infusion rates that can attenuate HGG growth in patients with different disease parameters. Our results suggest that following T cell therapy, tumour will either be eradicated or grow uncontrollably, depending on initial size and cell infusion schedule. This work provides an insight and practical guidelines for improving the efficacy of brain cancer immunotherapy by T cell infusion, which should be further studied clinically.

Keywords: immunotherapy, mathematical model, glioma, T cell, dynamical system

Suggested Reading:

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Abstract number: ABS-339-ISABS-2011

ANTENNARY FUCOSYLATION OF PLASMA PROTEINS IS A RELIABLE SCREENING TOOL FOR HNF1A-MODY: TRANSLATION OF A GLYCOME-GWAS HIT INTO A CLINICALLY USEFUL DIAGNOSTIC MARKER

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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 performed the first genome wide association analysis of the human plasma N-glycome and identified *HNF1A* as a master regulator of plasma protein fucosylation. Since even non-coding polymorphisms in the *HNF1A* gene have clearly detectable multiple effects on plasma protein fucosylation, we hypothesized that deleterious coding mutations in *HNF1A* should have even more profound effects and that antennary fucosylation of plasma proteins could be significantly decreased in HNF1A-MODY patients. *HNF1A*-MODY was found to be associated with a significant decrease in several HPLC peaks containing mainly antennary fucosylated glycans and the increase in peaks containing mainly glycans without antennary fucose. The proportion of HPLC peak DG9 in the sum of DG8 and DG9 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. Diabetic patients with DG9/(DG8+DG9) index indicative of HNF1A-MODY were sequenced and in two probands (with clinical labels of T1DM and T2DM respectively) HNF1A mutations consistent with a diagnosis of HNF1A-MODY were found.

Keywords: maturity-onset diabetes of young, antennary fucosylation, hepatocite nuclear factor 1 homeobox A, plasma proteins, genome wide association study

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DNA EVIDENCE IN FAMOUS CASE

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The value of DNA evidence has been demonstrated in all aspects of criminal investigations. As science and technology continue to advance, the importance and the value of DNA evidence in the protection of our society will also continue to grow. However, forensic scientists do not usually make the decisions about the extent of DNA evidence involvement in criminal cases however. Police officers, detectives, crime scene investigators, or evidence technicians usually are the group involve in recognition and collection of DNA evidence at the initial investigative stages. In the litigation stages, prosecution and defense attorneys direct the utilization of DNA evidence. In the adjudicative stages, the judges control the admission and legal ruling of DNA evidence. There is no guarantee that either of these groups will sufficiently understand the potential and limitation of DNA evidence and make the proper decisions. It is also possible that the integrity of some of the members of these groups is questionable. A review and analysis of many such instances will demonstrate the complexity and sensitivity of the investigations into these kinds of case. Several famous cases, such as; JFK assassination, Vincent Forster Death, Taiwan President Shooting case, the homicide of Nicole Simpson, the kidnapping of Elizabeth Smart, the murder of Laci Peterson, Monaca and Clinton case, the Kobe Bryant sexual Assault caseand the death of Jon Benet Ramsey will be discussed. The objective of this presentation will not be to convince the audience of the truthfulness or validity of one theory vs another, but rather to clarify skeptics and cynics about some issues related to the DNA evidence in those cases. In addition, the new developments and DNA techniques could be used in investigation those cases will also discussed.

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GALECTIN BINDING GLYCOFORMS OF SERUM PROTEINS -- A FUNCTIONAL BIOMARKER IN CANCER AND INFLAMMATION

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Most serum proteins carry carbohydrate side chains, mainly as N-glycans. Changes in these glycans have long been observed in conjunction with disease such as inflammation and cancer, and in a few cases are used in routine diagnosis, but almost nothing is known on what biological roles these glycan changes have. We have now discovered that specific glycoforms of major serum proteins are bound by galectins, a family of b-galactoside binding proteins found in tissue cells. Mechanisms. Using a newly developed fluorescence anisotropy assay, it can be demonstrated that the selective binding of galectins to glycoproteins involve more specific aspects of common N-glycans and neighboring proteins parts than previously assumed. This has been studied with asialofetuin and fetuin as a model glycoproteins, e.g. showing dramatically different binding of galectin-1 compared to galectin-3, and with haptoglobin and transferrin as relevant serum glycoproteins. Functions. The binding of glycoproteins to galectins in tissue cells results in different intracellular sorting after endocytosis. This has been shown with uptake of transferrin in various cells and with uptake of haptoglobin-hemoglobin complexes in alternatively activated macrophages. Relationships to disease. Increase or decrease of specific galectin-binding glycoforms in serum correlates in different ways with diseases. An increase in galectin-1 binding glycoforms and decrease in galectin-8 binding glycoforms correlates with breast cancer, whereas the reverse is true in IGA-nephritis. Galectin-8 is the only galectin binding IgA, and galectin-8 non-bound IgA correlates with IgA-nephritis.

Keywords: N-glycan, glycoprotein, serum, biomarker, galectin

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REGENERATION OF CARTILAGE DEFECTS USING GENE-BASED APPROACHES AND TISSUE ENGINEERING OF CARTILAGE BASED ON GENETICALLY MODIFIED CELLS

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Articular cartilage defects do not regenerate. Transplantation of autologous articular chondrocytes which is clinically being performed since several decades laid the foundation for the transplantation of genetically modified cells, which may serve the dual role of providing a cell population capable of chondrogenesis and an additional stimulus for targeted articular cartilage repair. Experimental data generated so far have shown that genetically modified articular chondrocytes and MSCs allow for sustained transgene expression when transplanted into articular cartilage defects in vivo. Overexpression of therapeutic factors enhances the structural features of the cartilaginous repair tissue. Significant benefits have been also observed in preclinical animal models that are, in principle, more appropriate to the clinical situation. Finally, there is convincing proof of concept based on a phase I clinical gene therapy study in which transduced fibroblasts were injected into the metacarpophalangeal joints of patients without adverse events. To realize the full clinical potential of this approach, issues that need to be addressed include its safety, the choice of the ideal gene vector system allowing for a long-term transgene expression, the identification of the optimal therapeutic gene(s), the transplantation without or with supportive biomaterials and the establishment of the optimal dose of modified cells. As safe techniques for generating genetically engineered articular chondrocytes and MSCs are available, they may eventually represent new avenues for improved cell-based therapies for articular cartilage repair. This, in turn, may provide an important step towards the unanswered question of articular cartilage regeneration.

Keywords: gene therapy, cartilage, transplantation, chondrocytes, animal models

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DNA ANALYSIS OF ANCIENT SKELETAL REMAINS FROM OLD BOSNIAN GRAVES

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Inheritable material preserved in archaeological skeletal material represents exceptional genetic potential. Analysis of this material could greatly improve understanding of former human population and could offer interesting results. The new approaches in DNA extraction, amplification and detection led to the more successful DNA analysis of skeletal remains. While working with bones and teeth forensic scientists are usually confronted by many problems (insufficient quantity of DNA, high level of DNA degradation, presence of PCR inhibitors etc.). Therefore, careful optimization of all the stages of the procedures employed in the analysis of this kind of samples is obligatory. Over the last decade, through the mission of DNA identification of victims from the last war in Bosnia and Herzegovina, a multitude of completely unforeseen difficulties in the DNA analysis of skeletal remains were solved. Now, that experience was used in a new challenge: DNA analysis/identification of skeletal remains from the ancient mass graves located in this country. Bone fragments and teeth were collected from ancient human remains found in several archaeological sites all over Bosnia. Double extractions were performed for each sample following optimized phenol-chloroform procedures and EDTA decalcification pre-extraction step. The QuantifilerTM Human DNA Quantification Kit was used for DNA quantification. PowerPlex ESX and PowerPlex® Y System were used in analysis of extracted DNA Fragment analysis was performed on an ABI PRISM 310 genetic analyzer. Obtained results offered interesting stories regarding potential familiar relationships between analysed skeletal remains.

Keywords: ancient DNA analysis, Bosnia and Herzegovina, phenol-chloroform extraction, EDTA decalcification, PowerPlex ESX system

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Abstract number: ABS-330-ISABS-2011

IN SEARCH OF NOVEL TYPES OF ANTIBIOTICS BY TARGETING BACTERIAL MEHANOSENSITIVE CHANNELS

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Multidrug-resistance in pathogenic strains of bacteria is increasingly a problem in the treatment of bacterial infections and diseases. Many strains of enterococci have acquired resistance to Vancomycin, the last antibiotic that was still able to fight them successfully. New types of antibiotics as alternatives for the treatment of bacterial infection are urgently needed. Mechanosensitive (MS) ion channels have been found in bacteria and many studies have now been reported characterizing their properties. Disruption of MS channel function causes slowing or impairment of bacterial growth. Previous work has demonstrated that parabens (a class of antimicrobial agents) and amphipaths (a chemically unrelated class of compounds with hydrophobic and hydrophilic properties) selectively interfere with bacterial MS channels and inhibit growth by opening the channels, thereby collapsing the cell turgor and causing leakage of cytoplasmic contents. Compounds that interfere with MS channel function are therefore exciting novel agents for inhibiting growth of bacterial pathogens. An important aspect of this work is to identify agents that specifically interfere with the bacterial MscS and MscL channels but have no effect on MS channels in human or animal cells. We have extended on the parabens results and found that Eriochrome cyanine R (ECR), a triphenylmethane dye (TPM), also selectively interferes with bacterial MS channels and inhibits growth by opening these channels. Since the link with the mode of action of selective interference with bacterial MS channels has not been made yet we aim to optimise the effectiveness of these compounds and rationally develop more effective analogues.

Keywords: mechanosensitive channels, bacteria, infectious diseases, multidrug-resistance, antibiotics

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GENETICS OF COAT PATTERNS IN THE DOMESTIC CAT

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The domestic cat is unique among mammals in displaying a range of phenotypic variation for coat pattern, thus providing the opportunity to identify genes associated with mammalian pattern variation. Two years ago we reported that multiple loci are involved with pattern formation in the cat, the “tabby” locus with two alleles specifying either a striped or whorled pattern, or the “ticked” locus, which is epistatic over all pattern. Utilizing genetic association and haplotype analysis, we have identified the gene responsible for tabby pattern variation in domestic cats. Molecular evolutionary analysis in 31 other felid species identifies sequence differences in the tabby locus as the cause of phenotypic variation in one other exotic felid. Genomic expression studies in felid skin reveals localized production of a paracrine factor, distinct from any of the classic paracrine factors involved with pigment type switching, as a likely mechanism for maintaining tabby markings. These results provide a new framework for understanding and studying mammalian color patterns.

Keywords: domestic cat, coat pattern, gene, mutation, pigment type switching

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A RETROSEPCTIVE ANALYSIS OF COLD-CASE INVESTIGATIONS

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This study will look at a series of unsolved, cold-cases that occurred between 1976 and 1984. Only a small percentage of cases analyzed any biological evidence. As DNA methods were developed and became available some of the cases in which biological evidence was preserved were re-examined with DNA methods. For the remaining cases this study attempted to understand why investigators did not seek DNA testing, and what avenues did they consider to try and solve these cases. For the cases highlighted in this study we will examine the likelihood that these cases can be solved with DNA testing given the limitations of evidence recognition, collection, and preservation. Finally, what are the lessons learned regarding the best use of biological evidence and DNA testing to bring about a timely and correct resiolution to the case.

Keywords: cold-case, investigation, physical, evidence, DNA

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VIRUS-HOST INTERACTIONS: THE ROLE OF MICRORNA IN TUMORIGENESIS AND IMMUNE EVASION

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MicroRNAs (miRNAs) are noncoding RNA molecules that post-transcriptionally regulate gene expression, thus modulating multiple cellular processes including development, immunity, and oncogenesis. MiRNAs have been identified in metazoan and plant species. More recently, DNA viruses were found to encode and express miRNAs during host infection. Although the functions of most viral miRNAs are not well understood, early analysis of target genes pointed to immune modulation suggesting that viral miRNAs are a component of the immune evasion repertoire, which facilitates viral persistence. Investigation of viral and host miRNA involved in viral disease, including human cytomegalovirus disease, chronic viral hepatitis, and human papillomavirus-related cancer. miRNA microarrays analysis of gene expression profile in in vitro models of viral infection and in clinical specimens. Bioinformatic prediction miRNA targets and validation by tandem array analysis, luciferase reporter assay, and protein expression analysis. Viral and host miRNA expressed during infection are mainly involved in immune evasion and in cell proliferation: (a) human cytomegalovirus express its own miRNAs, such as miR-US25-2, that target host genes, such as interferon response genes and histone deacetylases, leading to increased viral replication and inhibition of innate immune response; (b) human papillomavirus E6 and E7 oncoproteins markedly upregulate cellular miR-146a and miR-196a, involved in immune evasion and cancer development, respectively, and inhibit tumor suppressor miR-34a, miR-145, and let-7c; (c) progression of chronic hepatitis to severe disease and cancer is characterized by changes in miRNA expression profile toward inhibition of interferon response genes and epithelial to mesenchymal transition.

Keywords: microRNA, viral infection, cancer, innate immune response, virus-host interaction

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Abstract number: ABS-378-ISABS-2011

A NOVEL APPROACH FOR PROPHYLAXIS OF ACUTE GRAFT VS HOST DISEASE USING STROMAL STEM CELL THERAPY: PRELIMINARY RESULTS AFTER SINGLE DOSE ADMINISTRATION IN A PHASE 1 TRIAL USING MULTISTEM®

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MultiStem, adherent multipotent adult progenitor cells (MAPC), are an immunomodulatory, bone marrow-derived adult adherent allogeneic stem cell product manufactured to large scale, assuring consistency of universal donor cell product during clinical evaluation. Pre-clinical studies have shown safety of IV infusion and survival benefit in a haploidentical acute GVHD rat model (Kovacsovics 2008, 2009). An open label Phase I clinical dose escalation study is being performed with the primary goal to assess safety of MultiStem as an adjunct treatment for adult hematological malignancy patients shortly after allogeneic HSCT. Patients have been enrolled for MultiStem administration as a single dose or in multiple weekly doses. Infusional toxicity and RRTs are assessed for 30 days following the last MAPC dose. Secondary endpoints include incidence of acute GVHD, infection and survival through day 100. Dose escalation is guided by the Continual Reassessment Method (CRM). In the single dose arm, 18 patients from 5 clinical centers were administered MAPC IV at 1, 5, or 10 million cells per kg at day 2 after allogeneic HSCT. There was no observed infusional toxicity. Two patients experienced Bearman RRTs (Grade 3 mucositis; Grade 3 renal and pulmonary failure) deemed unrelated to study product. Engraftment occurred in all 18 patients. The median time to neutrophil engraftment was 14 days (range, 12-16 days) and 16 days (range, 11-25 days) for matched related and matched unrelated transplants, respectively. Evaluating data available from the first 15 patients, the 100-day cumulative incidence of Grade II-IV and III-IV GVHD was 36% and 9%, respectively (n=11), with no observation of GVHD in the highest dose group (n=6). Completion of this study will determine safety of repeated MultiStem administrations within the first month following HCST. Subsequent studies will assess if stromal stem cell therapy can be harnessed as a novel therapeutic option for GVHD prophylaxis following HSCT.

Keywords: cell therapy, stem cells, GVHD, prophylaxis, transplantation

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CELL THERAPY FOR MYOCARDIAL REFRACTORY ISCHEMIA WITH BONE-MARROW-DERIVED CD133+ CELLS: TRANSLATIONAL PATH AND PRELIMINARY RESULTS

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The clinical grade application of cell therapy products needs a fully good manufacturing practice (GMP)-compliant procedure. CD133+ BMCs isolation and release process was performed according to a two-step experimental program in agreement with quality standards as well as proof of principle of their phenotypic integrity and biological efficacy. In 12 patients with untreatable angina pectoris, myocardial segments with stress-induced ischemia as assessed by SPECT were injected with 3 to 14 x 106 autologous CD133+ BMCs. Cells were injected into the myocardium (6 anterior, 3 lateral, 3 inferior wall) through minimally invasive approaches. At baseline and 1 year follow-up, stress-SPECT, 2-D echocardiography and coronary angiography were performed to assess exercise capacity, myocardial perfusion, LV function and coronary anatomy. GMP implementation of currently available protocols for CD133+ cell selection enables the production of cells that can be safely translated to patients. Intramyocardial injection of CD133+ BMCs led to a significant improvement of CCS class (from 3.8 to 1.8 at 6 months) and serial SPECT documented improvements of rest and stress perfusion in the injected territories at 6 months and 1 year from operation. In 3 cases, coronary angiography showed an increase in the collateral score of the target areas. Clinical improvements still persist unchanged in 10 out of 12 cases at a mean of 25 months postoperatively. After stand-alone CD133+ BMCs transplantation for refractory MI, we observed long-term clinical and perfusion improvements in the absence of adverse events.

Keywords: cell therapy, angiogenesis, GMP, refractory ischemia, bone marrow cells

Suggested Reading:

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FROM DUST TO DUST: FORENSIC DNA ANALYSIS

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The primary value of DNA profiling significantly increased over the last fifteen years due to introduction of short tandem repeat (STR) loci in routine paternity testing, forensic work and identification of victims of mass disaster. Different methods of forensic DNA testing (known as DNA fingerprinting) have been widely established and accepted as standard procedures of investigation. This lecture presents the experience of the last eighteen years of identification of missing persons in Bosnia and Croatia. The data show that current protocols and procedures optimized for relatively fresh bones and teeth can be used in analysis of much older samples without significant modification. Also, introduction of new technologies (e.g., miniSTR assay) can help recover information from degraded DNA samples that typically result in partial profiles and total loss of information from regular STR amplicons. This approach has already been used in the analysis of highly degraded samples like those from the victims of World Trade Center terrorist attacks and WWII. Now the use of this scientific approach has been proven even for the two millenia old bone samples. In collaboration with Roche Molecular Systems recently we used a new generation of mtDNA strips and were able to get full profiles from several century old bones even in a cases where STR amplification completely failed.

Keywords: identification, bones, STRs, Croatia, Bosnia and Herzegovina

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TOOLS FOR THE GLYCOME ANALYSIS IN THE POST-GENOMIC ERA

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Glycosylation is a post-translational modification that enriches protein complexity and function. Dysregulation of glycosylation is associated with a wide range of diseases, including cancer, diabetes, as well as congenital, cardiovascular, immunological and infectious disorders. With regard to biotechnology, proper glycosylation of biologicals is important, as deviations in glycosylation are known to be associated with adverse drug reactions and reduced therapeutic efficacy. Therefore, glycomics is a rapidly emerging field that can be viewed as a complement to other „omics“ approaches including proteomics and genomics. There is a dramatic dynamic increase in the demand for sophisticated databases and analytical tools in glycobiology respectively glycobiotechnology. However, glycomics is significantly lagging behind genomics and proteomics, mainly due to the absence of high-throughput analytical methods which can reliably quantify a multitude of glycan structures in complex biological samples. For that reason, the EU-wide collaborative project “Methods for high-throughput (HTP) analysis of protein glycosylation” is funded within the FP7 of the European Union and will be started in 2011. The project is designed to fill that technology gap through development of relevant, reliable, and affordable commercial HTP glycoanalysis tools. This modular glycoprofiling system – to be developed and established within the project – is based on three orthogonal analysis platforms (UPLC, MS and CGE-LIF). Project and analysis platforms will be presented, focussing on the glycoanalysis approach, based on multiplexed capillary gel electrophoresis with laser induced fluorescence detection (CGE-LIF) utilizing a DNA-sequencer, which shows high potential for high-throughput (HTP) glycoprofiling of glycoconjugates.

Keywords: glycosylation, glycome analysis, high-throuput, capillary gel electrophoresis, CGE-LIF

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Abstract number: ABS-204-ISABS-2011

SYSTEMS GLYCIOBIOLOGY: FROM GENOME TO GLYCOME AN INTEGRATED STRATEGY FOR IDENTIFYING AND SCREENING POTENTIAL CLINICAL MARKERS

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As more therapeutic options become available there is an increasing need for clinical markers that will provide more sensitive and specific early detection of disease. At the same time, improved technologies for monitoring disease progression and response to therapy are required. In many cases, single assays of existing biomarkers are neither sensitive nor specific enough for use as sole screening methods and in general a combination of markers is required. Many systemic diseases, particularly cancer, have been linked with many systems, from genomics to glycomics. Therefore we have developed an automated 96-well plate based strategy for identifying, quantifying and screening potential glycans released from proteins in body fluids as clinical markers. We have constructed a data base of the serum glycome of healthy controls to compare with that of clinical controls and of patients with various diseases including schizophrenia, rheumatoid arthritis, breast, ovarian, lung, stomach, prostate and pancreatic cancers and compared the specificity and sensitivity of the glycan markers with the current markers used in the clinics. Automated data analysis is subsequently fine tuned for each disease opening the way for undertaking large scale clinical trials that may prove useful for diagnosing disease and monitoring progression and therapy. Importantly, the technology has enabled links to be made from the serum glycome to individual glycoproteins, glycoprocessing pathways, signaling transduction pathways and to the genome itself, demonstrating the possibility of probing a whole system for disease associated changes and providing a deeper insight into pathogenesis.

Keywords: clinical markers, genomics, glycomics, glycoproteins, glycoprocessing

Abstract number: ABS-352-ISABS-2011

POST MORTEM PHARMACOGENETICS - CONCEPTS, CASES AND RESEARCH

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Medico-legal autopsies are performed to determined the cause(s) and manner of death in cases where the death is suspected to be unnatural. Additional investigations such as histology and toxicology are often performed rountinely.However, some of the autopsies do not reveal the cause (and subsequently the manner) of death unambigously even after thorough investigation. One type of unambiguous cases are represented by drug-related deaths, where interpretation of the results of forensic toxicology are not clear. Post mortem genotyping and the analysis of parental drug and its metabolites may prove usuful in those cases. The lecture aims to clarify the concept of post mortem pharmacogenetics, show its potential by cases, and discuss the future reseaech needed in the field.

Keywords: post mortem, forensic genetics, forensic pathology, forensic toxicology, pharmacogenetics

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1. Druid H et al. Cytochrome P450 2D6 (CYP2D6) genotyping on postmortem blood as a supplementary tool for interpretation of forensic toxicological results. For Sci Int 1999
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Abstract number: ABS-327-ISABS-2011

A REVIEW AND UPDATE OF FORENSIC STATISTICS

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Forensic statistical analysis loosely falls into two groups identification and parentage testing. Identification only uses the distribution of genotypes based on allele frequencies and the Hardy Weinberg Equilibrium, wile parentage testing relies on the laws of Mendelian inheritance and population genetics. This presentation is an extension of the 2000 Croatian Medical Journal on DNA in the courtroom. It reviews the basic theory of Mendelian inheritance and Hardy Weinberg Equilibrium and population genetics, with the addition of the concept of linkage disequilibrium, since many new markers on mtDNA, Y chromosome and X chromosome are inherited in blocks that do not recombine and represent examples of linkage disequilibrium. Forensic identification assumes that all of the loci tested are in linkage equilibrium and the joint likelihood of multiple loci can be generated by simply multiplying the individual locus genotype frequencies together. However, the addition of mtDNA, NRY and some X chromosome markers alters the internal calculations for multiple loci, which is explored. Also, the genotype frequencies for these markers do not have the same rules of inheritance and therefore they do not have the same expected genotype frequencies as seen with normal autosomal SNP or STR loci, requiring additional explanation. Finally, parentage testing in its many forms can be complicated by the addition of mtDNA, Y and X chromosome markers. This presentation brings up to date the changes in the calculation of forensic statistics created by the addition of mtDNA, Y and X chromosome markers to our regular autosomal markers.

Keywords: forensic statistics, STR testing, autosomal markers, X and Y chromosome markers, mtDNA markers

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Abstract number: ABS-340-ISABS-2011

EARLY PRENATAL DIAGNOSIS – AN OVERVIEW

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Prenatal diagnosis is aimed to provide reliable information to parents and physician on the risk of having babies affected by anomaly or genetic disorder. Early diagnosis allows, if the patient so desires, for early pregnancy termination, not to forget that early pregnancy termination is associated with decreased maternal morbidity. Currently, available tests for early prenatal diagnosis of chromosomal abnormalities are limited. Although non-invasive, most efficient screening tests, such as maternal serum screening combined with ultrasound nuchal translucency measurement and ultrasound 3D surveillance, gives only calculated risk for the abnormality and final diagnosis still requires additional invasive test. These, being chorionic villus sampling and even amniocentesis are associated with risk for abortion. Therefore, the introduction of early non-invasive tests that will yield final diagnostic results would be an important advancement in prenatal care. In the past few years, taking advantage of placental passage of circulating fetal nucleated red blood cells, CD34+ hematopoietic progenitors, trophoblasts and Cell-free fetal DNA and RNA to the maternal circulation, considerable attention has been given to this new method of prenatal diagnosis. All these modalities theoretically allow early non-invasive prenatal diagnosis of an increasing number of genetic conditions both for pregnancy management and to aid reproductive decision-making. Only time will tell, how these modalities will be implemented into routine clinical practice.

Keywords: prenatal diagnosis, nrbc, free fetal DNA, nuchal translucency, 3D ultrasound

Suggested Reading:

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Abstract number: ABS-364-ISABS-2011

GENETIC ASSESSMENT OF PREGNANCIES WITH INCREASED NUCHAL TRANSLUCENCY AND NORMAL KARYOTYPE

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Increased nuchal translucency (NT) (>3mm) in pregnancy is associated with a wide range of congenital abnormalities, including chromosomal abnormalities, structural defects (mainly cardiac), and large number of genetic syndromes. There seems to be a direct correlation between the thickness of the nuchal fold and the risk for chromosomal aberrations, ranging between 21% when the nuchal fold is 3.5-4.4 mm, to 65% if it greater than 6.5 mm. Conventional chromosomal analysis fails to reveal chromosomal abnormalities smaller than 5-10 Mb, which are presently estimated to affect significant portion, around 5-10% of these pregnancies. Using advanced technologies, such as multiplex ligation-dependent probe amplification (MLPA) for subtelomeric regions, and high-resolution comparative genomic hybridization (HR-CGH) appear as potentially useful tool for prenatal cytogenetic analysis of high-risk pregnancies. Whereas array-CGH provides significant advantages, such as a more reliable detection of subtle abnormalities, detection of abnormalities that cannot be revealed by conventional karyotype and being a rapid test, is not free of limitations. Depending of the platform used, copy number variants of unknown significance requiring additional analyses might be found, and detection of lesions leading to conditions such as azospermia of the fetus might complicate the emotional burden of the couple. Long list of monogenic disorders were found to be associated with increased nuchal translucency, and high through-put technologies are expected to develop, allowing a rapid and low-cost molecular analysis of conditions such as Noonan syndrome, skeletal dysplasias, or Spinal Muscular Atrophy and others, in these pregnancies.

Keywords: nuchal transluency, ultrasound, trisomy 21, prenatal diagnosis, genetics

Suggested Reading:

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NUTRIGENETICS AND NUTRIGENOMICS IN PERSONALIZED NUTRITION AND MEDICINE

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Studies on genetic variants and their response to diet in health and disease (Nutrigenetics) as well as studies on the role of nutrients in gene expression (Nutrigenomics) lead the way in the development of personalized nutrition and in the definition of a healthy diet and development of Novel Foods. To continue in its role as an integrator of genomic and environmental processes, Nutritional science is adjusting its focus to include the microstructure of the genome, the metabolome, the proteome, the epigenome, etc. Ultimately we will be able to understand nutritional metabolism at levels of discrimination sufficient to permit individual dietary prescription. Genetic variation bears on the development of public health policy as well as on the delivery of healthcare at the individual level. When nutritional recommendations are made, genetic variability among individuals in the population will need to be taken into consideration. If the number of individuals affected by the genetic variation becomes important for policy setting, their benefit must be considered from the standpoint of both society and the individual. As we advance our knowledge of gene nutrient interactions, society will need to create or utilize appropriate social, ethical, legal, educational, economic, agricultural and industrial (i.e. development of novel foods) frameworks to gain the benefits of such knowledge. Public health and regulatory processes will need to be established to define when genomic discoveries such as gene/nutrient disease associations are ready to be evaluated as potential tools to improve health screening and recommended dietary values.

Keywords: nutrigenetics, nutrigenomics, personalized nutrition, personalized medicine, omega-3 fatty acids

Suggested Reading:

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THE NEXT GENERATION SEQUENCING REVOLUTION

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The sequencing of the human genome was accomplished by technological advances in both technolgies to cheaply sequence DNA and in sufficiently fast computers that could all the resulting sequencs generated. The reagent costs for the sequencing of a single human genome were in excess of 200 million dollars in the year 2000. Technologies for sequencing DNA has advanced considerably in the last ten years and now advanced DNA sequencers utilize massively parallel sequencing to generate sequence data on billions of DNA molecules simultaneously. Over the past four years the sequencing output on these new machines has increased over 1, 000 fold and this has resulted in dramatic decreases in the cost of sequencing genomes. I will review the current status of sequencing and discuss what to expect in the next few years from this technology. This technology will herald the dawn of an age of individualized medicine and will introduce a group of speakers who will discuss their use of this powerful technology to address important questions i both basic research and its clinical translation.

Keywords: massively parallel sequencing, individualized medicine, genome sequencing, transcriptome sequencing, bioinformatic challenges

Suggested Reading:

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NEXT GENERATION SEQUENCING OF OROPHARYNGEAL CANCERS

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Oropharyngeal cancers are one sub-type of head and neck cancers. There are distinct etiologies responsible for the development of these cancers, one caused by smoking and drinking induced DNA damage and the other by viral-induced genomic instability. In order to better understand the alterations that occur in different oropharyngeal cancers we have been using Next Generation sequencing to characterize the transcriptional output of both oropharyngeal tumors and matched normal tissue from the same patients. We have also been analyzing genome-wide methylation in these same samples with the Illumina methylation arrays. We demonstrate the power of these technologies to characterize alterations that occur during cancer development. We have also focused on genes and pathways that are differentially targeted in oropharyngeal cancers depending upon whether they are cause by smoking damage or viral instability. We have found that there are many more genomic alterations in the oropharyngeal cancers caused by smoking than viral exposure and have identified a group of genes in multiple pathways, including those involved in DNA repair that are different in smoking-induced cancers. Finally, we demonstrate that RNAseq is a powerful clinical tool which could provide useful information for clinical management.

Keywords: oropharyngeal cancer, human papillomavirus, next generation sequencing, transcriptome sequencing, methylome microarrays

Suggested Reading:

1. Tuch BB et al. Tumor transcriptome sequencing reveals allelic imbalances associated with copy number alterations PLoS One, 2010; 19: e9317.
2. Fox S et al. Applications of ultra-high-throughput sequencing. Methods Mol Biol 2009; 553: 79-108.

Abstract number: ABS-331-ISABS-2011

HIGH-THROUGHPUT SEQUENCING OF COMPLETE HUMAN MTDNA GENOMES: INSIGHTS INTO POPULATION HISTORY AND HETEROPLASMY

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The traditional Sanger-sequencing approach for obtaining complete human mtDNA genome sequences is both time-consuming and expensive, as numerous PCR and sequencing reactions are required to obtain the full 16.5 kb sequence. Because of these limitations, the typical approach in such studies is to first determine mtDNA haplogroups via sequencing the first hypervariable segment (HV1) of the control region and/or genotyping haplogroup-informative SNPs, and then select a subset of individuals of interest for complete mtDNA genome sequencing. However, such an ascertainment introduces a bias into the resulting complete mtDNA genome sequences that can influence subsequent analyses. To avoid these limitations, we have developed and implemented a rapid, cost-effective means of obtaining complete human mtDNA genome sequences using a parallel-tagged approach and high-throughput sequencing platforms. This approach enables unbiased population samples of mtDNA genome sequences to be obtained, thereby enabling new insights into population history. Additionally, because each position is sequenced many times with high-throughput sequencing, it is possible to obtain insights into the extent and nature of heteroplasmy (the existence of multiple mtDNA types within an individual) across the entire mtDNA genome. However, it is important to distinguish true heteroplasmy from sequencing errors, and I will describe the criteria we have developed to ensure accurate distinction of sequencing errors from true heteroplasmy, as well as the new insights we have gained into mtDNA heteroplasmy.

Keywords: mtDNA, heteroplasmy, next-generation sequencing, population history, demography

Suggested Reading:

1. Gunnarsdottir ED, M Li, M Bauchet, K Finstermeier, and M Stoneking 2011 High-throughput sequencing of complete human mtDNA genomes from the Philippines Genome Research 21:1-11.
2. Gunnarsdóttir ED, MR Nandineni, M Li, S Myles, D Gil, B Pakendorf and M Stoneking 2011 Larger mtDNA than Y-chromosome differences between matrilocal and patrilocal groups from Sumatra Nature Communications 2:228.
3. Li M, A Schönberg, M Schaefer, R Schroeder, I Nasidze, and M Stoneking 2010 Detecting heteroplasmy from high-throughput sequencing of complete human mitochondrial DNA genomes American Journal of Human Genetics 87:237-249.

Abstract number: ABS-357-ISABS-2011

A Y CHROMOSOME PERSPECTIVE OF POST-GLACIAL HUMAN MIGRATIONS IN SOUTHEAST EUROPE

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Exposing the emergence of genetic diversity in contemporary populations and its distribution across the geographic landscape provides one record of pre-historic and historic population movements. Subsequent organic 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 sensitivity of haploid genomes to genetic drift and founder effects encapsulates the molecular trajectory of population formation and mingling. These phylogeographic patterns of Y chromosome genetic diversification provide a molecular perspective to migratory routes, genetic barriers and polarity of net gene flow. Environmental conditions during the time of the Last Glacial Maximum about 18.000 years ago impacted modern human populations do to events associated with contraction of peoples to climatic refugees, genetic isolation, local genetic differentiation and founder effects associated with re-settlement range expansions as landscapes exposed with retreating ice sheets. Illustrative examples concerning Europe will be presented.

Keywords: Y chromosome, phylogeography, southeast Europe, human migrations, genetics

Suggested Reading:

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Abstract number: ABS-196-ISABS-2011

FAMOUS DNA LINEAGES FROM THE PERSPECTIVE OF ARCHAEOGENETICS AND GENETIC GENEALOGY

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Historically first DNA study performed on an ancient material was the 229 base pair sequence of mitochondrial DNA from the 140 year old museum specimen of quagga skin. The aim of this study was to determine if the sample of an extinct zebra-like creature will be analyzable by the current techniques of pre-PCR era. The results of the first aDNA analysis of the human origin appeared in 1985 when S.Paabo described his successful attempt to retrieve and analyse nuclear Alu repetitive sequence family DNA from an 2400 years old Egyptian mummy of a child. The analysis has shown that pieces of mummy DNA can be cloned, sequenced and identified. The invention of PCR boosted aDNA studies, but the majority of studies stick to the sequences of the mitochondrial genome and only a minority focuses on much more difficult studies of nuclear DNA. Last decade improvements in molecular-biological technologies helped to overcome some of the restricting problems that limited the scope of analysis mainly to the mitochondrial DNA that is abundant in the mammalian cells. Especially the transfer of improvements from the field of forensic genetics (inhibitor-free DNA extraction, multiplexes, short STR amplicons, qRT-PCR) enabled to extend the testing also on the nuclear DNA, including Y-chromosome STR typing. The results from the testing of lineage markers on the famous ancient artifacts can be further used for the genealogical analysis.

Keywords: degraded DNA, ancient DNA, archaeogenetics, genetic genealogy, lineage markers

Suggested Reading:

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Abstract number: ABS-335-ISABS-2011

MOLECULARLY-DEFINED CANCER SUBTYPES USING NEXTGEN SEQUENCING

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The rapid advancement of Genomics technologies (ESTs, Microarrays, Next Generation Sequencing, Mass Spectrometry etc.) enables clinical and basic researchers to address clinically relevant questions in biological systems and model organisms that they were unable to address in the past. These high-throughput technologies are capable of profiling the genome, transciptome and proteome of normal as well as of disease cells and provide valuable molecular information that could elicit important components of underlying molecular mechanisms responsible for the disease state. However, investigators have been facing many pitfalls in the application of these technologies and the appropriate design and experimental steps required have been challenging Additionally, the complexity of the subsequent bioinformatics steps are often underestimated and the difficulty in the analysis of the massive datasets have steered many researchers away from Genomics. This talk will address how our laboratory dealt with these challenges and provide examples of clinically relevant applications were Genomics research have been successfully applied. The value of the bioinformatics algorithms developed in conjunction with these examples and the need of integrating the bioinformatics field with clinical and biological science will be discussed. Finally, my laboratory is also particularly interested in developing protocols to interrogate complex specimen sources, such as small population of cells collected by laser capture microdissection, or previously disregarded paraffin-embedded tissue.

Keywords: masive sequencing (NextGen), expression profilling, mate-pair, translocation, gene fusions

Suggested Reading:

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NEW SYSTEMS BIOLOGY TOOLS FOR CANCER DIAGNOSTICS

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Innovations in “omic” technologies have led to exciting new methods for the identification of new targets for the treatment of devastating diseases such as cancer. Yet despite an enormous amount of data, novel therapies have been slow to emerge. Recent advances indicate that a new era of personalized medicine is emerging, giving way to better methods of cancer detection, more granular stratification into risk groups, more sensitive means of gauging responses to therapy, and novel patient and tumor-specific treatments. Neuroblastoma is a common pediatric tumor that is often fatal despite aggressive chemotherapy, surgery, and radiation. Several new diagnostic and therapeutic modalities have emerged that offer hope to children suffering from this aggressive tumor. Using neuroblastoma as a paradigm, these important areas of progress will be explored. Genomic studies have the potential to identify patients at the highest risk for disease progression, providing the opportunity at diagnosis to offer alternative forms of treatment. Identification of tumor-specific antibodies and other surface markers have led to innovative modes of therapy targeted specifically at neuroblastoma cells. The recent discovery of a gene mutated in a subset of patients and the development of a drug targeting this mutation has led to clinical trials for patients harboring the altered form of this gene. Finally, emerging research is focusing on defining host-specific factors that may influence how chemotherapy and other treatments affect tumor cells. Continued advances in genome and proteome-wide technologies will further refine personalized approaches to cancer diagnostics and treatment, ultimately improving outcomes and survival.

Keywords: genomics, proteomics, personalized medicine, neuroblastoma, cancer

Suggested Reading:

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Abstract number: ABS-191-ISABS-2011

THE FALL AND RISE OF IMMUNITY

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For discussion at this workshop it is relevant that immunity in cancer patients is suppressed. Suppression not only diminishes the success of immunotherapy, but also evokes malignancy and immunity as mutually dependent and co-evolving. As the average lifespan of contemporary humans by far exceeds the span expected from allometry, the outcome of tumor–immunity co-evolution is likely to depend on age-dependent status of immunity at the time of initiation of oncogenesis. While tumor–immunity interactions during clinically non-manifest phases of malignancy are largely unknown, immune defects in manifest disease are under current and intense scrutiny. One goal of these studies is to establish the phenomenological basis for the understanding of tumor–immunity interactions within the framework of complex systems and provide the tools to affect the natural history of the system. As it is widely believed that immune cells are the natural effectors of elimination of cancer, most studies have focused on cellular components of immunity. The talk will briefly review the major components affected in/by cancer (and age): T regulatory cells characterized as $CD4^{+}CD25^{hi}$ or $CD4^{+}CD25^{+}CD127^{lo}Foxp3^{+}$; myeloid derived suppressor cells–monocytes specified as $CD14^{+}HLA\text{-}DR^{lo/neg}$; incompletely differentiated ($CD83^{neg}$) dendritic cells; and recently revealed plasmacytosis accompanying solid tumors. Finally, we will discuss how the known tumor–associated alteration of each component of cellular immunity affects other components and the natural history of disease. These relationships render themselves to mathematical modeling to describe and quantify the system, guide immune reconstitution and predict the effects of immunotherapy.

Keywords: cancer, immunotherapy, immune suppression, tumor-immunity interactions, defective immune cells

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GLYCOSYLATION PROFILING FOR BIOMARKER DISCOVERY OF AUTOIMMUNE AND ALLOIMMUNE DISEASES

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Glycosylation features of human plasma proteins are known to depend age and gender as well as genetic factors. Moreover, glycans reflect the health status of a person. Disease-related glycosylation changes are being analyzed using high-throughput (HTP) technology such as multiplexed CGE-LIF on a DNA analyzer for glycan profiling [1] and mass spectrometric IgG glycosylation analysis [2]. Results will be presented emphasizing the role of glycosylation in the (dys-)regulation of humoral immune responses in autoimmune and alloimmune diseases such as rheumatoid arthritis and fetal-maternal alloimmune thrombocytopenia (FNAIT). In addition, a shot-gun glycan microarray approach for the characterization of autoantibodies in cancer will be introduced [3].

Keywords: autoimmune disease, rheumatoid arthritis, cancer, glycosylation, mass spectrometry

Suggested Reading:

1. Ruhaak et al (2010) J Proteome Res, 9: 6655.
2. Selman et al (2010) Anal Chem, 82: 1073.
3. Lonardi et al (2010) Expert Rev Proteomics, 7: 761.
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Abstract number: ABS-351-ISABS-2011

FIRST TRIMESTER SCREENING FOR TRISOMY 21 WITH ULTRASOUND AND BIOCHEMICAL MARKERS VS. FETAL CELL FREE NUCLEIC ACIDS IN MATERNAL SERUM

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With combined screening for fetal aneuploidies with maternal biochemical markers and nuchal translucency measurement, and the identification of myriad other ultrasound "markers" for aneuploidy, the ability to identify fetuses with sufficient risk of Trisomy 21 to warrant invasive testing, has increased exponentially. These screening approaches have been widely tested and validated, and have known specificity and false-positive rates. Fetal ultrasound has many additional advantages beyond aneuploidy screening. However, ultrasound with biochemical markers screening is not without cost. Integrated screening costs ~$700.000 for each T21 fetus identified. About 4% of affected babies would be missed by screening, with estimated societal cost of ~$760.000. Not included is the emotional toll on women of spontaneous abortion of healthy screen-positive fetuses following amniocentesis. It is recognized that fetal cell-free nucleic acids are available in maternal serum. This finding and the technical advances that enable identification and analysis of fetal DNA and RNA in maternal serum, have opened potential avenues of direct diagnosis of aneuploidies from maternal plasma, very early in pregnancy. These achievements have been rapid and impressive. However, there are certain drawbacks to these techniques: the currently available degree of enrichment is insufficient, methods are very costly and not validated for clinical use, and only a few investigators dominate the field. This advance has also opened potential problems. Will widespread use of these methods lead to an eugenics approach to T21 individuals? Will these methods be abused to allow an even greater number of gender preference terminations? These and other issues remain controversial.

Keywords: aneuploidy, biochemical screening, cell free fetal nucleic acids, trisomy 21, ultrasound

Suggested Reading:

1. Tong YK et al. 2010. Noninvasive prenatal detection of trisomy 21 by an epigenetic-genetic chromosome-dosage approach. Clin chem, 56: 90-8.
2. Chiu RW et al. Maternal plasma dna analysis with massively parallel sequencing by ligation for noninvasive prenatal diagnosis of trisomy 21. Clin chem, 56: 459-63.
3. Cuckle HS et al. 2008. Contingent screening for down syndrome--results from the faster trial. Prenat diagn, 28: 89-94.
4. Fan HC et al. 2008. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing dna from maternal blood. Proc natl acad sci u s a, 105: 16266-71.
5. Fan HC, Quake SR 2010. Sensitivity of noninvasive prenatal detection of fetal aneuploidy from maternal plasma using shotgun sequencing is limited only by counting statistics. Plos one, 5: e10439.

Following abstracts were not provided:

Henry Erlich ● *Roche Molecular Systems, Pleasanton, CA, USA*
"HLA polymorphism, disease susceptibilty, and pharmacogenetics"
"Resolution of DNA mixtures and degraded DNA by Next-Generation Sequencing"

Christopher Evans ● *Center for Advanced Orthopaedic Studies, Harvard Medical School, Boston, MA, USA*
"Gene therapy for arthritis"
"Gene therapy for bone and cartilage healing"

Matthew Ferber ● *College of Medicine, Mayo Clinic, Rochester, MN, USA*
"Clinical NextGen Sequencing of hereditary colon cancer genes"

Dennis Gastineau ● *College of Medicine, Mayo Clinic, Rochester, MN, USA*
"A day at the Human Cell Therapy Laboratory, Mayo Clinic"

Carol Henderson ● *Stetson University College of Law, Gulfport, FL, USA*
"Legal and Ethical Aspects of Bloodstain Pattern Evidence"

Gunnar Kvalheim ● *Norwegian Radium Hospital, University of Oslo, Oslo, Norway*
"Dendritic cell based cancer vaccines targeting the tumor stem cells"

Doron Lancet ● *The Weizmann Institute of Science, Rehovot, Israel*
"Variogenomics: human individual variation as the cornerstone of next-generation genomics"

Daniela Steinberger ● *Institute of human genetics, Justus Liebig University, Giessen and biologis, Frankfurt am Main, Germany*
"Personal genomics services (PGS): Digitalization of expert genetic knowledge and possible consequences"

Ron Tepper ● *Sapir Medical Center, Kfar Saba, Israel*
"Early Fetal 3D Ultrasound Examination"

Andre Terzic ● *College of Medicine, Mayo Clinic, Rochester, MN, USA*
"Stem cell platforms for heart repair"

Carmen Terzic ● *College of Medicine, Mayo Clinic, Rochester, MN, USA*
"Prediction of pathophenotype through transcriptome deconvolution"

Richard Villems ● *University of Tartu and Estonian Biocentre, Tartu, Estonia*
"An attempt to place the Balkans on the canvas of genetic variation of West Eurasia and North Africa"

Eske Willerslev ● *University of Copenhagen, Copenhagen, Denmark*
"What is to be learned from ancient DNA?"

George Wu ● *University of Connecticut Health Center, Farmington, CT, USA*
"A practical immunocompetent animal model of hepatitis C viral infection"

Kristin Young ● *University of Kansas Medical Center, Kansas City, KS, USA*
"The Basques in the Genetic Landscape of Europe"

TECHNICAL WORKSHOP

Abstract number: ABS-166-ISABS-2011

SORENSON FORENSICS INVESTIGATIVE LEADSM: DETERMINATION OF SUSPECT GENETIC ANCESTRY FROM CRIME SCENE EVIDENCE

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The utilization of many worldwide DNA databases is an essential tool in modern criminal investigations. Unfortunately, when an evidentiary DNA profile does not provide a viable suspect subsequent to a database search, the investigator may be left with little forensic direction. To assist in these critical situations, Sorenson Forensics introduces Investigative LEADSM; a single nucleotide polymorphism (SNP) based DNA test designed to estimate genetic ancestry. This test utilizes 190 autosomal ancestry informative markers (AIMs), specifically chosen from public databases of over 1million SNP's to best differentiate five, genetically diverse, worldwide populations; European, West African, Southeast Asian, Indigenous American, and India Subcontinent. Determination of an individual's gender and affinity to these inferred population samples provides indication of genetic ancestry and may help estimate a suspect's physical appearance. SNP data are generated utilizing the TaqMan® Open Array® Genotyping System, fluorescence-based polymerase chain reaction (PCR) assays, to provide qualitative detection of SNP targets. Test results are analyzed with a proprietary algorithm utilizing statistical tools such as Principal Component Analysis (PCA) and frappe analysis to calculate affinity levels of an individual DNA sample toward each of the 5 reference populations. Validation data showed the Investigative LEADSM test is a viable, robust and adequately sensitive test, capable of functioning on a variety of different forensic samples and DNA extract types. We believe this test will provide law enforcement investigators valuable information regarding the genetic ancestry of potential suspects. This test can be a great benefit for solving cold cases and other criminal investigations.

Keywords: forensic, DNA, SNP, ancestry, profile

Abstract number: ABS-285-ISABS-2011

OPTIMIZATION OF THE FORENSIC WORKFLOW WITH INNOVATIONS FROM PROMEGA: AUTOMATED SAMPLE PREP AND NEW KITS FOR STR-ANALYSIS

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"No good deed goes unpunished…" goes the saying about being noticed for effective performance. The state and national DNA databasing program demonstrates increasing value to law enforcement and has subsequently seen rapid growth in workload. Development of single-amplification STR kits, multicapillary electrophoresis and automation has provided great efficiency gains over the past decade. Still, many database laboratories seek further process improvements to keep pace with the dramatic growth in submissions. Currently, sample processing and thermal cycling are two of the longest steps in the STR analysis process for databasing laboratories. The PowerPlex® 18D System has been developed to significantly reduce the STR analysis process with direct amplification and rapid PCR technology. Additionally, simplifying or removing sample preparation also reduces manipulations and saves extraction reagents. Performance and workflow impact of PowerPlex® 18D will be shared for processing of common sample types including GE/Whatman FTA® cards, Omni Swabs and Bode DNA Collectors™. Improved automated setup of the STR reaction has also been optimised with the new Identity Automation software package allowing for faster DNA purification, Quantification and Normalisation of Casework samples.

Keywords: STR, database, automation, direct-amplification, innovation

Suggested Reading:

1. Implementation of the PowerPlex® 18D System in a Databasing Laboratory, Profiles in DNA 2011.
2. Analysis of PowerPlex® Systems using the Applied Biosystems 3500 and 3500xL Genetic Analyzers, Profiles in DNA 2011.
3. Automating the DNA IQ™ System on the Biomek® 3000 Laboratory Automation Workstation, Profiles in DNA 2008.
4. High-Throughput Processing of Samples on Solid Supports Using the Slicprep™ 96 Device, Profiles in DNA 2005.
5. PowerPlex® ESX and ESI Systems Technical Tips, Profiles in DNA 2010.

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SELECTED LECTURES

Presentation number: FG 1 Abstract number: ABS-233-ISABS-2011

CONCEPT, DESIGN AND APPLICATION OF GENOME-WIDE 200, 000 SNP ARRAY FOR PROFILING OF EXTERNAL VISIBLE TRAITS, KINSHIP AND ANCESTRY

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Technologies used to create one of the main Genome Wide Association Study (GWAS) platforms encompassing upto several million SNP markers have also been used to create numerous custom products. One emerging application of such technologies, with data gleamed from GWAS, is to ascertain the kinship relationships between samples from individuals in appropriate settings such as aircraft crashes, where investigators may have limited access to primary sources of DNA from suspected deceased individuals. Compared to STRs large-scale SNP efforts allow identification of upto 4th degree relatives. Such arrays also allow ancestry determination which may have utility in assessment of anonymous samples under appropriate settings such as identification of the origins of trafficked infants. Furthermore, since 2005 GWAS have unveiled thousands of associations between SNPs and traits. Large-scale meta-analyses across larger numbers of appropriately harmonized cohorts are facilitating larger portions of the genetic variance of a number of traits to be explained. Large international efforts such as the 1000 Genomes Project has created even richer datasets and allows for finer mapping of polymorphisms which impact such traits. Recent and pending data human external visible trait (EVT) phenotypic data from GWAS now allows prediction of such phenotypes, although legislation to use such approaches is currently only permitted in Holland. In 2009 we formed the VisiGen academic consortium encompassing large established population-based studies with GWAS and phenotypes related to EVTs. Visigen has designed a genome-wide 203, 000 SNP array for application in the EVTs, kinship and ancestry prediction arena for we present pilot results.

Keywords: genomics, external visibile traits, phenotype prediction, ancestry, low copy number

Presentation number: FG 2 Abstract number: ABS-258-ISABS-2011

MITOCHONDRIAL DNA POLYMORPHISMS IN 312 INDIVIDUALS OF CROATIAN POPULATION DETERMINED BY 105 PROBE PANEL TARGETING 61 HYPERVARIABLE AND CODING REGION SITES

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Aim was to detect mitochondrial DNA (mtDNA) sequence variation in 312 Croatian individuals by the use of multiplex polymerase chain reaction (PCR) amplification of informative sites of genome and subsequent hybridization to a linear array of 105 immobilized probes targeting 61 hypervariable and coding region sites. 5-plex (amplicons from 314 bp to 444 bp) and 10-plex (amplicons from 102 bp to 183 bp) PCR amplification of the mtDNA hypervariable regions I and II (HVI and HVII), coding region (CR) and variable regions I and II (VRI anf VRII) was used. Subsequent linear array typing targeted 25 HV sites, 25 CR sites and 11 VR sites within the human mtDNA genome. mtDNA types (mitotypes) of 312 randomly selected Croatian individuals was determined. One hundred ninety seven different mitotypes were observed (145 unique). Most frequent mitotype occurred 10 times; all other mitotypes occurred 13% or less. The corresponding genetic diversity value for this database was 0.9940. Mitochondrial DNA typing with an array of immobilized 105 probes is a powerful identification tool which can be benefit to forensic DNA analysis of mass disaster remains and identity testing of single and mass graves, as well as for criminal casework testing.

Keywords: mitochondrial DNA, polymorphisms, linear array, oligonucleotide probe panel, Croatian population

Presentation number: MG 1 Abstract number: ABS-155-ISABS-2011

HYPERTROPHIC CARDIOMYOPATHY: FROM MUTATION TO FUNCTIONAL ANALYSIS OF DEFECTED PROTEIN

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The main aim of this study was to comprehensively analyze genesis of hypertrophic cardiomyopathy (HCM) in Czech patients afflicted with this disorder from molecular genetic point of view to functional analysis of the 3D molecular model of defected ?-myosin heavy chain protein (β-MHC) in silico. A total of 170 probands from different parts of the Czech Republic were enrolled in this study. DNA samples were analyzed for mutations in each of the 2 protein – coding exons of MYH7 gene that are associated with severe forms of HCM development. The 3D model of human β-MHC was built using the X-ray structure of Nucleotide-free scallop myosin S1 as the structural template. We performed de novo structure prediction of two peptides (mutant and wild type variant) spanning the 769-788 region of the β-MHC. The Asp778Val amino acid alteration was found in patient with severe form of HCM. This variation was chosen for subsequent 3D molecular modeling in silico. Mutation of the Asp by Val not only changes the character of the interaction pattern with other amino acids or ions but Val being a small hydrophobic amino acid can completely change stability of the region. We hypothesize that it can change the dynamics and flexibility of the long helical part or it can modify its interaction property. Mutation location in the MYH7 gene and therefore changes in amino acid composition may have crucial negative impact on the disease outcome in patients with HCM.

Keywords: hypertrophic cardiomyopathy, MYH 7, β-MHC protein, mutation, 3D molecular modeling

Presentation number: AG 1 Abstract number: ABS-273-ISABS-2011

ANTHROPOLOGICAL, GENETIC AND CHEMICAL ANALYSIS OF ANCIENT SKELETONS FROM SOUTHERN CROATIA

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Early Croatian inheritance dates from the first centuries after the Croats arrived in the Southern Europe (7th -9th century). Although there are a lot of varied historical, written or archeological sources that contribute to our knowledge about the Croatian life during the early Middle Ages in the Southern Croatia region, insufficient attention has been paid to the exploration of the concrete human population who left the traces of their living in a considerable amount of human bones and skeletons. Our scientific program was a certain integrality of several scientific disciplines such as archaeology, anthropology, modern genetics and chemistry with intention to illuminate the biological and social profile of the early Croatian population. The program included osteological analysis of human skeletons who inhabited the regions of the early medieval Klis and Bribir Counties in 9th an 10th centuries in order to synthesize all relevant bioarcheological information derived from the analized osteological remains like precise anthropological measurements, genetic characteristics and status of different chemical elements. The findings of the program brought a new approach in elucidating the oldest early medieval population of Southern Croatia with complete anthropological profile and facts about their genetic characteristics as well as how they lived and which food consumed. This kind of approach should serve as a model for all other similar investigations and, beside scientific application, it could be used in approving museum presentation about Croatian history and making virtual models which have direct impact on tourist offer in presenting our tradition and history.

Keywords: Southern Croatia, early medieval population, anthropometric analysis, DNA analysis, chemical analysis

YIA

Presentation number: FG 3 Abstract number: ABS-186-ISABS-2011

INVESTIGATION OF NEXT GENERATION SEQUENCING FOR MITOCHONDRIAL DNA APPLICATIONS IN FORENSICS

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The development of forensic-quality mitochondrial DNA (mtDNA) reference profiles is laborious and costly, though essential to the use of mtDNA in case work scenarios. So-called “next generation sequencing” technologies, now employed routinely in many genetic disciplines, promise a low-cost, high-throughput alternative to Sanger sequencing protocols, but their utility for the generation of data appropriate for forensic applications is unproven. The aim of this study, therefore, was to investigate the use of next generation sequencing for the production of forensic-quality mtDNA profiles from reference samples. Entire mitochondrial genome (mtGenome) consensus sequences were developed from eighty-five anonymous population samples using parallel tagged sequencing in a single Illumina GA-IIx run, and the resulting profiles were compared to Sanger sequence data for portions of the mtGenome. The results indicated that even with high depth of coverage, standard secondary analysis of the Illumina reads will produce some ambiguous positions, as well as discrepancies with the Sanger consensus sequences in regions prone to length heteroplasmy. However, sequence ambiguities due to point heteroplasmy could be accurately distinguished from an excess of sequencing error in the short-read data, and in most instances of length heteroplasmy the majority molecule could be correctly inferred by applying region-specific alignment parameters. The opportunity to process tens or hundreds of samples in parallel using next generation sequencing technologies should eventually accelerate the production of mtGenome reference data for forensic purposes, provided effective alignment and data evaluation strategies are developed to ensure forensic data quality standards are maintained.

Keywords: mtDNA, next generation sequencing, illumina, heteroplasmy, sequence alignment

Presentation number: FG 4 Abstract number: ABS-238-ISABS-2011

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) INVOLVED IN THE DETERMINATION OF FACIAL MORPHOLOGY

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Forensic DNA profiling is a rapidly evolving field and in the last few years the concept of Forensic Molecular Photofitting has emerged. This new area seeks to obtain additional valuable information from a DNA sample regarding the physical appearance of a person, such as skin, eye and hair pigmentation and more recently, facial morphology. The goal of this study is to identify a set of single nucleotide polymorphisms (SNPs) involved in normal craniofacial variation and subsequently develop a robust, phenotypically informative forensic assay. In order to achieve the project goals, DNA samples and 3-D images will be collected from approximately 500 individuals. The samples will be genotyped at the set of candidate SNPs and subsequently evaluated for statistically significant associations with anthropometric craniofacial measurements, including cephalic, facial and nasal indexes. In the current stage of this research, more than 200 genes and 800 SNPs, proposed to be involved in the craniofacial development have been selected, based on literature review and various web resources. The SNP list consists of markers, showing high Fst values as well as non-synonymous SNPs, SNPs located in splicing sites and transcription binding sites of genes shown to be associated with craniofacial development in human or model organisms. This research will result in development of a molecular identikit and to enable 'a face to be put to a DNA sample. This will, lead to greater success in solving criminal cases, identifying mass disasters victims, investigating historical skeletal remains and better understanding of embryonic craniofacial development.

Keywords: forensic DNA phenotyping, facial morphology, pigmentation, 3-dimentional imaging, ancient DNA

Presentation number: MG 2 Abstract number: ABS-227-ISABS-2011

ROLE OF THE GLUTATHIONE S-TRANSFERASE GENE POLYMORPHISMS AS MARKERS IN COMPLEX DISEASES

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Many common human diseases are believed to be influenced by several genetics and environmental factors. In our study we focused our attention on that diseases characterized by oxidative stress. In this scenario the study of xenobiotic metabolizing gene polymorphisms may be investigated to lead to an increased knowledge of allelic variants that may result in a differential susceptibility toward complex diseases development. We focused our attention on Glutathione S-transferases (GSTs), phase II metabolizing enzymes, that play a fundamental role in cellular detoxification. Three different association studies were performed in the Italian population using case-control groups (a: 200 asthmatics and 200 controls; b: 193 hypertensives and 210 controls; c: 119 patients with Alzheimer disease and 114 controls). All participants to these studies were recruited from “San Giovanni Calibita” Fatebenefratelli Hospital of Rome. We screened single nucleotide polymorphisms at the GSTA1, the GSTO1, the GSTO2 and the GSTP1 loci. The effects of GSTM1 and GSTT1 null genotype were also investigated. In these studies conducted in our laboratory we have found significant associations among some GST gene polymorphisms and the analyzed complex diseases. We hypothesized that these results are probably due to an interaction between gene variations and environmental risk factors, suggesting that the alteration of expression or catalytic activity of GST enzymes may make individuals more susceptible to environment. For these reasons GST gene polymorphisms could be clinically used as markers of susceptibility, so allowing earlier prediction and diagnosis of complex diseases and providing an efficient means of prevention.

Keywords: detoxification enzymes, complec disease, gene-environment interaction, markers of susceptibility, oxidative stress

Presentation number: MG 3 Abstract number: ABS-192-ISABS-2011

LOW CYP2E1 GENE EXPRESSION PERMITS PHARMACOLOGICAL SELECTION OF TRANSPLANTED HUMAN HEPATOCYTES IN VIVO

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To induce proliferation of transplanted human cells in immunocompetent rats by administration of hepatotoxic doses of a selection agent. GFP-Huh7, a hepatocyte-derived cell line with low CYP2E1 expression, was used. Expression of CYP2E1, an enzyme responsible for conversion of acetaminophen (APAP) into hepatotoxic compounds, was quantitated by real-time PCR. To prevent rejection, immunological tolerance was induced by introduction of GFP-Huh7 cells into fetal rats in utero, prior to development of the immune system. After birth, GFP-Huh7 cells were transplanted and treated with pharmacological doses of APAP. ALT levels and histology were to detect liver damage. GFP (+) cells in rat livers were detected by real-time PCR and fluorescence microscopy. Expression of CYP2E1 in GFP-Huh7 cells was only 4% compared to control cells. Animals treated with APAP increased ALT to 210 IU/ml on day 1, followed by resolution by day 3. Histology confirmed patchy necrosis corresponding in time to the ALT peak. Fluorescence microscopy of livers from transplanted, APAP treated rats showed an increase of GFP (+) cells/field from 4±2 on day 2 to 45±10 on day 7. Real-time PCR confirmed a 10-fold increase of GFP mRNA in APAP treated rats. Control animals had no significant changes of GFP (+) cells or mRNA levels. In vivo, the difference in CYP2E1 gene expression between GFP-Huh7 and host hepatocytes provides a convenient and efficient pharmacological selection resulting in enrichment of GFP-Huh7 cells. Down regulation of CYPs by siRNA may permit application of the selection to other liver cell lines. This work was supported in part by grants from Herman Lopata Chair in Hepatitis Research (GYW) and the Croatian Science Foundation (MS).

Keywords: humanized animal model, liver transplantation, cytochrome P450-2E1 (CYP2E1), pharmacological selection, acetaminophen (APAP)

ABSTRACTS – POSTER PRESENTATIONS

POSTER PRESENTATIONS
Forensic Genetics

Presentation number: FG 5 Abstract number: ABS-177-ISABS-2011

AN STR DATABASE ON THE VOLGA-URAL POPULATION

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This work develops a reference STR database on the Volga-Ural population, Russia, comprised of 640 individuals that were sampled from eight ethnic groups. The database is composed of DNA profiles of =640 unrelated individuals chosen randomly from eight ethnic groups, living in the Volga-Ural region, Russia. Among those are the Turkic Bashkir (N=100), Tatar-Mishary (N=94), Chuvash (N=99), and the Finno-Ugric Mordva-Moksha (N=47), Mordva-Erzja (N=52), Mari (N=52), Udmurt (N=93), Komi-Permjak (N=103). They were all typed with ten autosomal STR markers: TH01, CSF1P0, FGA, vWA, D3S1358, TPOX, D16S539, D8S1179, D13S317, FES. The groups differentiate in allele frequencies, and therefore we computed ?-values between allele frequencies in each ethnic group and those in the database as a measure of their differentiation. The average heterozygosity per locus is 0.76, and the total exclusion probability 0.99976. There is no deviation from genetic equilibrium; the minimal p-value from Hardy-Weinberg tests is 0.11, and p=0.011 for the least among linkage disequilibrium tests which is insignificant with the Bonferroni correction. Between-ethnic variation occurred to be significant statistically, with average ?-value of 0.005 and the 95% confidence interval (0.0038, 0.0064) that was obtained by bootstrapping across loci. The Volga-Ural ethnic groups form a relatively compact cluster that greatly deviate from the Romanic Moldovans and the Turkic Yakuts, taken for comparison, and are closer to the Slavic Russians, Belarusians, and Ukrainians, although significantly differ from those as well.

Keywords: microsatellite, Volga-Ural region of Russia, population differentiation, STR database, ethnic groups

Presentation number: FG 6 Abstract number: ABS-173-ISABS-2011

PIGMENT ASSOCIATED SNP MARKERS IN A NEW ZEALAND FORENSIC CONTEXT

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Forensic DNA techniques and eye witness accounts have long been integral tools that aid criminal investigators in identifying a person of interest. The forensic applications for DNA found at a crime scene may now include the inference of externally visible characteristics (EVCs) of the donor, particularly in instances where a DNA database match or an eye witness account is unavailable. Loci associated with particular variations of a trait can be identified using specific DNA markers. Likelihood probabilities can be calculated, indicating the phenotype for a particular trait using a multiplex of SNP markers. Potential characteristics could include pigmentation, ancestry, facial characteristics, height and even chronological age. Knowledge of the likelihood of a donor's appearance through a collection of these characteristics may prove to be vital clues to their identity, helping to exonerate inappropriate suspects and improve investigative efficiency. A SNP multiplex reaction designed to reveal pigmentation haplotypes of an individual's probable hair and iris pigmentation could prove to be a new and valuable tool to add to the spectrum of forensic DNA analysis in New Zealand. To investigate this new form of DNA analysis, we have assessed the association of known pigment markers in a New Zealand population. Using this data, we are assembling these associated markers within robust multiplexes to analyze pigmentation haplotypes of a New Zealand population and access the accuracy of marker revelations and pigmentation probabilities.

Keywords: pigmentation, SNP, association, New Zealand, phenotype

Presentation number: FG 7 Abstract number: ABS-189-ISABS-2011

GENETIC DIVERSITY OF PORTUGUESE STRAINS OF ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is a saprophytic mold that is involved in recycling the environmental carbon and nitrogen. The fungus has a wide distribution and it may colonize all environments. In order to evaluate the genetic diversity of Aspergillus fumigatus isolates, we employed the multilocus sequence typing (MLST) that is a technique very powerful for genotyping isolates based on single nucleotide polymorphisms. This technique has a very good discrimination power, however, still lower when compared to short tandem repeats (STR). MLST was employed to a group of A. fumigatus isolates obtained from clinical and environmental samples in Porto, Portugal. Those strains were previously genotyped and considered distinct by a microsatellite based multiplex PCR (Araujo et al. 2009). Primers suggested by Bain et al. (2007) were employed for amplification and sequencing. The sequences obtained in this study were compared with the polymorphic strains published on MLST website. The number of polymorphisms ranged from 2 to 8 polymorphisms in each gene, considering both online available and personal collections. In our local collection the most polymorphic gene was MAT1-2 (2.9%), in contrast with published MLST sequences - ANXC4 (2%). There were no significant differences between clinical and environmental strains. Through this study it was possible to find a set (2 to 6) of polymorphisms unique to the Portuguese isolates. At this time it is imperative that MLST online database be enriched with strains from several sources/countries to get correct assumptions on the geographic distribution of fungal strains.

Keywords: microbial forensics, fungi, genetic diversity, Aspergillus fumigatus, endemic strain

Presentation number: FG 8 Abstract number: ABS-190-ISABS-2011

GENETIC DIVERSITY OF PORTUGUESE STRAINS OF PSEUDOMONAS AERUGINOSA COLLECTED FROM CYSTIC FIBROSIS PATIENTS

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Pseudomonas aeruginosa is a gram-negative rod-shaped bacterium, ubiquitous in soil and water, and it is responsible for serious infections in cystic fibrosis patients. Genomic diversity of P. aeruginosa may be caused by mosaic genes, point mutations and inversions. Such diversity is very dynamic and displays a fast turnover of haplotypes over time. Multilocus sequence typing (MLST) is a powerful tool that allows studying population structure and analyzing epidemiological relationships by comparing sequences of nucleotides of 5 to 7 housekeeping genes. The aim of this study was to evaluate the genetic diversity of Portuguese populations of P. aeruginosa. Bacteria were characterized by employing 5 loci (acsA, guaA, nuoD, ppsA and trpE) suggested by Curran et al. (2004). Some unspecific bands were found when performing the amplification of target genes using the primers suggested by Curran et al. In order to solve this problem, new primers were designed for some MLST genes. The proportion of polymorphisms in MLST genes ranged between 1.1 and 2.6%, considering just a few Portuguese bacteria. The gene acsA was found to be the most variable locus in the sequenced Portuguese strains, while trpE locus was the most variable in the sequences at on-line database entries. Few polymorphisms were found uniquely in the Portuguese collection of isolates, supporting the existence of endemic and local microbial populations. The wide spread of sequenced loci throughout the genome allows us to make very trustworthy assumptions about the identity of a given strain by screening only a small number of nucleotides.

Keywords: microbial forensics, bacteria, Pseudomonas aeruginosa, genetic diversity, endemic strain

Presentation number: FG 9 Abstract number: ABS-232-ISABS-2011

APPLICATION OF THE EPIGENETIC METHOD TO IDENTIFY FAMILY RELATED SKULLS IN AN ARCHAEOLOGICAL CONTEXT

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The modern epigenetic identification method is based on the morphological variation of minor skeleton traits on the human skull, and of their observed familial concentration. The term epigenetic was applied to these variants to define the epigenetic control which modify skeletal differentiation. Such control is exerted by factors, which are external to the responding cells and modify the environment in which the connective tissues develop. The authentication of the epigenetic traits in relation to the genetics is not always straightforward, since one is mainly dealing with bone material, and there are few possibilities for researching interpersonal family or kindship relations. The excavated mausoleum of necropolis Collatina, in Rome (I-III AD), allowed us to test the usefulness of this epigenetic identification method to ascertain the relatedness of people buried in a familial tomb of the imperial age. This approach encompassed the scoring of 49 epigenetic traits of the human skulls in 53 individuals, whose sex and age at death were estimated according to classical bio-archaeological and forensic methods. The aim to explore the relationships between individuals buried inside and outside the mausoleum was carried out by the distribution of the epigenetic traits using Multiple Correspondence Analysis, where each non metric trait represents a variable for every individual. The results confirmed the mausoleum represent a familial tomb but some individuals did not clustered as genetically linked people, highlighting the suggestion that they were extraneum heredem as liberti or slaves who achieved the burial consent inside the funerary building.

Keywords: epigenetic traits, archaeological context, relatedness, Rome, imperial age

Presentation number: FG 10 Abstract number: ABS-112-ISABS-2011

PREDICTION OF EYE COLOR FROM GENETIC DATA SHOULD ASSUME GENE-GENE INTERACTIONS

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Prediction of phenotypic features from genetic data will be applied by forensic science in suspect-less cases as well as in some cases of identification of human remains. Recent GWA studies revealed multiple genes and polymorphisms explaining a large proportion of variation in human pigmentation. These findings have been applied by forensic science and the first tests for accurate prediction of blue and brown eye colour have been developed. Improved prediction of intermediate eye colors may need additional data concerning genes and polymorphisms with a weaker influence on iris coloration. It has also been proposed that the final phenotype can be significantly affected by nonlinear effects between genes. We further investigated this issue and studied interactions between the most important polymorphisms associated with pigmentation. Our research involved analysis of variation in 11 known pigmentation genes in a cohort of 718 individuals of European descent from Poland. The obtained genetic data were subjected to detailed analysis for epistatic effects using multifactor dimensionality reduction and logistic regression methods. The performed study revealed significant interactions of a redundant character between the HERC2 and OCA2 genes affecting determination of hazel eye colour and between HERC2 and SLC24A4 affecting determination of blue eye colour. Furthermore, interactive effects of a synergistic character between HERC2 and OCA2 and also between HERC2 and TYRP1 were indicated, both affecting determination of green eye colour. Further study should explain if this knowledge will improve prediction accuracy, especially in the case of intermediate eye colors.

Keywords: forensic DNA phenotyping, pigmentation genes, eye color prediction, epistasis, prediction accuracy

Presentation number: FG 11 Abstract number: ABS-231-ISABS-2011

COMPARATIVE ANALYSIS OF THREE DIFFERENT STR MULTIPLEX SYSTEM APPROACHES IN FINGERPRINT DNA ANALYSIS

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The multiplex detection and analysis of mini-STR and STR markers is a common tool used in the forensic DNA analysis. Nowadays, available commercial kits are allowing almost routine analysis extremely low amounts of DNA presented in biological samples. However, analysis of LCN samples, especially those one that could be described as “touch” samples”, is still very challenging. We have collected 30 personal fingerprint samples (by the standard collection methods) and processed them using 3 commercial kits (PowerPlex® S5, PowerPlex® 16 and PowerPlex® ESI 17). PCR amplification was carried out in PE GeneAmp 9700, PCR System Thermal Cycler. Detection of the amplificated products was performed on ABI PRISM 310 Genetic analyser (ABI, Foster City, CA), while RT-PCR DNA quantification was performed using Quantifiler DNA Identification kit. Minimal concentrations of nuclear DNA were registered in only 8 (27%) of 30 fingerprints. DNA was not detected within other 22 (73%) of 30 fingerprints. Concentrations of detected isolated DNA ranged from 9, 33 x 10-3 pg/µl to 1, 41 x 10-2 pg/µl. DNA profiling of LCN samples faces many problems and depends upon many factors. Therefore, problems such as allele drop-out, allele drop-in, increased risk of contamination, locus drop-out and peak imbalance can occur. Results of our study confirmed the presence of artificial alleles after use of all of the three commercial kits (PowerPlex® S5, PowerPlex® 16 and PowerPlex® ESI 17). This suggests possible contamination during sample collection. Consequently, futher studies should investigate the optimization of DNA extraction, PCR protocol and usage of other miniSTR multiplexes.

Keywords: mini-STR, STR, DNA profiling, DNA fingerprint, epithelial cells

Presentation number: FG 12 Abstract number: ABS-181-ISABS-2011

OPTIMIZATION OF FORENSIC DNA ANALYSIS PROCESS FOR BOTANICAL TRACES: A COMPARATIVE ANALYSIS OF PLANT DNA EXTRACTION FROM FRESH AND HERBARIZED TILIA PLATHYPHYLLOS SCOP. LEAVES

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Forensic botany is a multidisciplinary science that investigates plant material as evidence collected from crime scene. Plant molecular biology is a potent tool in applied forensic botany and plant DNA extraction is one of the important steps in all further analysis. Plant DNA extraction is crucial issues in plant molecular biology. One of the major problems with plant DNA extraction lay in the fact that plants contains high amounts of secondary metabolites which could inhibit DNA extraction and amplification process. Also, plant material from crime scene is mainly in degraded form, so optimal extraction protocol has to be utilized to produce high amounts of good quality DNA. In our study we have compared three extraction protocol for DNA extraction from fresh and herbarized specimens of Tilia plathyphyllos Scop. leaves. Presence and quality of DNA obtained with these protocols were determined with agarose gel electrophoresis and by measuring the A260/280 absorbance ratio using spectrophotometer. DNA samples were amplified using trnL primers. In or study the highest amounts of DNA were extracted from fresh leaves but all three used protocols gave good results for both specimens. Also applied primers, as well as the PCR protocol, were optimal for chloroplast DNA amplification. This preliminary study presents the basis of all further plant molecular analysis that has to be routinely applied in our laboratory.

Keywords: forensic botany, plant DNA, fresh specimens, herbarized specimens, trnL primers

Presentation number: FG 13 Abstract number: ABS-143-ISABS-2011

INSERTION/DELETION POLYMORPHISMS (INDELS): NEW DIPPLEX KIT FOR FORENSIC IDENTIFICATION AND PARENTAGE TESTING

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INDELs are diallelic markers derived from a single mutation and have low mutation frequency, which makes them suitable for parentage testing. INDELs combine advantages of both STR and SNP. They have as small amplicon size as SNP (about 100 bp), but could be analyzed by techniques used for routine STR analysis. In present study we genotyped 55 unrelated members of Czech population and we also genotyped 11 families to analyze suitability of the DIPplex Kit for parentage testing. DIPplex Kit allows co-amplification of 30 diallelic markers located on autosomes. Selected loci are at minimum 10Mbp apart from each other, so they could be treated as independent markers. We used DNA isolated by QIAmp Blood Mini Kit (QIAGEN, Germany). DNA was amplified using DIPplex Kit and then analyzed on capillary electrophoresis ABI 310 (Aplied Biosystems, USA). Acquired data were analyzed using Genotyper software. All 30 loci fulfill Hardy-Weinberg equilibrium. There are several significant differences between Czech and African populations, but no significant differences between Czech and German or general European population were found. Probability of a random match in Czech population was 1 in 1, 4*10-13, combined power of discrimination was 99, 99992481%. Average paternity index was 1, 13-1, 77 for each locus, combined paternity index was approximately 21000 for a set of 30 loci. We can conclude that DIPplex kit is useful as an additional panel of markers in complex paternity cases. For application on degraded or inhibited samples further optimization of buffer and reaction conditions is needed.

Keywords: INDEL Insertion/deletion polymorphism, parentage testing, population study, forensic genetics, PCR multiplex

Presentation number: FG 14 Abstract number: ABS-365-ISABS-2011

Allele frequencies of the New European Standard Set (ESS) loci plus SE33 locus in Dalmatian Human Population

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In response to the ENFSI and EDNAP groups' call for new STR multiplexes for Europe, Promega developed a suite of four new DNA profiling kits. One of them is PowerPlex ESX 17 that we have used in this study. In previous population studies of Dalmatian (Southern Croatian) human population, 15 STR loci included in the AmpFlSTR®Identifiler® and Powerplex 16 were used. Additionally, we have decide to analyze 6 additional new STR loci (D22S1045, D1S1656, D10S1248, D2S441, D12S391, SE33). This study is based on an observation of representative sample of Dalmatian human population. A total of 120 unrelated individuals from different partss of Dalmatia participated in this study. Qiagen MicroTM Tissue Kit was used for DNA extraction from whole blood, Quantifiler® assay for quantification and PowerPlex ESX 17® System for amplification and detection. The total volume of each reaction was 5 ml. The PCR amplifications have been carried out in ABI 9700 Thermal Cycler. Electrophoresis of the amplification products was performed on an ABI 310 PRISM. Numerical allele designations of the profiles were obtained by processing with Gene Mapper® IDv 3.2 Software. Deviation from Hardy-Weinberg equilibrium, observed and expected heterozygosity, power of discrimination and power of exclusion were calculated. Also, we have compared Macedonian data with data obtained from other neighboring populations with available data for observed loci. Deviation from Hardy–Weinberg equilibrium, observed and expected heterozygosity, exact test of population differentiation and pairwise Fst were calculated within Powermarker v3.25, since power of discrimination and power of exclusion within PowerStats.

Keywords: New European Standard Set, PowerPlex ESX, Dalmatia

Presentation number: FG 15 Abstract number: ABS-129-ISABS-2011

SENSITIVITY, STABILITY AND SPECIFICITY OF KASTLE-MEYER AS AN INDICATOR TEST FOR BLOOD

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The presence of blood is studied in different ways and various matrix for Specificity, Sensitivity and Stability of reagents using Kastle-Meyer blood presumptive test. In this study is used blood samples in different dilution, blood in various matrix, blood in different fabrics and blood stains from different fabrics washed with water, soap and 3% hydrogen peroxide, blood stains in fabrics handling with heat and flame, also are used different fruits and vegetables for specificity of the test. The methods used were with rubbing and cutting of different matrix, the liquid blood is used as well. The Kastle-Meyer blood presumptive test or the phenolphthalein test for blood has been shown to be very sensitive to the presence of hemoglobin in blood, it has been shown to be so specific to hemoglobin and the reagents used with this test has been shown to be very stable for long time. The KM test is safe, easy and fast to use. Based on the results as conclusion we suggest using the Kastle-Meyer test in all forensic laboratories, serology part.

Keywords: Kastle-Meyer, blood presumptive test, phenolphthalein, hemoglobin, matrix

Presentation number: FG 16 Abstract number: ABS-109-ISABS-2011

CROATIA LAUNCHES NATIONAL REFERENT Y-STR HAPLOTYPE DATABASE

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Creation of a national referent Y-chromosome short tandem repeat (STR) haplotype database is one of the principal steps in a detailed genetic characterization. The aim of this study was to create a comprehensive Y-STR haplotype database of the Croatian contemporary population and to analyze substructure between the five Croatian regions. 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. A total of 1100 unrelated men from eastern, western, northern, southern and central Croatia were included in this study. Genomic DNA was extracted from FTA® cards using Chelex. 17 Y-STR loci were amplified using the AmpFISTR Yfiler PCR amplification kit. The haplotype frequencies were determined by direct counting and analyzed using Arlequin 3.1. AMOVA analysis calculated with the YHRD online analysis tool included 16 population samples with 19926 haplotypes. A total of 947 haplotypes were recorded, 848 of which were unique. Haplotype diversity was 0.998, with the common recorded haplotype found in 9 of 1100 men. Locus diversity varied from 0.267 for DYS392 to 0.772 for DYS458. Discrimination capacity was 86.1%. The results suggest high level of similarity among regional sub-populations within Croatia, and high level of resemblance with some south-eastern population groups from Bosnia and Herzegovina and rather strongly expressed difference from the north-western neighboring countries. The database will serve not only as a source of relevant information for forensic purposes, but also as a source of useful information in population genetics.

Keywords: Y chromosome, short tandem repeat, population genetics, haplotype, Croatian population

Presentation number: FG 17 Abstract number: ABS-252-ISABS-2011

HUMAN IDENTIFICATION FROM THE GUT OF BLOWFLY LARVAE (CALLIPHORIDAE)

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Entomological evidence is most commonly used to estimate the postmortem interval, where it is crucial to identify species of insect feeding on the corpse. Analysis of human DNA isolated from insects enables identification of human remains: in cases of identification of corpse displaced from the crime scene, in cases where larvae had alternative food source, or in cases of questionable reliability of collected evidence. The aim of this study was to recover suitable human DNA from the gut of blowfly larvae (Calliphoridae) at different larval stage. Different decontamination, preservation and extraction methods were compared. Obtained DNA profiles were matched to cadaver reference sample, using commercial forensic kits (AmpFlSTR® Identifiler™ and AmpFlSTR® MiniFiler™). The results show that it is possible to obtain human STR profile sufficient for human identification from fly maggot gut at different larval stage, while several technical precautions should be considered.

Keywords: STR, human DNA, DNA extraction, forensic entomology, fly larvae

Presentation number: FG 18 Abstract number: ABS-247-ISABS-2011

EXCESS OF LINKED MARKERS CAUSES WRONG CONCLUSIONS

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Analysis of SNP (single nucleotide polymorphism) markers on DNA microarrays may be used to solve issues of relatedness since hundreds of thousands of SNP's can be analysed. However, the statistical evaluation of these large amounts of data is a challenge. We have used several approaches to evaluate close and distant relationships based on SNP data retrieved from the Affymetrix GenomeWide SNP Array 6.0. Statistical evaluation of relatedness was performed using pair-wise comparisons in the R-package FEST as well as pedigree comparisons in Merlin. Relationships as far as 2nd cousins could be readily distinguished from unrelated and other genetically closer relationships with a selection of 5, 774 markers. When considering 3rd cousins, and more distant relationships, the number of markers needs to be extended. But the inclusion of an excess of markers presented new challenges. In a real-case family analysis we have shown that the use of more than 20, 000 SNP markers always yields the highest probability for the genetically closest relationship hypothesis. This is most probably caused by the fact that the computational model assumes linkage equilibrium between markers. We will show how the number of markers affects the probability calculations. We will also show how LD instead can be an advantage by using clusters of tightly linked markers.

Keywords: SNP, arrays, kinship, linkage, clusters

Presentation number: FG 19 Abstract number: ABS-251-ISABS-2011

SERIAL KILLERS IDENTIFIED WITH DNA

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Serial murders occurred in the last 6 years in two different cities in the Republic of Macedonia. Four males were killed on a similar way in the city of Ohrid and only females, same age, in the city of Kicevo. All of the bodies were in a process of decomposition or were sceletonized. Autopsy was performed on all victims, and DNA from bones of the victims, vaginal swabs, fingernail debris and biological traces on clothes were analyzed. Extraction of bones was performed by using of phenol-chlorophorm method, and out of the other materials we used Qiagen Micro kit. For amplification were used Indentifiler ABI, Y-filer ABI and Power Plex 16 Promega kits. Post amplification products were analyzed by using 310 Genetic Analyzer ABI. DNA View program was used for making statistical analyzes. One sceletonized body of the victims was identified in the city of Ohrid and the mechanism of injuries on his body was proved. In the second case, in the city of Kicevo, in the vaginal swabs of all female victims, DNA of the perpetrator was found. DNA was the only material evidence in those two cases, so the perpetrators of the crimes were arrested.

Keywords: serial killers, STR, sceletons, identification, evidence

Presentation number: FG 20 Abstract number: ABS-244-ISABS-2011

PROTOCOL DESIGN FOR FORENSIC AUTOSOMAL TESTING USING SNP SPECIFIC PRIMERS

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Use of the autosomal SNPs can be a supplement to the regular STR testing, which is the method of choice in present forensic genetics. Their usefulness can particularly show itself when dealing with highly degraded samples, due to their manipulation of the shorter fragment during the analysis. On the other hand, sensitivity and specificity issues arise with SNP analasys. This work will follow testing of four SNPs, from their selection, through primer design and analasys through SNP specific primer design. The aim of the presentation is to, through presentation and discussion of the laboratory results, explore the potential for autosomal SNP testing in forensic genetic.

Keywords: DNA, SNP, primer , specificity, sensitivity

Presentation number: FG 21 Abstract number: ABS-110-ISABS-2011

NFI APPLIES NEW DVI SOFTWARE SOFTWARE BASED ON BAYESIAN NETWORKS ASSISTS IN DVI FOLLOWING THE CRASH OF AFRIQIYAH AIRWAYS FLIGHT 8U771 NEAR TRIPOLI, LIBYA

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On Wednesday the 12th of May 2010, Afriqiyah Airways Flight 8U771 crashed on landing near Tripoli International Airport. One child survived the crash, 70 of the 103 victims were Dutch nationals. A large number of victims were blood-relatives. For DNA research/matching the NFI received soft and hard tissue samples from the bodies/body parts, personal items belonging to missing persons, reference samples from relatives of missing persons and DNA-profiles typed in foreign labs. The samples were received between the 8th and 28th day after the crash. DNA typing was performed using the QiaAmp-kits (Qiagen), Quantifiler-duo and Identifiler kits (ABI). DNA profiles were obtained within a period of 3-5 days after delivery of the samples. New software for the screening and matching of the DNA profiles was used. This software - called Bonaparte DVI - uses Bayesian networks. As a result less false hits are obtained compared to methods which do not take complete pedigree information into account, but only consider one relative at a time. An additional advantage is that the analysis tool is transparent and flexible, allowing one to incorporate other relevant factors. The Bonaparte DVI software proved to be a reliable and time-saving tool which significantly simplified and sped up the large-scale DVI process. The total DNA identification investigation took 26 days, 129 bodies/body parts were matched using the Bonaparte DVI software to a missing person. In total, 122 reports were written. Further information about Bonaparte DVI can be obtained via www.dnadvi.nl

Keywords: disaster victim identification, Bonaparte DVI software, DNA, kinship analysis, airplane crash

Presentation number: FG 22 Abstract number: ABS-183-ISABS-2011

USAGE OF THE POWERPLEX S5 MINISTR SYSTEM IN ANALYSIS OF HUMAN TELOGENIC HAIR SHAFTS

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The primary value of DNA typing has been significantly increased over the last fifteen years due to introduction of short tandem repeat (STR) loci. Nowadays, it is well known that even extremely highly degraded nuclear DNA may be successfully analyzed using miniSTR approach. We have examined possible application of miniSTR loci in forensic DNA analysis of 65 telogenic hair shafts that collected from various locations. All samples were briefly washed in absolute ethanol and extracted using Qiagen DnaeasyTM Tissue Kit. DNA concentration was determined using Quantifiler Human DNA Quantification Kit as described previously. The reaction was carried out in AB 7300 Real-Time PCR System according to the manufacturer's recommendations. The PowerPlex® S5 system has been used to amplify 4 miniSTR loci in 10µl total reaction volume. PCR amplification was carried out in AB GeneAmp PCR System Thermal Cycler. Electrophoresis of the amplification products was performed on an ABI PRISM 310 Genetic Analyzer. Raw data have been compiled, analyzed and numerical allele designations of the profiles were obtained using the accessory software: ABI PRISM® Data Collection Software v3.0 and GeneMapper® ID Software v3.1. As the result of these analyses, 19 telogenic hair shafts were completely or partially profiled and for 49 of them no profiles were detected. Results of this study, imply that PowerPlex® S5 System may be useful in forensic analysis of the samples with very low amount of nuclear DNA such as telogenic hair shaft. However, its application has certain limitations that should also be considered in the future application.

Keywords: miniSTR, telogen, hair shaft, DNA analysis, PowerPlex S5

Presentation number: FG 23 Abstract number: ABS-223-ISABS-2011

CHALANGES IN APPLICATION OF POWERPLEX® ESI 17 AMPLIFICATION KIT IN LCN (LOW COPY NUMBER) DNA ANALYSIS

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Development of a low copy number (LCN) DNA profiling method allows detection of very low levels of DNA. Given its increased sensitivity, LCN can be a particularly useful tool for investigating crimes where standard profiling techniques are exhausted or when options for forensic evidence appear to be limited. Issues concerning the PCR optimization protocols for LCN DNA were considered in this study. We have examined application of combined STR loci in forensic DNA analysis of 20 LCN DNA forensic samples. All samples are processed in Institute for Genetic Engineering and Biotechnology, in Forensic Laboratory. DNA extraction was performed by using Qiagen DnaeasyTM Tissue Kit. DNA quantification was determined using Quantifiler Human DNA Quantification Kit. The reaction was carried out in AB 7300 Real-Time PCR System according to the manufacturer's recommendations. The PowerPlex ESI17® system was used to amplify 16 STR loci in 7µl total reaction volume. PCR amplification was carried out in AB GeneAmp PCR System Thermal Cycler. Electrophoresis of the amplification products was performed on an ABI PRISM 310 Genetic Analyzer. Raw data have been compiled, analyzed and numerical allele designations of the profiles were obtained using the accessory software: ABI PRISM® Data Collection Software v3.0 and GeneMapper® ID Software v3.1. Results of this study imply that PowerPlex® ESI7 System may be useful in forensic analysis of the samples with very low amount of nuclear DNA such as telogenic hair shaft and fingerprint as well. However, its application has certain limitations that should also be considered in the future.

Keywords: LCN DNA, forensic samples, multiplex STR system, PowerPlex® ESI 17, DNA analysis

Presentation number: FG 24 Abstract number: ABS-158-ISABS-2011

FOUR SPERMS ENOUGH FOR IDENTIFICATION OF A RAPIST? INTRODUCTION OF LM IN FORENSIC PRACTICE

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Laser microdissection is a new technique used in forensic DNA analysis that enables precise separation of target cells from mixed forensic samples. This method is non-contact and contamination-free. The obtained cells can be used for a subsequent genetic analysis with the aim to determine single DNA profile via STR analysis that leads to individual identification of contributors to the mixed sample. The goal of our project is to optimize methods of cell separation from mixed forensic samples such as vaginal smears after rape (epithelial cells, blood and sperm) and placental tissue obtained from abortion material (maternal decidua and fetal chorionic villi). We have tested and compared several DNA extraction methods from the mentioned samples as well as several PCR protocols and post-PCR purification. We plan to utilize this technique for recovery of fetal cells from placental tissue after abortion in the near future.

Keywords: laser microdissection, sperm, STR, DNA, forensic

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ALLELE FREQUENCIES OF 15 STR ON SIX SUB-POPULATIONS IN PROVINCE OF ENTRE RíOS, ARGENTINA

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Fifteen STR´s (Short Tandem Repeats) loci present in AmpF/STR Identifiler kit (Applied Biosystems), CSF1PO, D13S317, D16S539, D18S51, D8S1179, D21S11, D7S820, D3S1358, TH01, D2S1338, D19S433, vWA, TPOX, D5S818 and FGA, were analyzed in a six subpopulation of Entre Ríos, a province located at the argentinean mesopotamian region. Allele frequencies as other statistical parameters are provided. The loci showed no significant deviation from Hardy-Weinberg equilibrium. A Neighbor-Joining tree that coincides well with their geographical and historical contributions was constructed based on frequencies distribution by Pairwise Fst Genetic Distance. This study demonstrates that Identifiler loci are a useful tool for forensic and parentage testing purposes on this subpopulations on this argentinean province.

Keywords: Entre Ríos, STR, Argentina, identifiler, forensic

Presentation number: FG 26 Abstract number: ABS-303-ISABS-2011

Y CHROMOSOME HAPLOTYPES AND SURNAMES IN THE SPANISH POPULATION

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In most societies, surnames are passed down from fathers to children, just like the Y chromosome. So, theoretically, men sharing the same surnames would also be expected to share similar Y chromosomes. Previous investigations have challenged such relationship but data has been collected so far only from the British Isles. In order to provide additional insights into the correlation between surnames and Y chromosomes, we focused our attention on Spain and investigated Y-chromosome SNP/STR variation by analysing a total of 1, 766 DNA samples from unrelated male volunteers belonging to 37 surnames and 355 controls. Our results suggested that the degree of co-ancestry within surnames was highly dependent on surname frequency. However, geographic distance between samples' place of origin influenced significantly this correlation. Within surname variation, number of descent clusters and TMRCAs correlated well with surname frequency, but exceptions were found. Studies evaluating the link between Y chromosome and surnames could be potentially helpful in forensic investigations as Y-chromosome profiles could be used to predict surname in forensic caseworks.

Keywords: Y-chromosome, haplotype, surnames, co-ancestry, Spain

Presentation number: FG 27 Abstract number: ABS-215-ISABS-2011

SPECIFICITY OF SIBSHIP DETERMINATION USING 15 STR LOCI WITHIN INHABITANTS OF BOSNIA AND HERZEGOVINA

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Aim of this study was to test the effectiveness of standard STR markers in assessment of sibship of human individuals within Bosnian and Herzegovinian population. Cumulative/Combined Sibship Index (CSI) together with sharing alleles between those pairs was method of distinguishing siblings from unrelated persons. CSI was calculated accordingly by multiplying Likelihood Ratio (LR) for all observed STR loci. CSI 0.05, 0067, 1, 3, 10, 10.3 and 63 as the CSI cut-off values for distinguishing were observed. LR+ for negative test result was defined in terms of determined sensitivity and specificity: LR+= sensitivity/1-specificity and LR-= 1-sensitivity/specificity. Two cut-off points, one associated with the minimal desirable value of LR (+) and the other maximal desirable value of LR (-) were identified delimiting the “grey zone”. By constructing a three-zone partition for CSI values, we got a gray zone, the area of inconclusive CSI values. Using the gray zone the most accurate result in terms of verifying real siblings from non-siblings has been shown at CSI cut-off 0.067. There was statistically significant difference (SSD) of CSI values between siblings and non-siblings. Using by these two methods is possible with very high reliability to separate siblings from non-siblings. However, in some cases from the “gray zone” it is necessary to implement and additional genetic test in order to confirm sibship more precisely.

Keywords: DNA typing, short tandem repeat loci (STR), cumulative / combined sibship indices (CSI), grey zone, distribution of sharing alleles

Presentation number: FG 28 Abstract number: ABS-317-ISABS-2011

DNA PERSISTENCE IN POST-MORTEM SOFT MUSCLE TISSUES IN RELATION TO ACCUMULATED DEGREE-DAYS (ADD)

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After the death of an organism DNA starts to degrade and as the organism's cell structure breaks down, nucleases are released and directly cause DNA degradation. Subsequent colonisation and action of insects and microorganisms also contributes to the DNA degradation. As the post-mortem interval (PMI) increases DNA continues to degrade until no high molecular weight DNA (HMW-DNA) remains. In order to assess DNA degradation in the model organisms chosen (pig and rabbit), two nuclear genes, Connexin 43 and RAG-1, were aligned to identify conserved regions. Primers were designed to amplify 70 bp, 194 bp, 305 bp and 384 bp amplicons. The primers were also designed to amplify human DNA, which allowed the use of commercially purchased DNA standards to be used as controls. Following DNA extraction PCR analysis was performed using the four primers sets in a multiplex (4-plex): the PCR was optimised so that it worked over a wide range of template amounts (0.1 ng to 75.83 ng). The multiplex PCR was found to work efficiently in triplicate samples of all three species down to 0.3 ng of DNA template. This multiplex has been used to assess whether DNA degradation can be predicted by accumulated degree-days (ADD), which provides a measure of both time and temperature. Full 4-plex profiles were generated until day 7 (ADD 111.93) from whole carcasses and body fragments and up to day 11 (ADD 172.48) from insect-activity-free muscle samples. Future work will include; development of real-time PCR quantification assays, DNA Fragment analysis and DNA preservation.

Keywords: accumulatd degree-days (ADD), post-mortem interval (PMI), multiplex reaction (4-plex), polymerase chain reaction (PCR), recombinase activating gene 1 (RAG-1)

Presentation number: FG 29 Abstract number: ABS-309-ISABS-2011

GENETIC POLYMORPHISMS OF 15 AMPFLSTR IDENTIFILER LOCI IN A SERBIAN POPULATION

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Allele frequencies of 15 AmpFlSTR Identifiler loci (D3S1358, TH01, D21S11, D18S51, D2S1338, D5S818, D13S317, D7S820, D16S539, CSF1PO, D19S433, vWA, D8S1179, TPOX, FGA) were obtained in a sample of 356 unrelated, autochthonous, healthy individuals of both sexes, born in Serbia. The agreement with HWE was confirmed for all studied loci with the exception of D21S11. The combined PD and the combined PE for the 15 studied loci were 4.67 x 10-18 and 0.99999837, respectively. According to the presented data, D2S1338, D18S51, FGA and D21S11 proved to be the most informative markers. After Bonferroni's correction, an interpopulation comparison between Serbians and five neighboring and ethnically close populations from the Balkan Peninsula – Bosnian, Croatian, Macedonian, Montenegrin and Slovenian, revealed significant differences in three loci with Montenegrins: D21S11, D18S51 and D19S433.

Keywords: STRs, AmpFlSTR identifiler, allele frequencies, population data, Serbians

Presentation number: FG 30 Abstract number: ABS-290-ISABS-2011

POSSIBLE APPLICATION OF GENETIC DISTANCE MEASUREMENT IN PATERNITY AND DNA TESTING

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Generally, genetic distance is mainly associated with interpopulation analysis as well as with interspecies analysis for phylogenetic studies. Interindividual genetic distance is not widely applied. The aim of this study is to test possible application of shared allele coefficient based on modification of classical shared allele distance as the measurement of human interindividual genetic relationship. Share allele coefficient is a measure of interindividual similarity which includes observation of reference group. It represents the degree of relatedness based on each observed STR locus. This measure represents proportion of shared alleles between individuals including the number of alleles for each observed locus within referent group (databases). Overall shared allele coefficient is average of these measures for each locus. Permutation approach has been used for testing of statistical significance. In this study, referent PowerPlex® 16 human database of Bosnia and Herzegovina has been used for control group. The results were compared with classical statistics for paternity testing as well as with maximum likelihood method of relatedness and relationship estimation. The simulations show that share allele coefficient is adequate method for comparisons between individuals and has possible application as additional statistical method in DNA paternity and forensic testing.

Keywords: genetic distance, STR database, forensic DNA statistics, paternity testing, human genetic relatedness

Presentation number: FG 31 Abstract number: ABS-298-ISABS-2011

POPULATION STUDIES AT 11 POLYMORPHIC STR LOCI IN A BOVINE SAMPLE FROM NORTHERN CROATIA

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Genotyping of polymorphic sites provides a unique DNA fingerprint. It is therefore possible to employ these methods to follow the meat samples along the retail chain, by generating a DNA profile that can be used to trace-back the identity of the individual animal from the carcasses or the meat cuts.DNA analysis from various type of samples originated from animal is still sort of challenge, especially when the meat cut or bone fragments are focal point of analysis. Applied Biosystems has developed STR kit that addresses the needs of the bovine DNA typing community and covers a common set of STR loci. Nevertheless, efficiency of existing commercial kit within Croatia still unknown and unexamined. Therefore we have performed Croatian cattle population study. Population studies included 111 randomly selected cattle (Bos taurus) from Northern Croatia. Genomic DNA was isolated from blood samples using BloodPrep™ Chemistry on the ABI PRISM6100 Nucleic Acid PrepStation™. Microsatellites were amplified using the StockMarks for Cattle® Bovine Genotyping Kit. The PCR amplification has been carried out in PE Gene Amp PCR System Thermal Cycler according to the manufacturer's recommendations. The PCR products were submitted to fragments analysis by capillary electrophoresis, with an automated sequencer ABI PRISM 3130 genetic analyzer according to the manufacturer's specifications. Results were read and interpreted using GeneMapper® ID Software v3.1., respectively. Microsatellite allelic frequency analysis was performed on these data using Cervus 3.0.3 The power of parental exclusion, expected and observed heterozygosity, probability of identity, and non-amplifying allele frequencies were calculated.

Keywords: bovine STR loci, Bos taurus, STR population studies, forensic DNA analysis, northern Croatia

Presentation number: FG 32 Abstract number: ABS-300-ISABS-2011

CONTRIBUTIONS OF THE FORENSIC MEDICINE IMPLICATIONS IN DIFFERENT TYPES OF ANIMAL BITES IN HUMANS

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This paper proposes the study of the legal medicine implications of injuries caused by animals to humans and presents specific aspects of the complications that arise in the evolution of these lesions, often leading to the death of the victim. The study was conducted from 2006 to 2010 at the County Department of Forensic Medicine of Oradea, Bihor. Animals leading to these injuries were both domestic (dog, horse, pig) and wild (fox, bear, wolf, snake). We had variable cases: 4 deceased cases and 28 living cases. In the deceased cases the inflicted wounds were in the form of crushed, bitten, torn and burst fractures of viscerocranium and the chest as well as rupture internal organs and blood vessels. In Bihor County, during the period of the study no cases of death resulting from bear or snake venom bite were registered. Thanatogenerating injuries occurred in the case of equine aggression. The bite wounds predominate in the living cases. When the bite was strong, there were amputations of the bitten area, tissue having a torn appearance. The lesions location included various topographical areas; in case the location is on the face the aesthetic problem of making the individual ugly occurs in the cases where surgery could not correct the aesthetic damage, this is still one of the parameters studied in 2 live cases. The results were given in graphs and tables, appreciating the percentage frequency based on age, gender, source of injury, anatomical-clinical, etiology, mechanism of trauma production, aesthetic aspect.

Keywords: animal aggression, aesthetic injury, domestic animals, wild animals

Presentation number: FG 33 Abstract number: ABS-302-ISABS-2011

ODONTONSTOMATOLOGICAL ASPECTS IN THE FORENSIC IDENTIFICATION

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This paper proposes to conduct a retrospective longitudinal study on some odontostomatological aspects in medical forensic identification.
The study was conducted in the period between 2008 and 2010, at the County Department of Forensic Medicine in Oradea Bihor. Cases of assault, rape and murder were included in our study in order to identify the aggressor. During the period of study 102 cases of aggression also presented bite wounds, 16 cases of rape and 5 cases of murder. Odontostomatological identification is an important element both in the anthropology of forensic medicine and in the biocriminal traseology. Biting occurred both in the context of aggression as well as in the context of defense-in our case 13 of the 16 cases of rape presented bite wounds. Of the 5 cases of murder, only in one case were identified bite wounds to the chest and upper limbs, the aggressor as the victim's boyfriend . Bite traces were produced by the front teeth in 84 of the cases and had different characteristics depending on the applied force, direction, the nature and the form depending on the surface area bitten, swallowing characteristics, environmental factors, thantological phenomena and changing position of victim. In 37 cases the dental items were erased or very small, in these situations histochemical examinations were performed. In rape and murder cases biological samples were collected for DNA identification of the perpetrator and fingerprints on the victim were analyzed. The results were given in graphs and tables.

Keywords: bite mark, bio-traseology, identification, odontostomatological identification, medical forensic identification

Presentation number: FG 34 Abstract number: ABS-210-ISABS-2011

IDENTIFICATION OF TUMOR SPECIMENS BY DNA ANALYSIS IN A CASE OF HISTOCYTOLOGICAL PARAFFIN TISSUE BLOCK SWAPPING

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A patient was diagnosed with high-grade breast carcinoma by all the pre-surgery clinical evidences of malignancy. Later, the histo-pathological reports did not reveal any tumor residue in the post surgical tissue block. This raised the suspicion, either exchange of block, labeling error or the technical error during gross examination of the tissue. The mastectomy residue was unprocurable to sort out the problem. So, two doubtful paraffin blocks were sent to DNA fingerprinting laboratory. The partial DNA profiles (8-9 /15 loci) were obtained from histocytological blocks. The random matching probability for both the paraffin blocks and the patient's blood were found to be one in 4.43E4, 1.89E6 and 8.83E13 respectively for Asian population. Multiplex STR analysis applied in this case determined that exact cause of absence of tumor was the error in gross examination of the post surgical tissue. Moreover, the analysis helped in justifying the therapy given to the patient. Thus, with DNA fingerprinting technique, it was concluded that there was no exchange of the blocks between the two patients operated on the same day and the treatment given to the concerned patient was in the right direction.

Keywords: forensic science, sample swapping, paraffin embedded block, short tandem repeats (STR), DNA profiling

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IDENTIFICATION BY DNA PROFILING FOR TRAGEDY EXPLOSION AIRPLANE IN IRAN

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In whole the world a lot of people are killed or lost by natural disasters such as floods, tsunamis, volcanoes, etc, and also intentionally or unintentionally unnatural disasters such as wars, car accidents, air accidents, etc. Among these victims large numbers of them are anonymous, because of intensive burning or other reasons. One of the most accurate methods of personal identification is molecular diagnostic, which includes short tandem repeats (STRs). After explosion of a tragedy explosion airplane full of 28000 litre gasoline in Iran, we cooperated with legal medicine organization of Iran to identify the corpses by comparing the profiles of family members of these corpses. The most important point in this event was taking samples of intensive burnt corpses. DNA was extracted from tooth samples (If there was any), otherwise from muscle tissue samples by RGDE method. After extraction of DNA from extremely burned corpses and their parents, identifiler kit (which has the ability of evaluating 16 loci of STRs in multiplex PCR) was used to prepare profiles. Nowadays, this system is used in identification centres such as police and crisis headquarters offices in the world, so it is unavoidable in our country too.

Keywords: DNA typing, molecular identification, explosion airplane, STRs, RGDE method

Presentation number: FG 36 Abstract number: ABS-260-ISABS-2011

DNA ANALYSIS FROM BURNED HUMAN REMAINS

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In a Hungarian animal shelter the owner who was a British-Hungarian citizen went missing, a few months later the police found some unidentified human remains. Later the investigation stated that an employee hit his boss with an axe handle several times, who succumbed to his injuries. The dead body was laid over a burning garbage and wood heap and covered with flammable material. A few hours later the fire was out, the remains of the corpse was buried at the yard of the shelter. The identification of the charred bones raised many technical difficulties. We cleaned the bones and subjected them to extensive anthropological examinations. To isolate sufficient amount of DNA from such charred remains, we had to find an intact spot for sampling and a suitable isolation method. We optimized Biorobot EZ1 workstation with EZ1 DNA Investigator Kit and Card to successfully purify DNA from charred bones. Modified DNA extraction showed improved performance in downstream STR analysis and the high sensitive STR kit which was able to obtained good quality STR profiles.

Keywords: STR analysis, identifiction, charred bones, modifeid EZ1 DNA investigator kit, antropology

Presentation number: FG 37 Abstract number: ABS-222-ISABS-2011

VALIDATION AND USE OF MTDNA LINEAR ARRAYS IN LARGE SCALE MISSING PERSON'S IDENTIFICATION AT THE ICMP

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The International Commission on Missing Persons (ICMP) was founded in 1996 to address the issue of persons missing as a result of the conflicts that occurred during the breakup of the former Yugoslavia in the 1990's. ICMP routinely types samples from both living family members and skeletal remains from mass graves using short tandem repeat (STRs). To date ICMP has successfully reported approximately 16, 000 DNA matches to person's missing from the region. Although the use of STRs has been largely successful in certain instances additional testing using alternative methods is required. The ICMP is currently validating a technique for mtDNA analysis based on next generation linear array hybridization. These new linear arrays target a total of 65 Single Nucleotide Polymorphisms (SNPs) in both the control region (40 SNPs) and the coding region (25 SNPs) which increases its power of discrimination and utility over the previous version of this assay. We will present an overview of the validation of this technique on both reference samples and challenging skeletal remains. We will also present some results of the utility of this tool with casework examples.

Keywords: mtDNA, linear arrays, SNPs, hybridization, missing persons

Presentation number: FG 38 Abstract number: ABS-296-ISABS-2011

THE IMPORTANCE OF THE PHYSICAL AND MORPHOLOGICAL TRAITS ANALYSIS IN FORENSIC MEDICINE-A COMPARATIVE STUDY BETWEEN TWO LOCALITIES (ORADEA AND NUSFALAU)

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Many physical and morphological traits are determined by the effects of genes and by the environment. In this research we purpose to show the high genetic homogeneous of some phenotypical traits in a female population of Nusfalau locality, Salaj county, comparative with a genetic heterogeneous control group from Oradea locality. These data are important to identify an unknown female dead body. There were studied 300 women of Nusfalau and 300 women of Oradea. We investigated the type of hair and eyes pigmentation, the aspects of lips and hair and the profile of nose, the cranial diameters, body and facial height, weight. We used the visual exploration, the Rudolph Martin schemes, the Topinard's scheme and a vernier callipers.The results are significant. They demonstrate a small variation of these traits in the group of Nusfalau. We already made a database with data from different localities. If we introduce the data of one unknown female dead body in the PC soft made by us, we can identify with a certain probability if she has the specific trait for certain area. Analysis of some physical traits is important in legal medicine to identify the geographic area from unknown dead body proceeds.

Keywords: physical traits, morphological traits, female dead body, Oradea locality, Nusfalau locality

Presentation number: FG 39 Abstract number: ABS-297-ISABS-2011

DATA ABOUT AN ANTHROPOMETRIC COMPARATIVE STUDY IN TWO FEMININE POPULATION OF BIHOR COUNTY, ROMANIA (ORADEA AND SEGHISTEA LOCALITIES)

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This paper is about the differences of fingers lenght in two feminine populations of two different localities. These localities are: Oradea and Seghistea, in Bihor county (Romania). Seghistea is a smaller locality than Oradea, so the genetic variation of some traits is smaller, too. We investigated 100 women from each locality. It were measured the lenghts of the 2nd, 3rd and 4th digits. We investigated 200 women. We measured the length of the 2nd, 3rd and 4th fingers of 100 women from each locality. We measured the length of the fingers, from the finger basis to the superior bound of the phalanx. Then we calculated the average, standard deviation, variances, variation coefficient. Also, we calculated F distribution and z-test. The results are important: the digit lenght are very different in the two studied populations. We may conclude the following: low variability of all fingers lengths in feminine population of Oradea, for both hands. We noticed that the averages are significant different. We can notice also the low variability of finger lengths in the feminine population of Seghistea. We also noticed that there are significant differences between the two hands. In the case of comparison of the two localities averages, the situation is very different than in each population, separately. After z-test calculation, we noticed that the two studied populations are very different, in all cases the obtained values exceeding the 1.96 value which is corresponding to p=0.05.

Keywords: fingers lenght, feminine populations, digit ratio, Oradea locality, Seghistea locality

Presentation number: FG 40 Abstract number: ABS-170-ISABS-2011

THE PROBLEM OF DEVELOPING FORENSIC DATABASES FOR THE URBAN POPULATIONS: INFERENCES FROM GENETIC DEMOGRAPHY

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European urban populations of Russia and CIS were shown to be homogeneous in forensic STR allele frequencies (Zhivotovsky et al.2007, 2009). However, in large city populations, specific genetic demography (population size, ethnic composition, migration parameters, marriage structure) results in peculiarities of allele frequency distribution, and, therefore, is important for developing forensic databases. In the samples from three large city populations (Kharkov, Minsk and Moscow), genetic demographic parameters were studied by special questionnaire data comparative analysis simultaneously with distribution of 18 STR (vWA, TH01, TPOX, CSF1PO, D5S818, D7S820, D13S317, D16S539, F13B, D18S51, D8S1179, D21S11, FGA, PentaE, PentaD, D2S1338, D19S433, D3S1758). For the Kharkov, Minsk and Moscow populations, in-migration coefficients were 0, 546, 0, 405 and 0, 546, correspondingly. For three cities, the main ethnic groups (Belorussian, Russian, Ukrainian) demonstrated differentiation with Fst = 0, 024, the most discriminative alleles being FGA, TH01 and D13S317 (Fst = 0, 062 – 0, 079). For the Minsk population (sample N = 450), 18 STR allele frequency distribution was compared not only with other Slavic urban populations, but, also, with large samples from several Belarus populations (77% of migrants to Minsk were from Belarus), demonstrating no significant differences. Accumulation of unique rare alleles in gene pools of the main ethic groups of the cities studied was in correspondence with values of migration coefficients and distances. Higher genetic variation of forensic STR was proved for the larger urban populations with higher migration coefficients and distances, and more differentiated ethnic composition of migrants, thus, demonstrating inference from genetic demography.

Keywords: STR, forensic database, urban population, genetic demography, migration

Presentation number: FG 41 Abstract number: ABS-264-ISABS-2011

THE POTENTIAL IMPACT OF SECONDARY TRANSFER AND PERSISTENCE OF DEOXYRIBONUCLEIC ACID (DNA) ON FORENSIC CASEWORK

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Due to increases in the sensitivity of DNA analysis it is now possible to recover DNA profiles from handled objects. However, alongside the improvements in the level of detection comes the potential increase in contamination, from known and unknown sources. The aim of this research was to undertake a series of experiments, designed to examine the potential for secondary transfer of DNA. DNA was extracted using the Qiagen Microkit followed by amplification with the AmpFlSTR® Identifiler® or SGM Plus® systems. These studies have indicated that secondary transfer does exist both when the vehicle for transfer is an object or another person's hand. Perhaps more importantly, the results indicated that when an object was handled by two different individuals, and partial profiles were retrieved, the dominant profile was not necessarily that of the final handler. This may suggest that the predominance of a subjects' profile from a handled object is dependent on how well the individual sheds their DNA, and not solely on the order in which the subjects handled the object. This could have a major impact on the information gained from seized objects at crime scenes, as it disputes the theory that the final person to handle an object would be the major contributor to a mixed DNA profile. This presentation will discuss the results of these experiments, further research into DNA transfer and persistence variables, as well as broadly examining the potential impact the findings may have on Forensic Casework.

Keywords: DNA, secondary transfer, persistence, touch DNA, low copy number

Presentation number: FG 42 Abstract number: ABS-154-ISABS-2011

UNUSUAL FINDING IN A PATERNITY TESTING CASE WITH AN XX ALLEGED FATHER

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During routine paternity testing case, a paternal exclusion was found. It called our attention that the alleged father was an XX individual with Amelogenin. A new sample was requested. We were informed that the alleged father had received a Bone Marrow transplantation (BMT) eight years before. His HLA identical sister was the donor. We contacted the alleged father in order to obtain a bucal swap. Once the sample was analyzed, a mixture XX/XY profile was obtained. In the mixture, the obligated paternal alleles that did not exclude the paternity were found. We thought that the sample was a traumatic sample and we proceeded to take new samples avoiding any trauma. Three different samples were obtained, one from the inner right chick, one from the inner left chick and one from the tongue. Two of the three samples showed an identical genetic XX profile as found in the first blood sample without evidence of the XY profile. In the third sample, an XY genetic profile was found with minor peaks for some alleles found in the XX profile. Our results indicate that the post-trasplant chimerism was not limited to the bone marrow derived cells. The stem cells had also replaced a good part of the mouth cells mucosa. Several reports had described genetic microchimerisms after kidney transplantation and blood transfusions. Our results indicate that in paternity testing with history of bone marrow transplantation, exhaustive studies should be carried out, even more, if genetic inconsistencies are found.

Keywords: paternity testing, STR, DNA, chimerism, stem cells

Presentation number: FG 43 Abstract number: ABS-248-ISABS-2011

DNA EXTRACTION FROM HUMAN REMAINS COMPARING AN ORGANIC EXTRACTION METHOD WITH A SILICA BASED METHOD FOR STR ANALYSIS.

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Human skeletal remains represent one of the most challenging samples for obtaining DNA for human identification. Previously, we had compared a standard organic extraction method with a silica-based method as described by Davoren et al. The results showed that the silica based method as published did not improve the quality or quantity of DNA obtained per gram of bone, and that decalcification with EDTA was required in order to obtain complete dissolution of the bone powder (JFS In press 2011). In a second phase we introduced EDTA decalcification steps for the silica based method using identical conditions as for the organic extraction method. Overall, the silica-based method recovered less DNA per gr of bone. Complete Identifiler STR profiles were obtained in 23 of 28 samples (82.1%) with the silica based method (DNA Blood Maxi kit, Qiagen) compared to 26 of 28 (92.8%) for the organic extraction method. In a third phase, we have reduced the amount of bone powder used for the silica based extraction to 0.5gr, and use a single EDTA decalcification step. With these modifications, complete STR profiles were obtained in the silica based method 8 of 24 samples (33.3%) compared to 23 of 24 (95.8%) for the organic extraction method. Our results indicate that EDTA decalcification steps are required in order to obtain higher yields of DNA suitable for STR analysis. Additional testing is required in order to determine if DNA can be obtained from smaller quantities of bone using the silica based method.

Keywords: STR, human remains, bone, DNA, silica

POSTER PRESENTATIONS
Anthropologic Genetics

Presentation number: AG 2 Abstract number: ABS-219-ISABS-2011

SKELETON CHANGES INDUCED BY HORSE RIDING ON EIGHT MEDIEVAL SKELETON REMAINS – KAMEN MOST - KALDRMA (CROATIA)

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Horse role in history was changing through years that contributed to development of morphological changes in horse riders' skeleton, especially if person was riding since childhood. The aim of this research was to confirm that 8 persons from Kamen Most were horse riders as well as 10 femora from Dominican's graveyard. In collaboration of Clinical Department for Forensic Medicine in Clinical Hospital Centre Split and two museums from Split, medieval location Kamen Most-Kaldrma and Dominican's graveyard from St Katarina's monastery (Split, 16th-19th century), were explored during 2008 and 2010. Anthropological analysis was performed by two independent anthropological teams. Sex determination by DNA analysis was done for eight persons from Kamen Most who were suspected to be horse riders. Average age of all eight persons from Kamen Most is estimated at 45.6 years while 70% femora from Dominican's graveyard indicates persons older than 35. Anthropological analysis from Kamen Most determined that all bone fragments, except pelvic bones, indicate male gender. All pelvis have characteristics of horse riders and all femoral fragments have male characteristics as well as characteristics of horse riders. DNA analysis confirmed male gender in seven persons. All 10 femora from Dominican's graveyard have main characteristics of horse riders' bones. Relatively high percentage of horse riders (22.86%) in population Kamen Most and 28.6% horse riders' femora from Dominican's graveyard indicate frequent horse riding, most probably from childhood when human skeleton is submissive to morphological changes. Reliefs on tombstones confirm that these populations probably used horses in economy and military purposes.

Keywords: late medieval, anthropology analysis, horse riders, changes on femur, changes on pelvis

Presentation number: AG 3 Abstract number: ABS-246-ISABS-2011

THE STUDY OF HUMAN SKELETAL REMAINS FROM THE EARLY-MEDIEVAL GRAVEYARDS IN DALMATIA

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Systematic anthropological analysis of the early medieval graveyards from southern Croatia includes 178 skeleton remains that were found within 136 graves. These graves, dated to the 9th century AC, were made from the stone blocks, rectangular-shaped and oriented east-west. Old jewellery and parts of cheramics characteristic for this region of Croatia were found in the graves along with skeletons. Smallest of analysed graveyards, Svećurje has total of 10 graves with 14 individuals while largest, Ostrovica, has 105 graves containing 128 skeletal remains. Long bones, partially destroyed and skull bones were mostly preserved (total of 63 skulls were found). The age analyses of the skeletal remains revealed that the most of them were younger than 45 years (36.05, STDEV 11.20). Anthropological analyses determined gender on 59 male and 47 female skeletons. DNA analyses was performed on skeletons on which sex determination was unsuccesful. Among the poorly preserved maxilla and mandibula remains caries was the most common dental disease as well as visible tooth abrasion. Cribra orbitalia, which points to anaemia and malnutrition, is evident on several skulls. Long bones (femur, tibia and humerus) showed visible signs of periostitis and osteoarthritis. Schmorl's defects were visible on some vertebrates as a sign of degenerative changes.

Keywords: anthropology, early medieval, Croatia, skeletal remains, DNA

Presentation number: AG 4 Abstract number: ABS-282-ISABS-2011

Y-CHROMOSOME GENETIC STRUCTURE IN SLOVAKIAN POPULATIONS BY SNP AND STR ANALYSIS

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Romanies are a peculiar ethnic group whose members are scattered throughout the world. The exact number of Romanies is not known, but it is estimated to be about 10 million in the world. 350, 000 of them living in Slovakia.This study provides additional population genetic data of the three different East Slovakian populations. Many Y-chromosomal single nucleotide polymorphisms (SNPs) are now available. The haplogroups which they define are highly non-randomly distributed among populations. 49 Y-chromosomal single nucleotide polymorphisms (SNPs) and 17 Y-chromosomal STR loci were tested in 645 unrelated male individuals from East Slovakia: Slovak population (n=355) and two Romany populations: Romany group from region Spiš (n=127) and Romanies from region Prešov (n=163). DNA was isolated from the buccal swabs using JetQuick kit according to the manufacturer's protocol. Samples of DNA was amplified with the AmpFlSTR Y filer PCR Amplification Kit and analyzed with a 3500 Genetic Analyzer (Applied Biosystems, USA).The Y-SNP loci were tested with the amplification of 10–12 ng genomic DNA, performed in ABI 7500 systems using TaqMan probes. Haplogroup diversity values were calculated and the populations were compared with G-test. The Slovakian Y chromosomal haplogroups were R1a1-M198, R1b1-P25. In the Romany population from Prešov it were haplogroups H1a-M82, R1a1-M198. In Romany group from Spiš the most frequent haplogroup was J2a2-M67. The H1a-M82 haplogroup was the most frequent in both Romany groups with the frequency as high as 50%. The Romany populations were significantly different in comparison with Slovakian population .

Keywords: Y-chromosome, SNP, STR, Slovakia, Romanies

Presentation number: AG 5 Abstract number: ABS-315-ISABS-2011

GENETICS OF SELECTED INDIGENOUS POPULATIONS IN MEXICO

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Detailed population-based genomic data are lacking for several indigenous groups living in Mexico. Such data are important for scientific and humanitarian purposes, including general studies of human genome diversity, human migration patterns, analysis of ancient DNA, and accurate identification and reunification of human remains. Mexico is an ethnically diverse pluricultural nation with a large percentage of Meztizos, Amerindians and European Caucasians. While Mestizos form the majority of the population in Mexico, modern genomic studies offer the best chance of defining the extent of admixture of those with Amerindian (indigenous) and European origins. The Comisión Nacional para el Desarrollo de los Pueblos Indígenas estimates that there are 62 indigenous groups in Mexico (~12 million individuals, ~10% of the population) living principally in central and southern regions of the country. Most of these individuals speak one of 20 distinct indigenous Mayan, Olmec or Aztec languages. Using contacts in these regions, anonymized buccal swabs were collected (using BODE Technology swabs) from over 1000 individuals belonging to one or more major Mexican language groups from one of several population centers in several major indigenous regions (including Chiapas, Oaxaca, Morelos, Guerrero, Michoacan, Guanajuato and Merida). DNA extraction and thirteen locus STR profiles were determined (ABI kits) at the Center for Genomic Sciences at UNAM in Cuernavaca, Morelos. In addition, mtDNA sequencing was performed on the majority of these samples. Detailed results will be presented comparing STR frequencies in the indigenous subgroup genetics to determine allelic frequencies and the extent of population admixture.

Keywords: indigenous, Mexico, Latin America, mtDNA, population genetics

Presentation number: AG 6 Abstract number: ABS-144-ISABS-2011

ANCIENT DNA ANALYSIS OF SKELETONS FROM A MEDIEVAL BURIAL IN THE ARAGONESE PYRENEES.

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Seven skeletons were found in a medieval burial site located in the Aragonese Pyrenees (Spain) and carbon dated between 780 AD and 985 AD. The results of a study aimed to characterize genetically the discovered human remains are presented. The bones varied in states of preservation as assessed macroscopically. The best quality bones in terms of potential DNA yield were selected from each skeleton (preferentially long bones). For genetic typing, special precautions for the work with ancient DNA were followed. The bones were cleaned thoroughly and pulverized under liquid nitrogen. The bone powder was demineralized with EDTA, and the DNA was extracted using silica-based methods. DNA yield was positively correlated with the collagen content in the bone. Autosomal STR analyses resulted in complete profiles of the samples with greatest DNA yield by using AmpFlSTR® MiniFiler, AmpFlSTR® Identifiler Plus and AmpFlSTR® NGM. Also, the Y chromosome haplogroup R could be determined in two samples based on ABI PRISM® SNaPshotTM analyses of phylogenetic Y-SNP markers. Analyses of the hypervariable segment I of mitochondrial DNA allowed the determination of the haplogroups (H and U5a) in five of the seven samples. The case presented here shows that despite the antiquity of the samples, the combined use of different genetic systems allows the retrieval of valuable genetic information.

Keywords: ancient DNA, STRs, mitochondrial DNA, Y chromosome

Presentation number: AG 7 Abstract number: ABS-174-ISABS-2011

DEVELOPEMENT OF 11 SINGLE NUCLEOTIDE POLYMORPHISMS (SNP) FOR FORENSIC APPLICATIONS

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In the present study, we developed a multiplexes typing mathod for analyzing 11 SNPs and validated with using forensic evidence samples. We analyzed following SNPs: rs41424948 (C/T), rs 2741083 (C/T), rs11082466 (A/G), rs3757853 (C/G), rs3760578 (A/G), rs2738077 (C/G), rs41397044 (C/T), rs45504994 (C/G), rs7617204 (A/G), rs8176044 (A/G), rs7026 (C/T). The forensic evidence samples were recruited from DNA Bank (Molecular Technique Unit of Wroclaw). DNA was isolated from blood samples using the QIAamp DNA Mini Kit (Qiagen). Targeted fragments of DNA were amplified in PCR reaction using commercial Qiagen Multiplex PCR Kit, according to the manufacturer's instructions. The PCR products were purified with shrimp alkaline phosphatase (SAP) and exonuclease I treatment (ExoSAP-IT, USB). Minisequencing reactions were performed using SNaPshotTM Multiplet Kit (Applaied Biosystems)according to the manufacturer protocol. Primer extensions products were analyzed in capillary electrophoresis together with LIZ 12O as an internal standard, on ABI PRISM® 310 Genetic Analyzer (Applaied Biosystems). Results, i.e. observed and expected heterozygosity, Hardy-Weinberg equilibrium (HWE) as well as statistical parameters of forensic interest were calculated. HWE for each SNPs was assessed using CHI2 test. No deviation from HWE was observed for any SNP. We tested 11-SNPs panel on two samples with LCN DNA (Low Copy Number DNA) from touched ojects and 3 samples with degraded DNA (old bones, teeth ect.). In all cases we obtain genetic profiles. The results showed that the 11-SNPs multiplex typing assay could applied in forensic work for analyzing degraded DNA, LCN DNA and provide supplementary data when STRs analysis was partial.

Keywords: SNP, degraded DNA, ancient DNA, forensic analyzing, human identification

Presentation number: AG 8 Abstract number: ABS-114-ISABS-2011

MOLECULAR DIAGNOSIS THROUGH GENETIC TYPING OF SKELETAL REMAINS IN HISTORICAL POPULATIONS OF SITUATED TURKEY

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Skeletal remains can supply important forensic information long after death occurs. Therefore, bone DNA can be invaluable in skeletal identification. For this, a set of burials from a cemetery used over a relatively short period of time was tested. Similar bone types (generally femur, rib, pelvis, and tooth) were collected from skeletons over a range of burial remain states. Methodically, the use of genetic technology in archaeometry is applied primarily to distinguish between individuals who may be the source of biological material associated with archeological remains. DNA sequences from ancient fossils have great potential for studies of phylogeny, biogeography and molecular evolution. For that reason, DNA was extracted from ancient human bones from Mugla in Turkey. Furthermore, all the bone samples which are obtained from burial place to be subjected to DNA isolation and then interspecific sequence polymorphisms in the mitochondrial cytochrome b gene were analyzed by PCR to determine the species origin of Bronze Age animal and human skeletal remains. DNA quantity was assessed using quantitative (real time) polymerase chain reaction (PCR), while DNA quality was estimated by attempting to amplify progressively larger pieces of DNA. The focus of the research was to use a more uniform set of skeletal remains to analyze burial region, historical period, grave type, identification of isolated ancient DNA (aDNA), species and gender. Finally, a series of experiments designed to better isolate and analyze DNA from samples with limited DNA quantity or quality was performed.

Keywords: archeaology, ancient DNA, gender determination, RTPCR, species determination

Presentation number: AG 9 Abstract number: ABS-208-ISABS-2011

Y-CHROMOSOMAL DIVERSITY OF VALACHS FROM THE CZECH REPUBLIC: MODEL FOR ISOLATED POPULATION IN CENTRAL EUROPE

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Evaluation of Y-chromosomal diversity of Moravian Valachs of the Czech Republic and their comparison with Czech population sample as well as other samples from Central and South-Eastern Europe. Effects of genetic isolation and sampling are evaluated. 94 male unrelated donors from Valach region in north-eastern Czech Republic border were sampled. Second sample set of Valachs consists of 79 samples that originate from 7 paternal lineages defined by surname. No close relatives were sampled. Third presented sample set consists of 273 unrelated males from the whole Czech Republic and is utilized for comparison, as well as published data from another 27 populations. Total number of samples is 3244. Y-STR markers were typed by standard methods using PowerPlex® Y System (Promega) and Yfiler® Amplification Kit (Applied Biosystems) kits, Y-chromosomal haplogroups were estimated from the haplotype information. Haplotype diversity and other intra- and inter-population statistics were computed. Our data reveal decreased genetic variability of Y-STR markers in Moravian Valachs compared to other Central European populations. This low diversity resembles isolated Balkan populations (Aromuns, Csango, Bulgarian and Macedonian Gypsies). Genetic distances significantly separate Valachs from surrounding populations (Czechs, Slovaks, Poles, Saxons). We illustrate effect of sampling on Valach paternal lineages, which includes reduction of discrimination capacity and variability inside Y-chromosomal haplogroups. Valach modal haplotype belongs to R1a haplogroup and it was not detected in the Czech population. Moravian Valachs display strong substructure and isolation on their Y chromosomal markers. They represent unique Central European population model for population genetics.

Keywords: Y-STR, Moravian Valachs, isolation, sampling, modal haplotype

Presentation number: AG 10 Abstract number: ABS-149-ISABS-2011

FIRST CONFIRMED EVIDENCE OF LEPROSY IN EARLY MEDIEVAL AUSTRIA

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Leprosy used to be a widespread, dreaded disease in Europe during the middle ages and it still remains an important health problem in some parts of the world today. Herein, we present data on the earliest "Austrian" (an adult female from the medieval period) proven to have suffered from leprosy. Manifestations of the disease were first identified during a systematic screening of pathological changes in human skeletons recovered from an archaeological site in Pottenbrunn (Lower Austria). Skeletal remains were investigated macroscopically, histologically and by the use of a µCT. Extracts from selected bones were subjected to PCR and DNA sequencing using primers specific to the M. leprae-repetitive element (RLEP). Traces of M. leprae DNA were detected in extracts from nasal and palatine bone samples, but not with other bones tested so far - which highlights the importance of careful sample selection for this type of investigation. Characterisation of the M. leprae strain was performed by sequence analysis of informative polymorphic sites: SNP-14676, SNP-1642875 and SNP-2935685. Results from SNP typing support previous reports indicating that European M. leprae strains fall into the SNP group 3. Notably, the lepromatous female had been buried among other individuals, suggesting that she may have not been confronted with strict social segregation during her lifetime and that others may have also been infected. Skeletons from the same population are currently re-screened for pathognomonic features and tested by RLEP-PCR. In summary, these findings put Austria on the map of confirmed leprosy cases in ancient Europe.

Keywords: leprosy, Austria, ancient DNA, RLEP-PCR, SNP typing

Presentation number: AG 11 Abstract number: ABS-142-ISABS-2011

SNP PCRS IN HVS-I REGION OF ANCIENT HUMAN MITOCHONDRIAL DNA REVEALS SPATIAL AND GENETIC DISCONTINUITY OF EAST-ASIAN HAPLOGROUPS IN HUNGARIAN NEOLITHIC

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Analysis of mitochondrial mutations in the HVS-I region is an effective and widespread method now for ancient human populational studies. Discontinuous haplotype data between the first farmers and contemporary Europeans was described before by several research groups. Our contribution is based on a survey initiated on the Neolithic skeletons from Hungarian archaeological sites in the Alföld region of Hungary. This Lowland, the Hungarian Plain, is well excavated as an important region for the spread of Neolithic culture from Near East and Balkans toward Central and Western Europe. This started cc. 8000 years ago and remarkably shaped the forming ancient farming system in Europe. HVS-I sequences from nt15977 to nt16430 of eleven Neolithic specimens from Alföld with sufficient mtDNA preservation were analysed for polymorphisms, identifying 23 different ones. After assigning all SNPs, a novel, N9a, N1a, C5, D1/G1a, M/R24 haplogroups were determined. On mitochondrial control mutations at nt16257 and nt16261, SNP PCRs were carried out to assess their distribution in remains. Neolithic data set was compared to contemporary Vác samples and references, resulting in higher frequency of N9a in Alföld as a remarkable genetic discontinuity. Our investigation is the first to study ancinet mutations from Neolithic of Hungary, resulting in the assignment of Far Eastern haplogroups, very rare in the contemporary European human populations. It is also a further proof of the non-descendant and genetic gap theory of Neolithic.

Keywords: polymorphic PCR, ancient DNA, European neolithisation, mitochondrial control mutation, N9a haplogroup frequency

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APPLYING ANCIENT DNA ANALYSIS TO POST-MEDIAEVAL SKELETONS FROM LATVIA

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Over the past decade molecular genetics approach to investigation of archaeological material has experienced significant increase. It is known that environmental conditions strongly affect ancient DNA (aDNA) preservation. Skeletal collections of Institute of Latvian History have remarkable historical value, making it worthy material for molecular genetics investigations. The aim of this study was to establish whether methods of aDNA analyses were applicable to material from these collections. To achieve the aim we chose to employ molecular gender determination, as gender is one of the first questions asked when investigating a burial. Thus, gender determination is widely used molecular analysis, about which definite experience has been accumulated. Seven human skeletons referring to 16th and 17th century were chosen for our study. DNA was extracted using guanidinium thiocyanate/silica protocol. Two regions of chromosomal DNA were amplified: firstly, fragment of SRY gene, which is located on Y chromosome and therefore only amplifies from male DNA and, secondly, fragment of amelogenin gene, which is located both on X and Y chromosomes and yields different sized PCR products. Gender of five skeletons (three males and two females) has been successfully determined. These results are consistent with gender determined by morphological features. We conclude that it is possible to acquire aDNA suitable for molecular genetics analyses from bones used for our study, and therefore it is worth to continue work in this area, e.g. with mitochondrial and Y chromosome DNA that could give an insight into genetics of ancient inhabitants of Latvia.

Keywords: ancient DNA, sex determination, post-mediaeval skeletons, amelogenin, SRY

Presentation number: AG 13 Abstract number: ABS-178-ISABS-2011

DIVERSITY OF NUCLEAR SHORT TANDEM REPEAT LOCI IN REPRESENTATIVE SAMPLE OF NORTH-EASTERN BOSNIAN POPULATION

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Diversity of nuclear microsatellite DNA markers were analysed in representative sample of north-eastern Bosnia population. It were processing 437 samples taken from unrelated persons and showed three examples of paternity. Effectively of profiles detection in this investigation confirmed valid of choice method extraction, amplification and genotyping STR loci by PowerPlex tm 16 commercial kit (PP 16 Promega) on ABI Prism 3100 Genetic Analyser. Genetic analysis of alleles variants on 15 STR PP 16 loci were detected 17 samples as rare alleles variants or microvariants. Samples were classified in 15 different alleles variants on 7 different loci: D7S820, D16S539, D3S1358 , D18S51, PENTA D, PENTA E and vWA Genetic analysis of mutation in paternity cases were determinated one step mutation in loci: FGA (26->27), Penta D (12->13) and D3S1358 (17->18). Genetic analyses of observed STR loci were determinated tri allelic patterns in D7S820 locus (Type II), which suggested about chromosomal duplication. Population genetic analysis of STR loci in representative sample of north-eastern Bosnia population involved application of tests inter and intra population diversity; genetic differences among population north-eastern Bosnia and representative population of Bosnia and Herzegovina, as like as population of Croation, Macedonia, Serbia and Slovenia. These results of this study are concurred with expected "regional STR date frame" for this part of Europe. This study showed that should to continue with testing a new DNA markers and investigate others local human population with larger numbers of samples

Keywords: STR, diversity, microvariants, tri allelic patterns, mutation

Presentation number: AG 14 Abstract number: ABS-151-ISABS-2011

MITOCHONDRIAL N1A HAPLOGROUP IN SOUTH – EASTERN EUROPE – A MATERNAL LEGACY FROM NEOLITHIC AND BEYOND

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It has been established, based on ancient DNA analysis of early Neolithic skeletons from various Central - European sites, that the frequency of N1a mtDNA haplogroup was 150 times more prevalent among first Neolithic farmers than in contemporary European populations (only 0.2 %), suggesting that first Neolithic farmers did not have strong impact on maternal genetic heritage of modern Europeans. This Neolithic type of N1a haplogroup belong to European sub - branch defined by control region 16147A variant. Here we present the influence of first Neolithic farmers in Southern – Eastern Europe due to finding of N1a mtDNA haplogroup in populations of Croatia and Adriatic Islands as well as Herzegovina, and complete lack of this haplogroup among populations of Serbia, Macedonia, Bosnia, Kosovo and Montenegro. We also present very interesting finding of even more ancestral lineage of N1a haplogroup in Croatian mainland and on two different Islands (Pag and Cres) belonging to African/South Asian branch characterized by control region motif 16147G. This branch is more prevalent in Arabian Peninsula and northern Africa and limitedly present also around Israel, Iran, Turkey and Greece and it is very rare in any European populations. On the Croatian island of Cres, this very unusual N1a lineage of the unknown origin has been recorded. Due to the influence of genetic drift the prevalence of this lineage is around 9.24% in total Island population. To the best of our knowledge this is the most prevalent finding of this haplogroup in any investigated population so far.

Keywords: neolithic farmers, N1a haplogroup, mtDNA, South - Eastern Europe, Croatian population

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MODELLING SEX-BIASED DISPERSAL IN THE PACIFIC

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Investigating the evolutionary processes that underlie the patterns of human genetic diversity seen today is crucial for building an integrative picture of human prehistory in the Pacific. Linguistic and archaeological evidence suggest a predominantly Asian origin for Austronesian-speaking people. In Polynesia, mitochondrial DNA supports this view, but Y-chromosomal markers suggest more indigenous origins. A cultural sex-specific dispersal process of matrilocality, i.e. men dispersing and marrying into their wife's community, has been proposed1 to integrate these contradicting findings. In support, phylogenetic analyses of postmarital residence have shown that early Austronesian societies were matrilocal2. However, these findings have not been tested. In our study we use sex-specific markers to test this hypothesis in a coalescent framework. In the Pacific there also has been a debate on the proper grouping of populations for analyses at a landscape level3. We test different groupings of populations for genetic analyses, based on history, culture, language and genes to find a model that best explains population history. We hope to explore the robustness of drawing inferences about past cultural processes from genetic diversity, and add to knowledge about the peopling of the Pacific.

Keywords: Pacific, Oceania, dispersal, sex-specific, coalescent

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CHARACTERISTICS OF EARLY MEDIEVAL INHIBITANTS OF NAKLICE (SOUTHERN CROATIA) REVEALED BY MITOCHONDRIAL DNA ANALYSIS

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In recent years, anthropology has been using modern genetic analysis in order to elucidate kinship inside ancient populations and to clarify pathways of their migrations and origin. Croatia has numerous archaeological sites from the early Middle Ages which have not been genetically analyzed. Excavation of 19 ancient graves at Naklice (Southern Croatia) has provided unique opportunity to investigate the gene pool ethnogenesis of ancient Southern Croatian population. The aim of this study was to determine the hypervariable region 1 (HV1) sequence of the ancient DNA extracted from the Naklice burial site skeletons and to assign them to appropriate haplogroups. Ancient DNA was extracted from skeletons' teeth/bones found in a graveyard. All samples were analyzed to determine the autosomal short tandem repeats (STR) profile of each skeleton. This was accomplished by sequencing the hypervariable region 1 (HV1) of the mitochondrial control DNA. Mitochondrial HV1 DNA region was successfully amplified from 27 out of 35 skeletal remains. Each of the 27 mtDNAs was compared to the Cambridge Reference Sequence and was assigned to an appropriate haplogroup. Haplogroup assignment revealed that 18 haplotypes belonged to haplogroup H (67 %), 5 (18%) belonged to haplogroup J, 3 (11%) belonged to haplogroup U5 and 1 (4 %) belonged to haplogroup HV. The frequency of haplogroups differs from the frequency of the same haplogroups in living population of Croatia.

Keywords: Southern Croatian, early medieval population, ancient DNA, mtDNA, HV1

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DNA PHENOTYPING OF ANCIENT SKELETAL REMAINS FROM GORZSA BURIAL GROUND

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We optimized Biorobot EZ1 workstation with EZ1 DNA Investigator Kit and Card to successfully purify DNA from 6700 year old bones. We used Qiagen Investigator Decaplex SE PCR Kit to analyze the extracted DNA. Modified DNA extraction showed improved performance in downstream STR analysis and the high sensitive STR kit which was able to obtained good quality STR profiles. We investigated ancient bone samples from the Late Neolithic burial ground of Gorzsa. Gorzsa located in South-East Hungary, the excavation site lies at the confluence of the Tisza and Maros rivers. In terms of absolute chronology, 14C calibrated dates place the occupation of settlement of Hódmezővásárhely-Gorzsa (Tisza Culture) roughly between 4970-4380 Cal BC. Soil and burial condition preserved the relatively stable DNA macromolecules in the skeletal remains, with varying degree of degradation. Our DNA testing method has added a valuable tool for anthropologists investigating ancient bone samples, and analyzing prehistoric extended family clans, family trees.

Keywords: ancient DNA, DNA extraction, STR markers, LCN DNA, Gorzsa

Presentation number: AG 18 Abstract number: ABS-131-ISABS-2011

SECULAR CHANGES IN CRANIOFACIAL DIMENSIONS AND SEXUAL SIZE DIMORPHISM INDEX IN CROATIAN POPULATION

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The aim of this study was to track temporal changes in sexual dimorphic craniofacial dimensions in a Croatian student sample. The research is part of a broader anthropometric research that is monitoring secular changes in craniofacial morphology of Croatian population. Although the study is based only on anthropometric data, it will discuss the impact of genetics and environment on secular changes in craniofacial morphology and sexual dimorphism, with special emphasis on the possible role of epigenetics. A total of 878 Croatian students aged 19-21 years, born between 1982 and 1990, were included in the research during a 10-year period (2000 - 2010). The variables investigated in the research were: cranial length, cranial breadth, cranial height, morphological facial height, facial breadth and the corresponding sexual size dimorphism indexes. A different pattern of changes in craniofacial dimensions was observed between sexes. In the male sample all craniofacial measurements except head circumference showed a significant change. In contrast, females showed significant changes only in head circumference and morphologic facial height. Sexual dimorphism did not display significant changes over time. The human craniofacial complex modifies and develops in response to environmental, hormonal and genetic factors. The study confirms the enduring process of craniofacial remodeling in Croatian population and shows a different response of the male and female craniofacial complex to temporal changes. Secular trends occur too quickly to be attributed to genetic changes. They are under strong environmental influence, and therefore should start to be considered in the context of epigenetics.

Keywords: anthropometry, epigenetics, secular changes, sexual dimorhism, Croatia

Presentation number: AG 19 Abstract number: ABS-278-ISABS-2011

EVIDENCE FOR A HUMAN ORIGIN OF JIVARO INDIANS SHRUNKEN HEADS ARTIFACTS IN POSSESSION OF THE POLISH MUSEUMS

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Novel genetic techniques used in forensic medicine help in resolving some historical myths or mysteries. The aim of the study was to confirm a human origin of the Jivaro Indians shrunken heads artifacts exhibited in two Polish museums. Shrunken heads, known as a Tsantsas, were trophies of headhunting South American Indians, specially prepared to keep facial appearance. However, it used to be very common to offer a fake shrunken heads made off animal ones such as sloths or monkeys, because of high prices and curiosity of travelers. A small samples of the skin were collected from the artifacts. Genomic DNA was isolated by enzymatic digestion and ion-exchange column extraction (A&A Biotechnology). Autosomal STR loci were genotyped using a commercial kit (AmpFlIdentifiler, Applied Biosystems) according to the manufacturer recommendations. Y-STR profile for the male samples was ascertained using Y-Filer (Applied Biosystems). Genotyping of amplifications products was completed using ABI Prism 377 genetic analyzer (Applied Biosystems). Each of the tested samples revealed a partial autosomal STR profile. Moreover, amelogenin products characterizied human sex, in these samples. Autosomal DNA-STR and Y-STR analysis proved that all the studied shrunken heads were of human origin. Three of four subjects were relatives and shared Y-chromosome haplogroup Q characteristic for Indigenous Americans. The fourth artifact was probably forged and its Y-STR haplogroup I2, suggested Southeastern European origin. A mitochondrial DNA study would be of value in confirmation Indian ethnicity of the victims.

Keywords: shrunken head, forensic sciences, Jivaro Indians, STR, authenticity

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THE REAL TIME PCR QUANTIFICATION AND SEX DETERMINATION OF ANCIENT DNA FROM BONES ORIGINATE FROM ZNOJMO- HRADISTE

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The skeletal material used in ancient DNA analysis originates from burial ground Znojmo – Hradiště. This historical site dates back to the age of Great Moravia (8th – 11th century). There are about 70 graves excavated by using sterile preparation methods so the risk of contamination of bones by recent DNA and ancient DNA degradation is minimized. Ancient DNA from tibial bones is extracted by means of the QIAamp DNA mini kit. The protocol for DNA purification is changed in order to get the best way for the highest DNA concentration efficiency. The Plexor HY system (Promega) is used for qRT PCR quantification and male sex determination.

Keywords: ancient DNA, Znojmo Hradiste, real time PCR quantification, sex determination, sterile excavation

Presentation number: AG 21 Abstract number: ABS-213-ISABS-2011

HAPMAP-BASED STUDY OF HUMAN SOLUBLE GSTS: THE ROLE OF NATURAL SELECTION IN SHAPING SNP DIVERSITY OF XENOBIOTIC-METABOLIZING GENES.

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Glutathione S-Transferase enzymes (GSTs; EC: 2.5.1.18) constitute the principal Phase II superfamily which plays a key role in cellular detoxification. GST genes are organized in chromosomal clusters; most of these genes are polymorphic, mainly due to single nucleotide substitutions. Different studies proved significant inter-ethnic differences in GST allelic frequencies but, at present, the role of natural selection in human genetic variability of GSTs is poorly understood. The aim of this study is to investigate the role of natural selection in shaping SNP diversity of soluble GST genes. Using the HapMap database, we analyzed the population differences in the soluble GST genes using the phasing data from unrelated individuals shared among eleven populations in the International HapMap project. Fst-based selection test was applied to HapMap data for detect cytosolic GST loci under selection. Comparisons on GST gene polymorphisms among HapMap populations highlight that ethnicity is an influencing factor of GST genetic variability. Applying a genome scan based on F-statistics, we identify nine SNPs which present F-coefficients that are significantly more different than that expected under neutrality (rs2239892, rs3814309, rs7483, rs1571858, rs929166, rs11807, rs4715344, rs4715354, rs3734431). Our study confirm that GST gene variation reflects human demographic history but it also demonstrate that natural selection could shaped the genetic profile of some GST SNPs. Moreover, the identification of human genome regions, targets of natural selection, may detected candidate genes for complex diseases. Analyzing the literature, we provide complex disease hypothesis (male infertility, embryotoxicity) on the identified GST SNPS.

Keywords: detoxification enzymes, evolution, complex disease genes, genome scan, F-statistics

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ORIGIN AND DISPERSION OF INDO-EUROPEANS: PHYLOGEOGRAPHY OF R1A1 Y-CHROMOSOMAL LINEAGE

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Recent discussion of the prehistoric spreading of the Indo-European language group has generally concentrated on two alternative hypotheses: so-called “Kurgan Culture” hypothesis, which places the homeland of proto-Indo-Europeans to the Steppe of Eastern Europe, and alternative hypothesis of the spread of farmers from the Near East (Anatolia) to Europe in the Neolithic times. Y-chromosomal haplogroup R1a1, lineage is thought to have originated in the Eurasian Steppes north of the Black and Caspian Seas, seems to be associated with the Kurgan culture. Three geographic areas with the highest frequency of R1a1 haplogroup were revealed: Eastern Europe; Southern Siberia and Hindustan where the highest diversity of microsatellite haplotypes was observed. Phylogenetic analysis of microsatellite haplotypes demonstrates the presence of three corresponding major clusters with the age of the generation of haplotytic diversity of 7.2-12.5 ky. The highest diversity in Hindustani is related to the presence of haplotypes of Indo-Pakistani and Southern Siberian clusters in the population from India and Pakistan, probably due to relatively recent migrations from Central Asia. The age of the cluster admittedly brought to Hindustan from Central Asia / Southern Siberia is 3, 9 +/- 1, 3 ky. Probably, the primary center of the generation of diversity and expansion of R1a1a was the territory of the Eastern European Steppe. With the spread of of R1a1 carriers, secondary centers of genetic diversity and population expansions were formed in the Southern Siberia and Hindustan.

Keywords: Y chromosome, phylogeography, R1a1 haplogroup, Indo-Europeans, genetic diversity

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ELEMENTAL STATUS ON EARLY MEDIAEVAL SKELETAL REMAINS: DIET RECONSTRUCTION

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The total absorbed metals dose could be objectively determined by checking the element status in biological samples. Content of heavy metals in a diet could correlate with heavy metals content in human bones; therefore, determining heavy metals concentrations, and their relationship in human bone, could be used to reconstruct the basic diet. One of the goals of this study was to testing metal concentration levels of the bone material excavated from ancient burials for better understanding of medieval living habits. The aim of our study was to determine metal content of 100 mediaeval individuals excavated from Ostrovica and Naklice burial sites (Southern Croatia) and 30 recent human bones. After microwave digestion (CEM, USA Model Mars 5-2004) element content of manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), calcium (Ca) and strontium (Sr) were measured with an Atomic Absorption Spectrometer. Mercury concentration was determined by direct consecutive measured by mercury analyzer. When comparing results from ancient to the modern bone heavy metal concentrations, it is obvious that Cu, Ca and Sr greatly exceeded the values while concentration of Pb, Cd and Hg was lower. Concentration of Sr and Ca was a few times higher than in modern bone samples. In correlation with elemental contents of various main food components known to have been used during the Early Middle Ages, we could reconstruct a menu. With exception of milk, all main dietary components, such as roots and tubers, meat, cereals, legumes and leafy vegetables are rich in Zn, especially the vegetables.

Keywords: ancient bones, mediaeval period, elemental status, diet, atomic absorption spectrometry

Presentation number: AG 24 Abstract number: ABS-148-ISABS-2011

MITOCHONDRIAL DNA REVEALS STRONG INFLUENCE OF EVOLUTIONARY FORCES ON THE GENETIC STRUCTURE OF ISLANDS MLJET AND LASTOVO

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Eastern Adriatic Islands are examples of Croatian population isolates with low genetic diversity resulting from geographic and reproductive isolation and high inbreeding level. We investigated the distribution of mtDNA haplogroups and their frequencies in two Croatian insular populations (Islands of Mljet and Lastovo) and one continental population (city of Dubrovnik). Since these two islands are part of Dubrovnik archipelago, it was interesting to compare the obtained results. We analysed 300 mtDNAs based on high-resolution analysis of SNPs from the control and coding region (68 from Mljet, 51 from Lastovo and 181 from Dubrovnik) and our results confirmed strong influence of evolutionary forces on the genetic structure of isolated populations. Although all typical European haplogroups were represented in the sample, a significantly higher prevalence of certain haplogroups and their subclades was detected - haplogroup H in general (73.5%) and H1b clade (3! 0.9%) on Mljet, U haplogroup in general (23.5%) and U1 clade (5.9%) on Lastovo, in comparison with the mainland sample of Dubrovnik, where haplogroup distribution and frequencies correspond to results from other European and Croatian mainland populations. Haplotype analysis revealed six possible founder lineages on Lastovo and only two on Mljet, accounting for almost half of its sample. Also, the Island of Mljet has the lowest reported gene diversity index among Croatian isolates. We can conclude that the observed impact of evolutionary forces in such small populations can cause significant departures from expected haplogroup frequencies and help us to understand still unrevealed parts of our demographic history.

Keywords: island isolates, mitochondrial DNA, mtDNA, haplogroups, genetic drift, gene diversity

Presentation number: AG 25 Abstract number: ABS-150-ISABS-2011

DIVERSE MATERNAL LEGACY OF TWO EASTERN ADRIATIC ISLANDS

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This study aims to portray genetic differences of mtDNA lineages between populations of two remote and isolated eastern Adriatic islands in order to show how microevolutionary processes can form different population structures. We investigated populations of two geographically distinct islands, the Island of Susak in north-eastern Adriatic which was compared to a mid-eastern Island of Dugi otok. Both islands are open-sea islands and remote to mainland, both are divided from mainland by other islands, and both have been influenced by nearest mainland and/or other islands at the time of peopling. We analysed 149 mtDNAs based on high resolution analysis of SNPs from the control and coding region (64 from Susak and 85 from Dugi otok). Results show a relatively high diversity of haplogroups on Dugi otok: 6 haplogroups and 24 subhaplogroups (41.2% of H haplogroups, 20% of J haplogroups, 18.8 of U haplogroups and 13% of other haplogroups). Susak, however, revealed only 3 haplogroups and 9 subhaplogroups (34.4% of H7 subhaplogroup, and 65.6% in total of H haplogroups, 32.8 of T haplogroups and 1.6% of W haplogroups). Haplotype analysis showed 40 different haplotypes on Dugi otok, and, consequently to haplogroup analysis, Susak showed only 16 different haplotypes. Also, results revealed some private haplotypes found only on these islands, and not in other populations of Croatia and South-Eastern Europe. We wanted to emphasise events such as founder effect, bottleneck, isolation and various historical, geographical and sociocultural factors as means in shaping of genetic history of contemporary populations on Eastern Adriatic islands.

Keywords: Island of Susak, Island of Dugi Otok, mtDNA, founder effect, isolation

POSTER PRESENTATIONS
Medical Genetics

Presentation number: MG 4 Abstract number: ABS-257-ISABS-2011

GENOTYPING OF TLR2 AND TLR4 GENES AND KIDNEY TRANSPLANTATION

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Toll like receptors (TLRs) are crucial molecules for activation of adaptive immune system. We analyzed the distribution of TLR2 (T/C1350) and TLR4 (Asp299Gly) SNPs among 53 kidney transplanted patients, determined by Real time PCR with TaqMan probes. According to the obtained genotype patients were grouped into wild type or heterozygous group. Regarding the TLR4 gene, there were 5 heterozygous patients and 48 wild-types and for TLR2 gene there were 6 heterozygous and 47 wild type patients. Data on history of kidney function and immunosuppressive therapy were taken from medical records. Median time after transplantation was 8 years (min. 3, max. 24). Univariate analysis showed significant difference in serum urea concentration between wild-types and heterozygous patients for TLR2 gene and for TLR4 gene, respectively. Patients with wild type TLR2 and those with wild type TLR4 had higher urea concentrations, for TLR2 median 9.9, 3.8-58.3 mmol/l vs 6.2, 4.9-12 mmol/l; z=-2.076, p=0.037, and for TLR 4 median 9.9, 3.8-58.3 mmol/l vs 5.5, 4.0-9.9 mmol/l; z=-2.313, p=0.018 (Mann-Whitney test). Those TLR2 and TLR4 gene SNPs related differences in urea were not confirmed by multivariate analysis, when taken urea and time after transplantation as covariates, even though the patients with wild type TLR2 and TLR4 did not differ from heterozygous in the time after transplantation by univariate statistical analysis, respectively. Heterozygosity of the analyzed SNPs of TLR2 and TLR4 gene might still be protective regarding urea level in kidney transplanted patients. Further research in larger group is needed to confirm these findings.

Keywords: genotyping, toll like receptors, kidney transplantation, immune response, kidney function

Presentation number: MG 5 Abstract number: ABS-130-ISABS-2011

GENETIC AND EPIDEMIOLOGICAL CHARACTERISTICS ATOPICAL DISEASES AT THE KHARKOV POPULATION

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To investigate correlation intersexual genetic and epidemiological parametres optimising clinical diagnostics of bronchial asthma (BA) and atopic dermatitis (AD). We have investigated 119 probands with BA from 46 (38, 7 %) men and 73 (61, 3 %) women at the age from 18 till 85 years; 297 patients with AD, from which 130 (43, 7 %) men and 167 (56, 3 %) women at the age from 0 till 80 years and older. The probability to be ill by the end of life was calculated as the cumulative diseasing (populations frequency). Was estimated angular transformation φ, χ2 on a significance value 0, 05. From 119 observed probands with BA at 66 (56 %) patients was revealed skin signs atopy. Calculated population frequencies have shown that for men of the Kharkov population they make 3, 87 %, and for women - 3, 12 %, (p <0, 05); for BA at men - 7, 7 %, and at women - 8, 7 %, (p <0, 05). Investigating of frequency AD among patients with BA has shown that among men it has made 4, 6 %, and among women - 5, 2 % that is authentic above populations frequencies AD (p <0, 05). Presence the AD is regarded as the factor of high risk of development BA. Taking into account genetic heterogeneity of atopy the alleles dose predetermines development of its forms and disease severity level. Women in most degrees, than man's requires of formation of heavy currents.

Keywords: bronchial asthma (BA) , atopic dermatitis (AD), population, genetics, epidemiology

Presentation number: MG 6 Abstract number: ABS-111-ISABS-2011

GENETIC VARIATIONS IN ACTN3 AND FTO GENES ARE ASSOCIATED WITH FAT ACCUMULATION

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Obesity is a complex process influenced by both social and genetic factors. While the polymorphism of FTO (rs9939609) was a subject of many research, the polymorphism of the gene ACTN3 (rs1815739) has never been studied in the context of obesity. Purpose To find associations of SNP of the genes FTO (rs9939609) and ACTN3 (rs1815739) with anthropometric characteristics of fat accumulation and distribution. Material 300 white European males were studied; mean age 23.0±5.8 years. Evaluation of fat development included measurements of skinfold thickness at 8 points and bioimpedance analysis ("ABC-1 Medass" was used (Nikolaev et al., 2004). Results Carriers of the genotype AA of FTO gene were characterized by much larger values of skinfolds at subscapular area (p=.05), triceps (p=.007), forearm, abdomen and thigh (p<.005), than genotypes AT and TT carriers. Also individuals with the genotype AA have much larger arm and thigh circumferences. Carriers of the genotype TT of ACTN3 gene demonstrated much larger values of subscapular skinfolds (p< .05), as well as triceps (p= .05), abdomen and thigh skinfolds (p< .05); they were also characterized by much larger body weight. Conclusions Polymorphisms of the genes ACTN3 and FTO are associated with fat accumulation. The presence of homozygous genotype at a mutant allele (AA of FTO gene; TT of ACTN3 gene) results in bigger fat accumulation as compared with heterozygotic genotype and homozygotes at a normal allele. Thus, humans carrying genotypes AA and TT have greater risk of obesity development.
Support of Russian Foundation of Basic Research (grant # 10-06-00582-a).

Keywords: ACTN3, FTO, obesity, polymorphism, bioimpedance analysis

Presentation number: MG 7 Abstract number: ABS-201-ISABS-2011

GENOME-WIDE META-ANALYSIS OF BRACHIAL CIRCUMFERENCE

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Brachial circumference (BC), also known as upper arm or mid arm circumference, can be used as an indicator of muscle mass and fat tissue, which are differently distributed in men and women. Analysis of anthropometric measures of peripheral fat distribution such as BC could help in understanding complex phenotypes such as obesity. The purpose of this study is to identify genetic variants associated with BC through a large-scale genome-wide association scan (GWAS) meta-analysis. To identify sex-specific effects underlying BC we used fixed-effects meta-analysis to synthesise summary results from 14 GWAS on 8,961 males, 9,792 females and on the combined set of 18,753 individuals. Individual studies carried out two sets of analyses using linear regression and an additive genetic model: adjusted for age and adjusted for age and BMI. Out of the 6 stratified meta-analysis, we observed an excess of signal for the female-specific age-and-BMI adjusted stratum (4 SNPs with p-values<1x10-6 observed, versus 2.4 SNPs expected under the null). We also found more signals for the age-and-BMI adjusted meta-analysis across males and females (37 SNPs with p-values<1x10-5) compared to the null hypothesis of no association (24 SNPs expected under the null). 24 SNPs have been taken forward for de novo genotyping in independent sample sets. In this first GWAS meta-analysis for BC to date, we have identified promising signals within the power constraints of this study, which we are following up by attempting replication in independent datasets.

Keywords: brachial circumference, mid arm circumference, genome wide association scan meta-analysis, single nucleotide polymorphism, quantitative trait

Presentation number: MG 8 Abstract number: ABS-113-ISABS-2011

GENE-GENE INTERACTIONS MAY CONTRIBUTE TO THE RISK OF DEVELOPING MELANOMA AND BASAL CELL CARCINOMA

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The risk of developing skin cancers is dependent on environmental factors such as UV radiation as well as personal genetic predispositions. It has been shown that some pigmentation genes may be associated with susceptibility to melanoma (MM) and basal cell carcinoma (BCC) independently of their influence on eye, hair and skin coloration. Our previous study showed that polymorphisms in the MC1R gene significantly affect risk of developing skin cancers. Besides the main effects of individual genes, interactions between genes should also be considered when assessing the general influence of the genetic factor on susceptibility to diseases. Here we assessed the role of 24 polymorphisms located in 11 known pigmentation genes, including MC1R, in cutaneous melanoma and basal cell carcinoma development in a Polish population. The study population consisted of 718 males and females and included 116 patients with melanoma and 112 patients with basal cell carcinoma. Logistic regression was used to test for significant associations between the analyzed polymorphisms and skin cancers. Gene-gene interactions were examined using the multifactor dimensionality reduction method and the detected epistatic effects were additionally evaluated by logistic regression. Polymorphisms within MC1R, TYR and SLC45A2 were found to be associated with MM, while variation within IRF4 and ASIP showed association with BCC. The study revealed significant synergistic interaction between MC1R and TYR and between SLC24A4 and KITLG, both affecting risk of MM. It was also detected that synergistic interaction between MC1R and SLC24A4 may affect development of BCC.

Keywords: melanoma, basal cell carcinoma, genetic risk, pigmentation genes, epistasis

Presentation number: MG 9 Abstract number: ABS-145-ISABS-2011

GENETIC POLYMORPHISM OF XENOBIOTIC BIOTRANSFORMATION ENZYMES AND PREDISPOSITION TO DIFFERENT ONCOPATHOLOGY

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Xenobiotic biotransformation enzymes (XBE) are involved in metabolism of chemical substances, for many of which an etiological relation to oncopathology is shown. As a result of reduced metabolite detoxication in xenobiotic biotransformation reactions, the probability of damaging “critical genes”, directly involved in carcinogenesis development, is increased. The aim of the study was to investigate the influence of genetic XBE polymorphism on forming of predisposition to emergence of lung- and ovarian cancer. The study comprised 118 lung cancer patients and 108 ovarian cancer patients and the case-control. PCR-RFLP and multiplex PCR methods were used for studying polymorphism of genes: GSTT1 (deletion), GSTM1 (deletion), NAT2 (C481T, G590A and G857A) and MDR1 (C3435T). The examined individuals were divided into “fast” and “slow” acetylators according to the results of genotyping for three NAT2 sites. The association of genotypes with predisposition to cancer was estimated by computing odds ratio and 95%confidence interval. Association of GSTT1(-)/3435CCMDR1 genotypes combinations with predisposition to lung cancer was detected in residents of Belarus, especially when the carrier of such a combination is a “slow” acetylator. An increased risk of ovarian cancer emergence was revealed in women with the haplotype 481CC/590GG/857GG of NAT2 gene (“fast” acetylators) and homozygous deletion in GSTM1 gene. Thus, genetic polymorphism of the detoxication system enzymes affects the risk of ovarian- and lung cancer emergence, with the genetic etiology component of these diseases being considerably different, i.e. predisposition to one or another cancer is characterized by their own combinations of polymorphic variants in the studied genes.

Keywords: genetic polymorphism of xenobiotic biotransformation enzymes , lung cancer , ovarian cancer, predisposition to cancer, PCR-RFLP and multiplex PCR methods

Presentation number: MG 10 Abstract number: ABS-159-ISABS-2011

GANGLIOSIDE GM3 SYNTHASE GENE IS DOWN-REGULATED IN WHITE BLOOD CELLS OF PTSD PATIENTS

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Gangliosides are sialic acid bearing glycosphingolipids and sialic acid residues have important role in recognition events in immune cell and immune response pathways. Blood immune cells are directly and indirectly under control of hypothalamus-pituitary-adrenal (HPA) axis. Alterations in HPA signaling and immune system are characteristic of the posttraumatic stress disorder (PTSD). Interaction between psychological processes, nervous and immune system in PTSD is poorly understood. We studied expression of genes for three ganglioside sialyltransferases: ganglioside GM3 synthase (ST3 beta-galactoside alpha-2, 3-sialyltransferase 5 - ST3GAL5), ST3 beta-galactoside alpha-2, 3-sialyltransferase 2 (ST3GAL2) and ST6 (alpha-N-acetyl-neuraminyl-2, 3-beta-galactosyl-1, 3)-N-acetylgalactosaminide alpha-2, 6-sialyltransferase 3 (ST6GALNAC3), in white blood cells of 117 PTSD-affected and 52 PTSD-unaffected veterans. We established down-regulation of ST3GAL5 gene in PTSD patients ($P<0.001$). ST3GAL5 primarily regulates amount of ganglioside GM3, ubiquitous ganglioside in different tissues cells' membranes and precursor of almost all complex gangliosides. Due to important role of ganglioside GM3 and complex gangliosides in immune cell recognition processes, down-regulation of ST3GAL5 may have important functional implications and reflect either predisposition, or be a pathophysiological consequence of PTSD. We further demonstrate that ganglioside GD1α, enzymatic product of ST6GALNAC3, might have important role in white blood cells.

Keywords: ganglioside, stress, sialylation, glycosylation, immune cell

Presentation number: MG 11 Abstract number: ABS-371-ISABS-2011

DENTAL AGE ESTIMATION USING ORTHOPANTOMOGRAMS FROM CHILDREN IN CROATIA AND BOSNIA- HERZEGOVINA

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The aim of this cross-sectional retrospective study was to compare the accuracy of three radiographic methods for age estimation using orthopantomogram radiographs (OPGs) from developing children. OPGs of 2652 children (1214 boys and 1438 girls, aged 5.22–14.92 years) with 1474 children from Croatia and 1178 children from Bosnia and Herzegovina were examined and seven mandibular teeth from left side of mandible were assessed using Cameriere's method by measurement of open apices in teeth, mineralization stages of four different teeth from right side of jaws using Haavikko's adopted method based on Finnish children and Demirjian's mineralization stages of seven teeth from left side of mandible for Willems' method with updated scoring based on Belgian children. The mean difference(±SD) in years between dental and chronological age (DA-CA) was calculated for each method. Results show that the most accurate method was by Cameriere (boys -0.08 ± 0.71, girls -0.03 ± 0.70 years), Haavikko's method underestimated age more (boys -0.09 ± 0.80, girls -0.34 ± 0.75 years), while Willems' method overestimated age (boys 0.51 ± 0.79, girls 0.28 ± 0.83 years). According to the analysis of variance, no statistically significant differences were found in results of DA-CA between children from Croatia and Bosnia and Herzegovina for each method. No statistically significant difference of DA-CA was found between boys and girls for Cameriere's method, for Haavikko and Willems method there were statistically significant differences between genders. Statistically significant differences were found in results of DA-CA among different age groups for each method. Published results could be used in clinical, forensic and anthropological purposes when sample of children was used from specified countries.

Keywords: forensic dentistry, age estimation, developing teeth, Croatia, Bosnia and Herzegovina

Presentation number: MG 12 Abstract number: ABS-147-ISABS-2011

FORENSIC DNA PROFILING OF HUMAN BONE MATERIAL BY DIRECT PCR

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Current standard protocols for DNA extraction from bone material are often time-consuming and labour-intensive and therefore not amenable to genetic screenings with large numbers of samples involved. Aim of this study was to evaluate the feasibility of a direct PCR approach for forensic investigations of skeletal remains. A part of a human femur was mechanically and chemically cleaned and grinded to fine powder. DNA was extracted after steps of decalcification and lysis by proteinase K. Aliquots from bone powder and post-decalcification material were subjected to direct PCR amplification of the mitochondrial hypervariable sequence 1 (HV1) applying either a so-called “direct” protocol or a “dilution” protocol. PCR products of the expected size were purified and analysed by DNA sequencing. Diluted post-decalcification material could be used for mitochondrial DNA (mtDNA) typing by direct PCR and nuclear short tandem repeat (STR) profiling by multiplex PCR. By combining direct PCR with “nested”PCR, it was further possible to perform mtDNA profiling of human bone powder without the need of prior DNA extraction or even decalcification. In recent years, novel commercial kits with recombinant DNA polymerases and improved buffer chemistry have recently been introduced for the analysis of samples such as blood, tissue (animal, plant), bird feathers and formalin-fixed paraffin-embedded tissues by direct PCR. Based on our findings, we conclude that the direct PCR approach may also constitute a real option for preliminary genetic testing of skeletal remains, especially for screening large numbers of bones (e.g. mass graves) or when given time-windows are narrow.

Keywords: direct PCR, skeletal remains, forensic DNA Profiling, mitochondrial DNA (mtDNA), short tandem repeats (STRs)

Presentation number: MG 13 Abstract number: ABS-168-ISABS-2011

ANALYSIS OF VHL ALTERATIONS IN CLEAR CELL RENAL CARCINOMA PATIENTS FROM BASHKORTOSTAN REPUBLIC OF RUSSIA

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Renal kidney is a heterogeneous group of malignant tumors, the vast majority of which are renal cell carcinomas of various morphological types. One of clear cell renal carcinoma (CC-RCC) features is the inactivation of tumour suppressor gene VHL (von Hippel-Lindau). The aim of investigation was to analyze somatic mutations in VHL gene in 72 CC-RCC patients from Bashkortostan Republic. SSCP analysis followed by direct DNA sequencing was used to investigate somatic VHL alterations. Mutations of VHL have been identified in 10 of 72 samples (13.9%) with CC-RCC. All the identified mutations were somatic and found only in tumor tissues that allowed us to exclude a hereditary von Hippel-Lindau syndrome and consider all cases as sporadic forms. Eight types of mutations were detected in 10 CC-RCC patients: 2 insertions and 8 point mutations in the heterozygous state. Insertions resulted in frameshifts. In the 2nd exon of the VHL gene we revealed: p.His115Tyr (in 1.4% of patients), p.Asp143Glu (1.4%) and p.Pro154Leu (2.8%), which change the structure of the binding of HIF-1α in β-domain. In the 3d exon of the VHL gene we revealed mutations: p.Val170Phe at 1.4% of patients, p.Arg176Trp (1.4%), p.179_Leu178dup (1.4%), p.Glu186_Asp187ins (2.8%), p.Glu186Lys (1.4%), which involve a violation of section interaction elongin C α-domain. We also revealed somatic mutations p.Trp117Arg (1.4%), p.Leu128Val (1.4%) and p.His110Pro (1.4%) that haven't been described previously. Mutations of VHL gene result in stabilization of hypoxia-inducible factors, and may contribute to cancer progressions, that allow to consider VHL gene as a prognostic marker of RCC.

Keywords: renal cell carcinoma, VHL gene, mutations, sporadic cases, tumour

Presentation number: MG 14 Abstract number: ABS-184-ISABS-2011

STUDYING FREQUENCIES AND INTERACTION OF ALLELES C677T AND A66G IN DISTRIBUTION FOR COMPOUND GENOTYPES MTHFR/MTRR OF FOLATE CYCLE GENES IN EARSTERN UKRAINE

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According to a pilot study (Matalon et. al., 2007), Ukrainian population may have a higher frequency (0.57) of allele 66G MTRR. Polymorphisms interaction C677T and A66G may lead to enhancement or compensation of phenotypic manifestations each of them individually. Thereby, we observed more extensive group of patients (1238) with inherited diseases and the polymorphisms, suggesting interaction on phenotype level. SNPs in MTHFR and MTRR were screened by allele-specific PCR. The gene fragments were visualized in 3% agarose gel. The being studied and expected frequencies of alleles and allele compounds of C677T MTHFR and A66G MTRR were calculated. Allele frequency 677T is 30.0% and allele frequency 66G is 57.0%. Difference between being studied and expected frequencies were observed for 2 from 9 compounds with confidence interval > 95 %. The being studied of compound frequency C677T Htzg/A66G Hmzg (17.0%) was higher than expected frequency (12.9±3.6%), and heterozygotes frequency C677T N/A66G Htzg (20.1%) was less than expected (25.8±4.0%). Compound C677T Htzg/A66G Hmzg may be maintained by natural selection due to mutual biochemical compensation of mutant alleles, since it is known, that decreased activity of MTHFR leads to low/normal level of folic acid, while MTRR is to high/normal level.

Keywords: folate cycle, MTHFR, MTRR, Ukraine, allele frequency

Presentation number: MG 15 Abstract number: ABS-175-ISABS-2011

SPECTRUM AND FREQUENCY OF MFN 2 GENE IN HEREDITARY MOTOR AND SENSORY NEUROPATHY PATIENTS FROM BASHKORTOSTAN REPUBLIC (RUSSIA)

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Hereditary motor and sensory neuropathy (HMSN) is a clinically and genetically heterogeneous disorder of peripheral nervous system. The HMSN frequency in Bashkortostan Republic (BR) is 10, 3:100000. We examined HMSN patients from BR and detected spectrum and frequency of specific mutations in mitofusin 2 (MFN2) gene - cause the axonal subtype of CMTIIA. The MFN2 gene (1p36.22) codes a protein mitofusin 2 - an important factor in the fusion of mitochondria. Molecular-genetic investigation of HMSN in 165 unrelated families showed 9 different nucleotide changes in the MFN2 gene. Four of them haven't been described previously. Four nucleotide changes are supposed to be disease causing mutations: c.776G>A (p.Arg259His) (0, 6% among all HMSN types in total of patients sample) and c.2113 G>A (p.Val705 Ile) (1, 2%) - previously described mutations, c.775C>T (p.Arg259Cys) (0, 6% in total sample of patients, 2, 1% - among patients of Tatar ethnic origin) and c.2171T>C (p.Ley724Pro) (0, 6% in total sample, 2, 1% - among Bashkirs) - new mutations, not detected among healthy family members and controls (n=80). Five revealed nucleotide changes appeared to be gene polymorphic variants: c.892G>A (p.Gly298Arg), c.957C>T (p.Gly319Gly) and c.1039-222t>c - described polymorphisms, c.175+28c>t and c.2204+15t>c - new nucleotide changes in gene introns. It is found that the contribution of HMSN IIA type to general structure of HMSN among patients from BR is 5, 1%. The received data will contribute to optimize medical and genetic consulting of HMSN families in our region.

Keywords: hereditary motor and sensory neuropathy, peripheral nervous system, mutations, MFN2 gene, polymorphisms

Presentation number: MG 16 Abstract number: ABS-135-ISABS-2011

EVALUATION OF ANTIOXIDANT TREATMENT AS A NEW PREVENTION MODEL FOR KIDNEY STONE FORMATION

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Urolithiasis is a disease characterized by formation of solid deposits in the urinary tract. The numerous causes may lead to kidney stone formation. However, most kidney stones are predominantly composed of calcium oxalate which predominantly generates free radicals that are damaging to the renal tubular cells. To study the toxic effect of oxalate to renal tubular cells and possible inhibition of oxalate caused oxidative stress by antioxidant treatment. Madine-Darby canine kidney cells (MDCK cells) were used as in vitro model of urolithiasis. Oxidative stress was induced by exposure of MDCK cells to sodium oxalate (NaOX). In order to prevent oxidative stress MDCK cells were pretreated with different concentrations of L-arginin, an antioxidant. The cytotoxicity of oxalate against MDCK cells was determined by MTT Assay and by light microscopy. The oxidative stress was evaluated by expression of superoxide dismutase (SOD) by RT PCR using RNAeasy kit (Qiagen) and standard protocol. In the cells treated with NaOX only, observed necrosis was proportional to the concentration of NaOX. Interestingly, cells treated with L-arginin prior NaOX exposure showed lover levels of necrosis by light microscopy as well as by MTT assay. Positive correlation of SOD expression was observed in all groups of cells by RT-PCR. Our results suggest that L-arginin is able to hamper oxalate induced oxidative stress generation in kidney cells and as such may play role in prevention of urolithiasis. More studies are needed to further evaluate its potential as a prevention agent of urolithiasis.

Keywords: urolithiasis, l-arginin, MDCK-II, prevention, oxidative stress

Presentation number: MG 17 Abstract number: ABS-167-ISABS-2011

MULTIPLEX LABELLING WITH ANILINE AND 2-AMINOBENZAMIDE ENABLE PARALLEL CHROMATOGRAPHY ANALYZES OF DIFFERENT N-GLYCAN SAMPLES

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The complexity of branched glycan structures composed of similar monomeric units hinders their analysis and the knowledge about biological functions of glycans is lagging far behind the knowledge about other macromolecules. HPLC is one of the principal analytical tools for glycan analysis, but complex biological samples require long runs that limit analytical capacity. One of the most widely used fluorescent labels for glycans is 2-aminobenzidine. Here we show that glycans labelled with aniline and 2-aminobenzamide can be simultaneously analyzed on hydrophilic interaction columns since they have non-overlapping excitation and emission spectra, and the attached labels have very similar influence on the retention times of glycans. This method can make glycan analyses faster and also enable introduction of internal standards.

Keywords: N-glycan, anilin, 2-aminobenzamide, parallel chromatography analyses, multiplex labelling

Presentation number: MG 18 Abstract number: ABS-118-ISABS-2011

STUDY ON MUTATIONS IN GENE OF BETA-MYOSIN HEAVY CHAIN IN HYPERTROPHIC CARDIOMYOPATHY PATIENTS IN BELARUS

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One of the widespread hereditary diseases of the cardiovascular system is hypertrophic cardiomyopathy (HCM). The gene MYH7, encoding the heavy chain of cardiac beta-myosin, is one of the major candidate genes of this disease. HCM was proved to have population specificity of genetic anomaly distribution. A spectrum of various mutations responsible for disease development is different in populations. This determines the necessity for studying the mutation spectrum of the gene MYH7 in every individual population. Therefore the aim of our work was to study mutations of the gene MYH7 in HCM patients in the Belarusian population. The study comprised 30 unrelated patients with a diagnosis HCM undergoing treatment in the Republican Scientific and Practical Center of Cardiology. Search for mutations was carried out in the 13th and 23rd gene exons of the beta-myosin heavy chain by the DNA sequencing method with automatic analyzer ABI Prism 3130. As a result of sequencing one missense-mutation was detected in the 23rd exon of the gene MYH7 resulting in amino acid substitution Glu->Lys in the 924 position. No mutations were revealed in the 13th exon in the HCM patients. Thus, mutation in the 13th and 23rd exons of the gene MYH7 occur very rarely in Belarusian HCM patients. We shall carry out further investigations including search for mutations in other exons of the gene MYH7. There is also the necessity for studying genes encoding other proteins involved in the cardiomyocyte sarcomere composition and responsible for development of the given disease.

Keywords: hypertrophic cardiomyopathy, beta-myosin heavy chain , sequencing method , mutation, gene MYH7

Presentation number: MG 19 Abstract number: ABS-214-ISABS-2011

GENETIC POLYMORPHISM OF DETOXICATION ENZYMES AND REDISPOSITION TO BRONCHIAL ASTHMA IN CHILDREN

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It is assumed that genetically determined differences in metabolism rate of substrates of exogenous and endogenous origin can underlie unequal susceptibility to some diseases including bronchial asthma (BA). In this connection the study on the gene system of xenobiotic biotransformation enzymes (XBE) is of current concern. The aim of the study was to investigate association of gene polymorphism of detoxication enzymes glutathione-S-transferases (GST) and N-acetyltransferase-2 (NAT2) with predisposition to BA in children. A total of 175 children suffering from atopic BA and 120 persons from the control group were examined. PCR-RFLP and multiplex PCR methods were used for studying polymorphism of genes: GSTT1 (deletion), GSTM1 (deletion), GSTP1 (A313G) and NAT2 (C481T, G590A and G857A). The examined individuals were divided into “fast” and “slow” acetylators according to the results of genotyping for three NAT2 sites. The association of genotypes with predisposition to BA was estimated by computing odds ratio and 95% confidence interval. A statistically significant protective effect for BA development was revealed in the carriers of the 481CT/590GA/857GA haplotype of NAT2 gene (“slow” acetylator) as well as in the carriers of the GSTP1(313AA)/GSTT1(+)/GSTM1(-) genotype combination. The combination of genotypes GSTP1(313GG)/GSTT1(-)/GSTM1(-) occurred only in the group of BA patients. An increased risk of BA emergence was also revealed in individuals with the 481CC/590GA/857GG haplotype of NAT2 gene (“fast” acetylators). The result obtained allow conclusion that, genetic polymorphism of the detoxication system enzymes affects the risk of childhood asthma emergence.

Keywords: childhood asthma, genetic polymorphism of detoxication enzymes, glutathione-S-transferases, N-acetyltransferase-2, genetic predisposition to BA

Presentation number: MG 20 Abstract number: ABS-243-ISABS-2011

PROGNOSTIC MARKERS BY INTERPHASE FLUORESCENT IN SITU HYBRIDIZATION (FISH) FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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CLL is clonal proliferation of B lymphocytes. The diagnosis is done by bone marrow biopsy and immunophenotyping. However, the prognosis can be obtained by cytogenetic changes found in the abnormal clone. Conventional cytogenetics is difficult in CLL because of low mitotic index. FISH is an excellent method to detect chromosomal changes in interphase of mitosis of abnormal cells. Chromosomal aberrations are present in up to 55% of all B-CLL cases. We have performed a preliminary study for 21 samples of CLL using 3 FISH probes for deletion of 11q23, trisomy for chromsome 12, and deletion of 13q14. The aim is to determine the cytogenetic abnormalities which harbor prognostic significance in patients' survival. Prognosis for trisomy 12 is intermediate survival, deletion 13q14 has favorable outcome, and 11q23 has unfavorable outcome associated with aggressive disease. Five peripheral blood and 16 bone marrow samples were directly harvested using standard cytogenetic methods. Nuclei from the direct culture were hybridized according to the manufacturer's instructions to 3 probes for regions: 11q23 (dual color, break apart), 12p11.1-q11 (centromeric probe), and 13q14 (LSI) (Abbott Laboratories, Abbott Park, Illinois, USA). Slides were analysed on Olympus BX61 and recorded on Cytovision software. Results: Five patients showed trisomy 21 (24%), 7 patients had deletion 13q14 (33%), and 2 patients had deletion 11q23 (9%). Compared to published studies, we got very similar procentages for the analyzed probes, even though our sample size was small. This study enables us to start the diagnostics for CLL patients at our institution.

Keywords: FISH, CLL, prognostic markers, in situ hybridization, molecular cytogenetics

Presentation number: MG 21 Abstract number: ABS-245-ISABS-2011

TWO-YEAR SUMMARY OF THE EFFICACY OF INTERPHASE FISH (FLUORESCENT IN SITU HYBRIDIZATION) ON PRENATAL SAMPLES AT THE CLINICAL CENTER OF THE UNIVERSITY OF SARAJEVO

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Prenatal diagnostics at the Clinical Center of Sarajevo, Bosnia include conventional karyotyping and FISH (fluorescent in situ hybridization) on amniotic fluid, products of conception, and rarely chorionic villi. Since mid 2008, when the prenatal diagnostics was established, 1028 prenatal samples were processed for conventional karyotype and 102 samples also included rapid, 2-day test, based on interphase FISH probes for chromosomes 13, 18, 21, X and Y. The rate of successful culturing was more than 99%. Methods: Karyotyping was done based on standard cytogenetics procedures and interphase FISH, AneuVysion kit, was used based on manufacturer's instructions (AneuVysion, Abbott, Illinois, USA). All probes were validated internally. Results: We will discuss the abnormal karyotype results, especially the derivatives of translocations, which will be analyzed together with the abnormal ultrasound findings. AneuVysion test showed 7 abnormal findings. Besides the use for rapid results, the AneuVysion test was often used to confirm a specific finding in the prenatal karyotype (not included in these results). Conclusion: The prenatal diagnostics at the Clinical Center Sarajevo utilizes two standard cytogenetics methods: karyotyping and FISH. The Clinical Center is the only center in Bosnia oferring the AneuVysion FISH test, which proves to be very beneficial in cases which need fast results.

Keywords: FISH, amniotic fluid, AneuVysion, molecular cytogenetics, karyotype

Presentation number: MG 22 Abstract number: ABS-182-ISABS-2011

ASSOCIATION OF NOS3 TAG POLYMORPHISMS WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY

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Perinatal hypoxic-ischemic encephalopathy (HIE) is characterized with impaired cerebral circulation and increased activity of nitric oxide synthase (NOS). Activation of the NOS3 in endothelial cells has a neuroprotective role. Aim of this study was to test the association of NOS3 gene with HIE. The study included 110 unrelated term or preterm born children (69 boys and 41girls) with HIE and 128 term and preterm born children (60 boys and 68 girls) without any perinatal problems. Children with perinatal HIE fulfilled the diagnostic criteria for perinatal asphyxia. All children were admitted to the Clinical Hospital Split between 1992 and 2008. We analyzed 6 tagging SNPs within NOS3 gene (rs3918186, rs3918188, rs1800783, rs1808593, rs3918227, rs1799983), in addition to previously confirmed NOS3-associated SNP rs1800779. Genotyping was conducted using “Real-time” PCR. Association analyses were performed under the additive and genotypic model. Allelic test did not observe any SNP association with HIE. Genotypic test detected association of rs1808593 tag SNP with HIE ($\chi 2$= 9.625, p=0.0081). We also observed rs1800783-1800779 TG haplotype association with HIE ($\chi 2$= 11.769, p=6x10-4). Our study had 80% statistical power to detect (at α=0.05) an effect of [OR]=2.07 for rs3918186, [OR]=1.69 for rs3918188, [OR]=1.70 for rs1800783, [OR]=1.80 for rs1808593, [OR]=2.1 for rs3918227, [OR]=1.68 for rs1800779, [OR]=1.76 for rs1799983, assuming an additive model. Despite the limited number of HIE patients that reduced statistical power of this study, we observed genotypic and haplotype associations of NOS3 polymorphisms with HIE.

Keywords: nitric oxide , endothelial nitric oxide synthase (NOS3), tagging polymorphisms, hypoxic-ischemic encephalopathy, children

Presentation number: MG 23 Abstract number: ABS-199-ISABS-2011

FORENSIC ENGINEERING ANALYSIS OF DOT APPROVED COMPARED TO NON-DOT OR NOVELTY HELMET PERFORMANCE AS A FUNCTION OF VELOCITIES EXCEEDING STANDARDIZED TESTING

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In the United States, consumer helmets can be generalized into two categories: DOT (Department of Transportation) approved and non-DOT approved, or novelty. For a helmet to be DOT approved it must pass a series of tests administered by the Department of Transportation and will display a DOT sticker. DOT approved helmets must meet a series of performance testing requirements including impact, penetration, and retention tests. In terms of impact performance testing, the helmet is dropped in a guided freefall at a range of velocities onto a hemispherical or flat steel anvil, where data collected must not exceed a critical acceleration (400 g's max, 200 g's for 2 ms, 150 g's for 4 ms). [Federal Motor Vehicle Safety Standard (FMVSS) No. 218] The question that arises is whether a DOT approved helmet is more effective at preventing head injury than those helmets which are not DOT approved for other testing configurations including increased impact velocity as well as lateral impact. Performance is quantitatively compared by each metric outside federal testing standards including peak g's (acceleration), delta t (duration of impact), and the Head Injury Criterion (HIC). When utilizing a DOT approved helmet over a non-DOT approved helmet, the impact testing reports significantly decreased peak accelerations, decreased HIC and increased delta t.

Keywords: helmet performance, biomedical engineering, accident reconstruction, impact biodynamics, motorcycle

Presentation number: MG 24 Abstract number: ABS-202-ISABS-2011

GENOTYPING COMMON POLYMORPHISMS ASSOCIATED WITH DEEP VENOUS THROMBOSIS IN BOSNIA AND HERZEGOVINA

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Susceptibility to deep venous thrombosis is associated with numerous polymorphisms, of which the most common polymorphisms are F5 (coagulation factor V Leiden) 1691G-A gene mutation, F2 (coagulation factor II, prothrombin) 20210G-A gene mutation and MTHFR (methylenetetrahydrofolate reductase) thermolabile polymorphism (677C-T mutation). In this study, we genotyped 30 individuals with no family history for deep venous thrombosis. For materials we used 6 ml of whole blood for DNA isolation. Samples were genotyped for MTHFR 667C-T, F2 20210G-A and F5 1691G-A mutation by Sybr green method. In our sample we found one heterozygote (GA) for F2 20210G-A and F5 1691G-A mutation. No F5 1691G-A mutant homozygote (AA) was found, but we did find one homozygote (AA) for F2 20210G-A mutation. Allele frequency for G allele in F5 gene was 0.9875 and for A allele was only 0.0125. We also calculated allele frequency for G allele in F2 gene which was 0.9409 and 0.0501 for A allele. Regarding MTHFR genotypes in our sample, 60% of CC – wildtype genotype, 36% of CT genotype and 4% of TT genotype were detected. Allele frequencies for C and T allele are 0.7833 and 0.2167, respectively.

Keywords: FV, FII, MTHFR, genotyping, deep venous thrombosis

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STEROID-INDUCED OSTEOGENESIS: FROM BENCH TO BED

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Clinical management of delayed healing or non-union of long bone fractures and segmental bone defects poses a substantial orthopedic challenge. Surgical advances and bone tissue engineering are providing new avenues to stimulate bone growth in cases of bone loss and non-union. The Reamer-Irrigator-Aspirator (RIA) device allows surgeons to aspirate the medullary contents of long bones and utilize the progenitor-rich “flow through” fraction in autologous bone grafting. Dexamethasone (DEX) is a synthetic steroid that has been shown to be a powerful inducer of osteoblastic differentiation. The aim of this lecture is to present a novel, intraoperative, one-step procedure that utilizes the RIA system to recover autologous bone along with progenitor cells from bone marrow. The recovered materials are treated with a high concentration of DEX and then implanted into the fracture site. The lecture will follow complete development of the method, starting with initial in vitro experiments with human bone marrow derived-mesenchymal stem cells (hMSCs), all the way until the method was successfully applied in the treatment of difficult clinical cases of delayed bone healing and established non-unions. Preliminary clinical results will be presented as well. Bone regeneration is a hot topic in translational clinical orthopedics research with both basic physiology and therapeutic implications. The ultimate goal of this paper is to present this novel method as an excellent example of the basic science information being adequately used in clinical applications, and vice versa, to show how clinical needs and observations are recognized and exploited by basic scientists.

Keywords: osteogenesis, bone graft, mesenchymal stem cells, dexamethasone, regeneration

Presentation number: MG 26 Abstract number: ABS-160-ISABS-2011

BCR-ABL KINASE DOMAIN MUTATIONS IN PRIMARY AND SECONDARY RESISTANT IMATINIB-TREATED CHRONIC MYELOID LEUKEMIA

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Mutations of BCR-ABL tyrosine kinase domain are the major cause of resistance to imatinib in patients with chronic myeloid leukemia. However, it is not known whether KD mutant clones that are detectable prior to therapy are selected in the presence of imatinib, and whether they may predict for therapy outcome. We sought to investigate the occurrence and dynamics of 4 common KD mutations (T315I, F311L, E255K, E255V) in imatinib-treated patients lacking predicted therapy response (by ELN criteria). Eleven of 41 imatinib-treated CML patients showed primary resistance to imatinib with inadequate CgR and consistently high levels of BCR-ABL transcripts monitored by real-time PCR. Using ASO-PCR we investigated the prevalence and the evolution of the above mentioned mutations in pretherapeutic diagnostic samples and in follow-up samples collected throughout imatinib therapy. In 3 patients F311L mutation was detected in imatinib-naive pretherapeutic leukemic samples. Eight patients showed no mutations at the time of diagnosis. After 24 months of imatinib therapy identical mutation status was identified in 5/12 patients (41.6%). Two patients acquired additional mutation (E255K, E255V, one each) and had approximately 10 times higher BCR-ABL transcripts compared to patients with single KD mutation. T315I mutation was not detected. We conclude that: (1) increased proportion of mutated sequences during treatment detected by ASO-PCR suggests clonal evolution of mutated cells, (2) KD mutations are not restricted to the accelerated phase of the disease, and in some cases, mutations occur in chronic phase of the disease and prior to imatinib therapy.

Keywords: CML, imatinib therapy, KD mutations, imatinib resistance, ASO-PCR

Presentation number: MG 27 Abstract number: ABS-291-ISABS-2011

CLINICAL AND GENETIC STUDY OF MENTAL RETARDATION OF UNKNOWN ETIOLOGY IN CHILDREN

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Mental retardation is not a specific disease or disability, but it is characterized with the insufficiency of intellectual (mental) function. These represent different genetic, clinical and social states whose common feature is significant, below-average intellectual functioning manifested in the period of early individual development, with a coefficient of intelligence below 70. In about 2/3 of cases, etiology of mental retardation is unknown. The main goal of this study is to perform analysis of frequency of mental retardation of unknown etiology among persons with special needs at the individual level of analysis, according to standard medical diagnosis and clinical characteristics. This is based on analysis of available documentation and on a survey conducted among the parents of these children. The degree and possible forms of participation of genetic factors in the development of mental retardation in the selectively defined sample is examined. Study included children with special needs, pupils of Center "Vladimir Nazor" in Sarajevo. Basic methods of this study are biostatistical and genealogical analysis. Genealogy tree was made for each of respondent and consists of two or three generations. Study included 100 respondents, 63 males and 37 of female gender, all with mental retardation. In this study, it was found that mental retardation of unknown etiology makes 34% of the overall etiology, with approximately equal frequency in both genders. Genealogical analysis determined more possible modes of inheritance and the most common one is the autosomal recessive mode.

Keywords: mental retardation, genealogy analysis, inheritance , clinical features, children with special needs

Presentation number: MG 28 Abstract number: ABS-117-ISABS-2011

EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS RECEPTOR IN HUMAN OVARIAN CANCER

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Angiogenesis plays an essential role in the proliferation and metastasis of tumor cells. Vascular endothelial growth factor (VEGF) is an important element in angiogenesis in normal and pathological tissue. The aim of our study was to determine the expression level of VEGFA mRNA and its receptors (FLT-1, KDR) by cells obtained from biopsy samples ovaries in ovarian cancer patients (OC). The study comprised 31 OC patients. The 1st stage of the disease (in FIGO classification) was revealed in 4 cases, the 2nd stage – in 2 cases, the 3rd stage – in 15 cases and the 4th – in 10 cases. Expression of VEGFA, FLT-1 and KDR was determined by RT-PCR semi-quantitative method in RNA samples. On patients with the 4th stage, a significantly high level of VEGFA (0, 93±0, 12) was shown as compared with this parameter in patients with the 1st - 2nd stages (0, 52±0, 07) and in patients with the 3rd stage (0, 61±0, 04). On patients with the 4th stage, a high level of FLT-1 (1, 40±0, 23) was also revealed as against this parameter in patients with the 1st – 2nd stages (0, 92±0, 13) and in patients with the 3rd stage (0, 86±0, 12). The level of KDR expression in patients with the 1st - 2nd stages was 0, 59±0, 06 and in patients with the 3rd and 4th stages this parameter was slightly lower - 0, 48±0, 002 and 0, 49±0, 003 respectively. The expression level of VEGFA mRNA and FLT-1 in patients with the 4th stage was significantly higher than that in patients with the 1st – 3rd stages.

Keywords: ovarian cancer, vascular endothelial growth factor, expression, angiogenesis, RT-PCR semi-quantitative method

Presentation number: MG 29 Abstract number: ABS-139-ISABS-2011

GENETIC PREDISPOSITION TO MYOCARDIAL INFARCTION, ASSOCIATED WITH GENES, RESPONSIBLE FOR BLOOD COAGULATION

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Diagnostics of genetic predisposition to myocardial infarction (MI) is very important because this disease is the leading cause of mortality nowadays. Genetic predisposition to MI is associated with genes, responsible for blood coagulation. The methods of polymerase chain reaction with specially designed allele specific primers were used. The frequencies of polymorphisms such as Thr312Ala of fibrinogen α-chain gene, Val34Leu of Factor XIII gene and 4G/5G insertion/deletion in PAI-1 gene (inhibitor of plasminogen activator), as well as FactorVLeiden and G20210A (prothrombin) mutations were examined in blood samples of 175 patients with MI and 270 people of the control group. The rates of FVL and G20210A mutations in the experimental group were shown to be 2.4 and 2.2 times respectively as high as in the control group. Double increase of allele 34Leu homozygote frequency in patients with MI as compared to the control group was obtained. This data proves that such gene variants are MI risk factors. As for PAI-1 gene, it was revealed that 4G/4G genotype can predispose to MI (OR=1.44), and 5G/5G one can be protective. Some significant correlation between genetic and clinics-biochemical parameters of patient blood was revealed. Comparison of risk genotype frequencies with MI rates in different European countries was conducted. 4G/4G genotype frequencies in different European countries were shown to correlate significantly with MI rates there in spite of the fact that predisposing genotypes interaction with environmental factors is of great importance. Thus some new MI predisposing gene variants were detected.

Keywords: myocardial infarction, genetic predisposition, gene polymorphisms, European countries, blood coagulation

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COMPLEX OF GENES RESPONSIBLE FOR HIGH ADAPTATION TO HYPOXIA

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Hypoxia resistance is a very important factor for highly-skilled sportsmen, especially for biathlonists whose sport competitions take place usually in mountains. DNA of the members from the National Olympic biathlon team of Belarus was studied for revealing genetic factors giving rise to the highest adaptation ability to hypoxia. Molecular – genetic analysis of 13 polymorphisms in 10 genes (HIF1A, ACE, eNOS, EPO, BDKRB2, PAI-1, VEGF, ENDT, MB, PPARG) was made using blood samples of sportsmen. The methods of polymerase chain reaction with specially designed allele specific primers were used. Five of the investigated genes were shown to make major contribution to hypoxia resistance. The frequencies of allelic variants defining increased functional activity of investigated genes in highly-skilled biathlonists were shown to exceed mean values, typical of the persons not playing sport. This corroborates the presence of a genetic hypoxia resistant components which are required for attainment of high sport results. Nevertheless some single unfavorable gene variants were revealed in biathlonists. The hypoxia resistance complex in the genotypes of the main stuff members of the Olympic biathlon team was shown to be better, than one in the genotypes of reserved stuff representatives. Detection of undesirable gene polymorphisms makes it possible to correct their negative effects by means of individual medico-biological provision.

Keywords: hypoxia, adaptation, olympic biathlon team, molecular - genetic analysis, gene polymorphisms

Presentation number: MG 31 Abstract number: ABS-255-ISABS-2011

EX VIVO DEVELOPMENT OF THE RAT LIMB BUD WITH 5-AZACYTIDINE

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The aim of this investigation was to explore the developmental potential of rat limb buds in a serum-supplemented organ culture system under the influence of the DNA demethylating agent 5-azacytidine. Fisher rat fore- and hind-limb buds were removed under the dissecting microscope from 13 and 14-days-old embryos and placed on a lens paper supported by a stainless steel grid where they spent three days or two weeks at the air-liquid interface. Eagle's Mininmum Essential Medium was supplemented with 50% rat serum and used alone or with 5 μm 5-azacytidine. Longer and shorter limb bud axes were measured by an ocular micrometer. Samples were processed by routine histology, embedded in parafin and uninterrupted serial sections were stained by HE, Masson trichrome or Azan stain. In isolated limb buds immature epithelium, mesenchyme, myotubes and different stages of angiogenesis such as single hemangioblasts, hemangioblast agreggates, blood islands and capillaries filled with erythroblasts were present. During the 3-day culture period, stratified epithelium and cartilage differentiated but differentiated striated muscle was not observed. In limb buds that spent two-weeks in culture, necrosis and absence of angiogenesis together with keratinization of the stratified epithelium was discovered. Limb buds treated with 5-azacytidine were smaller than controls. It can be concluded that developmental parameters such as overall growth and differentiation of specific tissues in this mammalian model system make it adequate to screen for embryotoxic substances such as the epigenetic drug 5-azacytidine used in this investigation.

Keywords: 5-azacytidine, limb bud, ex vivo, development, embryotoxicity

Presentation number: MG 32 Abstract number: ABS-207-ISABS-2011

ROBUSTNESS TESTING OF THE HIGH THROUGHPUT HPLC-BASED ANALYSIS OF PLASMA N-GLYCANS

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Analysis of plasma protein glycosylation by high throughput HPLC-based 96 well platforms is becoming a routine tool for the analysis of large sample sets in studies comprising thousands of individuals. Analytical method which is routinely used to obtain a large amount of data should be well characterized and all potentially critical steps should be identified. Robustness of the high throughput method was tested by Plackett Burman two level, 11-factor, 12 experiment screening design. For initial screening eleven potentially important factors were chosen. The response variable was calculated as a coefficient of variance between area percent of each peak in the each reaction and the area percent obtained after performing the procedure according to the laboratory standard operating procedure with standardized plasma samples. Thirteen out of 16 glycan groups separated by HPLC revealed statistically significant changes with varying response to 11 factors, which was expected due to their structural and chemical differences. Time of exposure to DTT (DTT), Time of storage prior to formic acid exposure (STORE1), time of exposure to formic acid (FORMIC), duration of labeling reaction (2AB) were identified as the most critical steps which should be closely monitored to avoid introduction of analytical errors in the analysis.

Keywords: screening design, Plackett-Burman, N-glycosylation, glycan analysis, HPLC based analysis

Presentation number: MG 33 Abstract number: ABS-307-ISABS-2011

EXPRESSION OF PANNEXIN FAMILY OF GAP JUNCTION PROTEINS IN INVASIVE DUCTAL BREAST CARCINOMA

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Breast cancer (BC) is the most prevailing malignancy among worldwide female population, with constantly increasing incidence and serious consequences on morbidity and mortality of affected patients. Genotypically and phenotypically BCs represent very heterogeneous group of tumours, diverse in their pathohistological features, expression of molecular markers, malignant/metastatic potential, clinical presentation, prognosis, and response to therapy. The purpose of this preliminary study was to determine the expression pattern of pannexin family (Panx 1/2/3) of gap junction proteins in invasive ductal breast carcinoma. A total of 27 female patients with diagnosed invasive ductal BC were included in the study. All cases were graded according to the modified criteria of Scarff Bloom-Richardson, as described by Elston and Ellis. Tumour tissue samples surgically removed from each patient (primary tumor and positive axillary lymph nodes) were embedded in paraffin blocks for immunohistological staining or snap-frozen in liquid N2 for RNA isolation. Immunohistochemical staining was performed with rabbit polyclonal anti-human pannexin-1, 2 and 3 antibody (AbCam ab78380, ab55917, ab98093) using DAB substrate and HRP reaction. RNA isolation, in vitro transcription and qRT-PCR were performed using TRI Reagent and RNeasy Mini kit, gene specific Quanti Tect Primer Assay (Qiagen) and 7500 qRT-PCR system (Applied Biosystem). Obtained results showed positive (protein, mRNA) expression pattern ranging from diffuse (Panx2) to granular (Panx1, Panx3) cytoplasmatic staining with slight or no membrane positivity in tumor cells of all analyzed samples. However, the significance, if any, of their expression for tumorigenesis of invasive ductal breast carcinoma has still to be elucidated.

Keywords: invasive ductal breast carcinoma, pannexin 1, pannexin 2, pannexin 3, gap junctions

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EXPRESSION OF PANNEXIN FAMILY OF GAP JUNCTION PROTEINS IN SPORADIC CARDIAC MYXOMA CASES

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Cardiac myxoma (CM) represents the most prevalent type of primary cardiac tumors in adults, constituting up to 50-85% of all benign lesions of the heart. Due to their strategic localization and inherent pathohistological characteristics, they are considered as quite malignant entities with very serious consequences for morbidity and mortality of affected patients. Surgical resection of cardiac myxoma is currently the only treatment of choice.
The purpose of this study was to determine the expression pattern of pannexin family (Panx 1/2/3) of gap junction proteins in sporadic cardiac myxoma cases. A total of 28 consecutive patients with sporadic cardiac myxoma diagnosed from 2002 to 2010 at Department of Cardiac Surgery, University Hospital Centre Zagreb were included in the study. Tumour tissue sections obtained from archival FFPE tumor tissue blocks (n=25) or fresh surgical tissue samples (n=3) snap-frozen in liquid N2 were used for immunohistological staining and RNA isolation. Immunohistochemical staining was performed with rabbit polyclonal anti-human pannexin-1, 2 and 3 antibody (AbCam ab78380, ab55917, ab98093) using DAB substrate and HRP reaction. RNA isolation, in vitro transcription and qRT-PCR were performed using TRI Reagent and RNeasy Mini kit, gene specific Quanti Tect Primer Assay and 7500 qRT-PCR system. Obtained results showed positive expression pattern for Panx 1- 3 in tumor cells (lepidic tumor cells arranged singly or in small clusters and vasiforme cell aggregations) of all analyzed samples on protein and mRNA level. The significance, of pannexin gene expression as a marker for cardiac myxoma has still to be elucidated.

Keywords: sporadic cardiac myxoma, pannexin 1, pannexin 2, pannexin 3, gap junction

Presentation number: MG 35 Abstract number: ABS-313-ISABS-2011

EXPRESSION OF PHOSPHORYLATED HER2 RECEPTOR IN DUCTAL INVASIVE BREAST CANCER

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HER-2/neu oncogene is amplified/overexpressed in 15-25% cases of invasive human breast carcinomas (BC), resulting with a high risk of relapse and poor survival. Due to reduction in BC recurrence and markedly improved survival, treatment of HER-2/neu overexpressing patients with humanized monoclonal antibody trastuzumab (Herceptin®) has become a worldwide treatment of choice. Unfortunately, in some patients responses to trastuzumab vary in magnitude and can be short-lived, with clinical limitations resulting from acquisition of trastuzumab resistance. We evaluated the expression of Tyr-1248 phosphorylated form of HER2 receptor (pHER2) among 83 patients diagnosed with ductal invasive BC. Immunohistochemically detected expression of pHER2 was correlated with known prognostic markers for BC. 42 selected patients were HER2 negative while 41 showed HER2 overexpression and were scheduled for trastuzumab therapy. Overexpressed HER2 was mainly in its phosphorylated/active form ($p<0.001$). Patients with higher pHER2 expression exhibited more positive axillary lymph nodes ($p=0.010$), poorly differentiated tumor ($p=0.003$), smaller primary tumor ($p=0.017$) and less expressed estrogen receptors ($p=0.011$). Low pHER2 values were detected in 22.0% of patients overexpressing HER2 while 7.1% of HER2 negative patients showed high pHER2 expression. High pHER2 expression was found in 67% (20/30) of patients sensitive to trastuzumab therapy compared to 27% (3/11) of drug resistant patients. Our preliminary data suggests that evaluation of pHER2 expression may be very important prognostic biomarker. It seems that higher expression of pHER2 determines BCs with increased metastatic potential but better response to trastuzumab therapy while low pHER2 expression among patients selected for trastuzumab, may predict resistance to therapy.

Keywords: pHER2, phosphorylated HER2, trastuzumab, breast cancer, herceptine resistence

Presentation number: MG 36 Abstract number: ABS-236-ISABS-2011

ISOLATION AND IDENTIFICATION OF CHROMOSOME BREAKPOINTS USING FACS SORTING AND DEEP SEQUENCING

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We have investigated the use of FACs sorted chromosomes and NGS sequencing to isolate chromosomes containing translocations from the unaffected partner chromosome and to establish the translocation breakpoint. Human chromosomes containing a balanced translocation attaching the chromosome 7q tip to the p arm of chromosome 2 were FACS sorted to isolate them. Chromosomes were isolated in sufficient quantities to prepare libraries for sequencing without needing a whole genome amplification step. Next Generation Sequencing of libraries prepared from normal and translocation containing chromosome 2 showed clear differential isolation and at least 4 fold enrichment of the individual chromosomes. The enriched chromosome sequencing allowed us to acquire 20x coverage of each chromosome providing sufficient depth that the breakpoints on the isolated chromosomes could be identified.

Keywords: next generation sequencing, FACs sorting, balanced translocation, chromosome breakpoint discovery, individual chromosome enrichment

Presentation number: MG 37 Abstract number: ABS-179-ISABS-2011

EXPLORING GENETIC OVERLAP OF FOUR AUTOIMMUNE RISK LOCI IN TYPE 1 DIABETES FAMILY TRIOS IN CROATIA

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Knowledge accumulated through genetic research of complex diseases emphasize that these diseases develop as a consequence of cumulative effects of polymorphisms in multiple genes. Many of these susceptibility genes overlap between various autoimmune diseases. The aim of this study was to determine whether four SNPs reported as risk variants in juvenile idiopathic arthritis (VTCN1 gene), sarcoidosis (ANXA11 gene), primary biliary cirrhosis (IL12RB gene) and celiac disease (LPP gene), are associated with susceptibility to type 1 diabetes mellitus (T1DM). We genotyped four SNPs (rs2358817, rs1049550, rs6679356, rs9865818) within VTCN1, ANXA11, IL12RB and LPP genes in 265 T1DM family trios in Croatian population. To test the association we performed transmission disequilibrium test (TDT) in all trios and stratified analysis in different age-of-onset groups. Transmission disequilibrium test did not detect an association of VTCN1, ANXA11, IL12RB and LPP gene polymorphisms with T1DM , but interesting trend in IL12RB2 rs6679356 minor allele C overtransmission in patients below 8 years was observed (p=0.075) and undertransmission in patients above 12 years of T1DM onset (p=0.063). We demonstrated that gene regions associated with several autoimmune diseases are not associated with T1DM. These results exclude investigated SNPs as possible common susceptibility loci between these autoimmune diseases and T1D in our sample set. We observed a trend towards overtransmission and undertransmission of IL12RB2 rs6679356 minor allele C in two groups of patients (below 8 years and above 12 years of T1DM onset, respectively) suggesting that common autoimmune loci might play different roles in disease development within different age groups.

Keywords: SNP, genotyping, type 1 diabetes, autoimmune loci, TDT

Presentation number: MG 38 Abstract number: ABS-268-ISABS-2011

IMPACT OF ANTIPLATELET THERAPY AND CARDIOPULMONARY BYPASS ON PLATELET FUNCTION IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFTING USING MULTIPLE ELECTRODE AGGREGOMETRY

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Aim: Antiplatelet therapy (APT) is known to substantially reduce mortality and rate of ischaemic complications after coronary artery bypass grafting (CABG). Rate of perioperative APT resistance varies widely and could be influenced by cardiopulmonary bypass (CPB). The purpose of the study was perioperative assessment of platelet function with respect to administered APT and CPB, and determination of patients with APT resistance who could benefit from more aggresive treatment strategy. Methods: In prospective study we enrolled 192 patients undergoing elective CABG. Patients were divided into 4 groups regarding preoperative APT management. All patients received Aspirin (ASA) 300 mg/ day postoperatively starting on the day of procedure. Platelet function was assessed prior to surgery and at fourth postoperative day (POD 4) using multiple electrode aggregometry (MEA). Adenosine diphosphate (ADP test) and arachidonic acid (ASPI test) induced platelet aggregation tests were used. Results: Group of patients exposed to ASA preoperatively had lower values of ASPI test ($P<0.001$) comparing to patients not receiving ASA. However, we registered 28.6% ASA resistant patients. Both ASPI ($P<0.001$) and ADP ($P<0.001$) test values increased significantly at POD 4, suggesting postoperative platelet hyperactivity. Postoperatively, we registered 33.3% ASA resistant patients despite higher ASA dosing regimen. Conclusion: MEA can recognize patients with ASA resistance during the both the pre- and post- CABG period. Postoperatively, ASA 300mg did not sufficiently inhibit platelet aggregation in 33.3% patients. In this subgroup, dual antiplatelet therapy with ASA and clopidogrel could be useful for maintaining graft patency, and preventing adverse ischemic events.

Keywords: coronary artery disease, antiplatelet therapy, cardiopulmonary bypass, platelet function, multiple electrode aggregometry

Presentation number: MG 39 Abstract number: ABS-304-ISABS-2011

RECURRENT APHTHOUS ULCERS AND POLYMORPHISM IN CORTICOTROPHIN RELEASING HORMONE RECEPTOR 1

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Recurrent aphthous ulcers (RAU) is common, but still poorly understood chronic inflammatory disease characterized by recurring painful oral ulcers. RAU are associated with different types of stress. One of the main stress-response systems is hypothalamic–pituitary–adrenocortical (HPA) axis, where corticotrophin releasing hormone (CRH) and receptor 1 (CRHR1) have integrative role in coordination of endocrine, autonomic and immune system. Genetic predisposition might play a role in RAU and role of polymorphisms of CRHR1 have not been investigated yet. The purpose of this study is to investigate (1) role of stress as a trigger for development of RAU, (2), association of selected genetic variations in CRHR1 with RAU and (3) relations between RAU, stress and genetic variations in CRHR1. Recruitement is going to be performed by oral surgeon, dentist and general practitioner. The examinees are going to have to comply the set criteria (clinical finding, questionnaire, informed consent and taking swab for genetic analysis). We plan to study association of RAU and two haplotype-tagging SNPs in CRHR1: rs242939 and rs1876830 (subsets of SNPs sufficient to reveal haplotype information for 14 SNPs in CRHR1) using TaqMan Pre- designed SNP genotyping assays. Genetic analysis has not yet been carried out.

Keywords: recurrent aphthous ulcers (RAU), hypothalamic - pituitary - adrenocortical (HPA) axis, CRHR1, SNPs, stress

Presentation number: MG 40 Abstract number: ABS-221-ISABS-2011

HIGH THROUGHPUT ISOLATION AND GLYCOSYLATION ANALYSIS OF IGG – VARIABILITY AND HERITABILITY OF THE IGG GLYCOME IN TWO ISOLATED HUMAN POPULATIONS

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All IgG molecules carry N-glycans which modulate their biological activity. Changes in N-glycosylation of IgG associate with various diseases and affect the activity of therapeutic antibodies and intravenous immunoglobulins. We have developed a novel 96 well protein G monolithic plate to rapidly isolate IgG from plasma of 1821 individuals from two isolated human populations. N-glycans were released by PNGase F, labeled with 2-aminobenzamide and analyzed by hydrophilic interaction chromatography with fluorescence detection. The majority of the structural features of the IgG glycome were consistent with previous studies, but sialylation was somewhat higher than reported previously. Sialylation was particularly prominent in core-fucosylated glycans containing two galactose residues and bisecting GlcNAc where median sialylation level was nearly 80%. Very high variability between individuals was observed, approximately three times higher than in the total plasma glycome. For example, neutral IgG glycans without core fucose varied between 1.4% and 19%, a difference that significantly affects the effector functions of natural antibodies, predisposing or protecting individuals from particular diseases. Heritability of IgG glycans was generally between 30% and 50%. Individual's age was associated with a significant decrease in galactose and increase of bisecting GlcNAc, while other functional elements of IgG glycosylation did not change much with age. An important observation is that competition between glycosyltransferases which occurs in vitro did not appear to be relevant in vivo, indicating that the final glycan structures are not a simple result of competing enzymatic activities, but a carefully regulated outcome designed to meet the prevailing physiological needs.

Keywords: immunoglobulin G, glycosylation analysis, variability of glycome, high throughput protein isolation, monoliths

Presentation number: MG 41 Abstract number: ABS-203-ISABS-2011

FREQUENCY OF HPV GENOTYPES IN GEOGRAPHICALLY LIMITED POPULATION OF EXAMINEES IN BOSNIA AND HERZEGOVINA AND ITS CORRELATION WITH GENERAL BIOLOGICAL PARAMETERS – PRELIMINARY RESULTS

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One of the aims of our research is to determine the incidence of HPV infection and the frequency of HPV genotypes in a limited population of women in Bosnia and Herzegovina. Vaginal and cervical swabs taken with DNAPapTM Cervical SamplerTM were used as a source of genetic material. Universal HPV primer, which is able to detect a wide range of HPV types, was used to test presence of HPV infection, while originally optimized method based on controlled multiplex PCR amplification of the most common HPV genotypes (11, 16, 18, 31, 33 and 35) was applied in HPV genotyping. Amplicons were analyzed on agarose gel and the DNA biochip on an automated electrophoretic detector of high resolution (2100 Electrophoresis Bioanalyzer system, Agilent, USA). The results of the HPV PCR test were correlated with general biological and sociodemographic data gathered through appropriate questionnaires. MedCalc v2 was used for the analysis of genetic data and calculation of derived indicators. Preliminary results represent the incidence of HPV infections and given HPV genotypes in the sample of 100 examinees, as well as the correlation of the obtained results with the general biological and sociodemographic factors. These results could provide a scientific basis for initiating a broader study on a larger and more relevant sample of the female population of Bosnia and Herzegovina, in order to develop the necessary strategies and screening programs for prevention of HPV infection and cervical cancers and thus reduce the high mortality rate of women suffering from this type of cancer.

Keywords: HPV infection, HPV genotypes incidence, HPV PCR test, biological and sociodemographic data-correlation, cervical cancer

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PROTEOMIC ANALYSIS OF INTERMEDIATE-GRADE PROSTATE TUMORS

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Differential protein analysis in carcinoma tissue, including protein expression level, and clinical and pathological parameters were correlated in four patients with intermediate grade prostate cancer. Tumor and health prostate tissue specimens from four patients were compared after protein isolation and analysis. Qualitative and quantitative analysis performed by usage of two-dimensional gel electrophoresis (2-DE) and mass spectrometry separated differentially expressed proteins in cancer and healthy tissues. Quantitative protein expression analysis was performed by VersaDoc Imaging System with PDQuest software v.7.4.0. Identification of 54 differentially expressed proteins was confirmed by the SWISS-PROT and NCBI databases. Selected differentially expressed proteins were cut from gel and digested by trypsin. Peptides isolated from the gel were purified by ZipTip and analyzed by mass spectrometry (AB 4800 plus MALDI TOF/TOF system). Differences in protein expression were evaluated through the Cross-database search of obtained proteome (NCBI, MSDB and SwissProt). Patients with Gleason score 7 (4+3) and tercial gradus 5 had expression of zinc finger protein 600, heat shock protein gp96 precursor, BIP protein, heat shock protein 60 kD, antigen of the monoclonal antibody Ki 67 isoform 1 and prohibitin. Expression of these proteins was in correlation with worse clinical and pathological parameters that might indicate need for further and more aggressive cancer treatment. Some of the identified proteins could be used as biomarkers in follow up of disease progression. Moreover, better classification of patients who need more aggressive further adjuvant oncological treatment may be achieved by the elucidation of prognostic and predictive biomarkers.

Keywords: proteomics, biomarkers, intermediate-grade prostate cancer, differential display, mass spectrometry

Presentation number: MG 43 Abstract number: ABS-169-ISABS-2011

ANTI-GLYCAN ANTIBODIES IN PLASMA OF PATIENTS WITH COLORECTAL CANCER

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Colorectal cancer is the third leading cause of cancer-related death in the Western world with 655, 000 deaths worldwide per year. Invasive cancers that are confined within the wall of the colon (TNM stages I and II) are curable with surgery, but if untreated, they spread to regional lymph nodes (stage III), and distant sites (stage IV) what is associated with poor prognosis. Since early diagnosis is essential for successful treatment, development of reliable screening tools is of utmost importance. Glycans are predominant surface components of eukaryotic cells and microorganisms. As such, they give rise to high levels of anti-glycan antibodies of all classes. Apart, so called natural antibodies to certain defined mono, di and oligosaccharides, common in bacterial, fungal and parasite cells, are pre-exist in human sera and can be profiled using glycan arrays. Alterations in metabolism of cancer cells result in the production of altered glycan structures, which are being recognized by the immune system and result in generation of novel anti-glycan antibodies. Using printed glycan array with preliminary chosen 48 glycan structures we screened 193 control samples from healthy donors and 197 samples of plasma from patients with CRC. Our results show that plasma anti-glycan antibodies from both healthy donors and CRC patients can bind up to 75 % of total number of plasma samples in each group, but in case of CRC the binding to some glycans is found to be more frequent.

Keywords: anti-glycan, antibodies, colorectal, carcinoma, glycans

Presentation number: MG 44 Abstract number: ABS-266-ISABS-2011

NEUROFIBROMATOSIS TYPE 1: THE FIRST GENETIC STUDY OF NOVEL AND RECURRENT MUTATIONS IN NF1 GENE IN CROATIAN PATIENTS

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Neurofibromatosis type 1 (NF1), is one of the most common autosomal dominant disorders in man. NF1-gene is a fully penetrant exhibiting a high mutation rate and as a consequence, a high number (50%) of sporadic cases caused by new mutations. To date, a number of different novel and recurrent mutations of NF1gene have been found. The aim of this study was to identify the spectrum of disease causing mutations in the NF1 gene in Croatian non-related patients. In 21 patients who fulfilled only one NIH diagnostic criterion (multiple café au lait spots) at the time of NF1 gene testing, the whole coding sequence and all splice sites were studied for NF1 mutations. All children with sporadic NF1 are continued to be followed-up prospectively. Of 21 NF1 gene analyses, 18 (85.71 %) were positive. In 16/21 patients the specific mutations were found by gene sequencing and in two children MLPA revealed the deletion of whole NF1 gene. Thirteen of 16 found NF1 mutations were novel: c.147C>G, c.205-2A>C (IVS2-2A>C), c.1260+1G>C (IVS9+1G>C), c.1393_1421dup, c.1659_ 1660dupTC, c.3144G>A, c.3445A>G, c.4076delC, c.4168C>T, c.4168C>T, c.4686delA and c.4756dupT, while 3 mutations have been previously identified: c.1721G>A, c.3827G>A, c.3827G>A. No mutational hot spots in NF1 gene were observed. The mutational spectrum showed mainly nucleotide substitutions, suggesting that the genotypic characteristics of Croatian NF1 patients may be distinct from other populations. We present this study as the first step in the routine diagnosis procedure for Croatian patients with NF1.

Keywords: neurofibromatosis 1, NF1 gene, mutation detection, gene sequencing, MLPA

Presentation number: MG 45 Abstract number: ABS-108-ISABS-2011

ASSOCIATION OF SEROTONIN 1A RECEPTOR GENE POLYMORPHISM WITH COMPLETED SUICIDE IN IRANIAN POPULATION

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Serotonin systems appear to play a key role in the pathophysiology of suicidal behavior. Many Studies have examined the association between a functional polymorphism of the serotonin receptor gene promoter (5-HT1A-1019C>G) and Suicide but have yielded inconsistent results. In the present study, we aimed to assess the association between this polymorphism and Suicide in the Iranian population. In our study we analyzed promoter polymorphism -1019C>G on 250 suicide victims and 150 unrelated age- and sex-matched healthy control subjects by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). There was no statistically significant difference in the genotype distributions or allelic frequency, in the serotonergic polymorphism between suicide victims and normal controls ($p>0.05$). We concluded that this polymorphism maybe not associated with susceptibility to suicidal behavior. More work is needed to replicate these findings. Our future studies aim at identifying other genetic associations.

Keywords: polymorphism, serotonin system, suicide victims, PCR, RFLP

Presentation number: MG 46 Abstract number: ABS-157-ISABS-2011

GENETIC VARIATIONS OF DRUG-METABOLIZING ENZYMES IN POPULATION FROM BOSNIA AND HERZEGOVINA

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Genetic polymorphisms of drug-metabolizing enzymes have been demonstrated between distinct ethnic groups, contributing to observed interindividual variation in drug response and disease susceptibility. This is the first study performed in a population from Bosnia & Herzegovina (BH), in which we analyzed the frequency of genetic variations in N-Acetyltransferase 2 (NAT2) and Cytochrome P450 (CYP) in population from Bosnia and Herzegovina (BH). Genomic DNA was extracted from blood samples collected from 218 unrelated subjects. A real-time PCR was used for the detection of NAT2 and CYP polymorphisms, with the application of the specific TaqMan® SNP Genotyping Assay (Applied Biosystems) for NAT2*5, NAT2*6 CYP2C9*2, CYP2C19*2, CYP2D6*4, and CYP3A5*3, while CYP3A4*1B was genotyped by the high-resolution melting analysis. Our results showed that frequencies of mutant NAT2 alleles in BH population were in line with the Caucasians genotype data reported earlier. Interestingly, CYP3A5*3 polymorphism was found to be predominant in the Bosnian population with an incidence of 94%, similarly to other European populations tested so far. However, frequency of CYP2C9*2 polymorphism seemed to be lower in this sample of BH population as compared to the Caucasians genotype data. In conclusion, our data demonstrated the distribution of specific variants of NAT2 and CYP in BH population and indicated a significance of this analysis in specific ethnic groups. Importantly, results of this study may lead to translation of pharmacogenetics and individualized therapeutic approach in current clinical practices in BH.

Keywords: drug-metabolizing enzymes, N-acetyltransferase 2, cytochrome P450, Bosnian, pharmacogenetics

Presentation number: MG 47 Abstract number: ABS-206-ISABS-2011

AMITOSIN AS TOOL FOR POSTGENOMIC MOLECULAR DIAGNOSTIC OF CANCER

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Recently, cancer is postgenomic disease and the new approach in molecular diagnostic of cancer links with postgenomic cancer detection. Evidently, the targeted epigenetic DNA methylation alterations in all cancer types open the postgenomic era in epigenomics carcinogenesis for the last decades. The global DNA hypomethylation is the early postgenomic event in cancer development. Amitosin is the origin modifier of alkaloid composition of Chelidónium május with antiviral, antiinflammatory and anticancer activities. We have studied that Amitosin is natural tool in the DNA hypomethylation detection for the molecular cancer diagnostic at the level of periferal blood lymphocytes in cancer patients. We have shown the specific Amitosine spectrum emmision during its binding to lymphocyte genomic DNA from the cancer patients in comparison with non-cancer genomic DNA. In addition, we elucidate the target Amitosine fluorescention in the culture lymphocytes from the cancer patients whereas the Amitosine fluorescention in the healthy lymphocyte culture was abcent. Moreover, the target Amitosine fluorescention was associated with the healthy lymphocyte culture exposed by 5-azacytidine DNA demethylation drug. On the base of data obtained we have resumed that Amitosine is powerful indirected methyl-sensitive tool for the detection of the postgenomic loss of DNA methylation in epigenetic molecular cancer diagnostic.

Keywords: cancer, DNA hypomethylation, postgenomic diagnostic, amitosine, 5-azacytidine

Presentation number: MG 48 Abstract number: ABS-299-ISABS-2011

OCT AND NANOG ESIRNAS INHIBIT EXPERIMENTAL MOUSE TERATOMA GROWTH IN VITRO

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Stemness genes Oct and Nanog were recently suggested by our and other studies to be involved in development of teratomas belonging to testicular germ cell tumours (TGCT). Teratomas can be experimentally obtained by cultivating gastrulating mouse embryos in vitro. EsiRNAs are constructs able to partake RNA interference pathway of the cell to induce mRNA degradation i.e. inhibit the expression of the genes of interest. The aim of this study was to determine whether the stemness genes Oct and Nanog have a role in teratoma development. Therefore, esiRNAs targeting the coding regions of mouse Nanog (transcript XM_132755.3), mouse Oct4 (transcript NM_013633.1) or GFP (132-591) were synthesized. 7, 5–days-old C3H mouse embryos were microsurgically isolated under the steromicroscope and cultivated at the air-liquid interface for 7 days in Eagle's MEM with 50% rat serum. Specific esiRNA (5pmol esiRNA) in transfecting agent Lipofectamine 2000 (0, 25µl) were added to the culture medium. Embryos/teratomas were measured at the beginning of cultivation and for consequent 7 days. Data were analyzed by the One sample t-test. EsiGFP treated group served as a negative control since no esiGFP target in a mouse cell exists. Indeed, esiGFP group showed no statistically significant difference in growth when compared to controls. Teratomas developed from esiOct and especially esiNanog treated embryos showed statistically significant reduction in growth when compared to controls. By this experiment we confirmed crucial role of stemness genes Oct and Nanog in teratoma development and the utility of intervening in tumour growth by esiRNA treatment.

Keywords: teratoma, esiRNA, Oct, Nanog, in vitro

Presentation number: MG 49 Abstract number: ABS-220-ISABS-2011

HUMAN LIVER CELLS CAN BE PROTECTED FROM HCV INFECTION BY CONTINUOUS INTRACELLULAR EXPRESSION OF HCV RNA ANALOGS

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Hepatitis C virus infection frequently results chronic liver disease and liver failure. It is the most common indication for liver transplantation. The incidence of re-infection is virtually 100% for new livers transplanted into HCV (+) recipients. That infection results in a rapidly progressive liver damage which can result in failure of the graft. Currently, such infection and subsequent progressive liver disease cannot be prevented. We have shown that HCV RNA structural analogs can inhibit HCV RNA replication in infectious HCV systems. To determine whether human liver cells continuously expressing HCV structural analogs would be resistant to HCV infection. HCV RNA structural analogs were constructed and tested in a HCV genotype 1b BB7 replicon, and a Japanese fulminant hepatitis-1 (JFH-1) HCV genotype 2a infection model. An Huh7.5 cell line continuously expressing HCV structural analogs were prepared by stable transfection by a retroviral vector. The efficacy in preventing HCV replication in JFH-1 infection model was determined by real-time PCR quantification of HCV RNA. In stable transfected Huh7.5 cells with HCV structural analogs after exposure to JFH-1 HCV, cellular HCV RNA levels were significantly lower than in controls, indicating that the JFH-1 HCV genome replicated with lower efficiency in cells stable transfected with HCV structural analogs than in control cells. HCV infection is inhibited in human liver cells that continuously produce HCV structural analogs. If such cells were introduced into HCV-infected patients, these cells would be predicted to decrease HCV re-infection, and therefore, increase long-term success rates. This work was supported in part by grants from Herman Lopata Chair in Hepatitis Research (GYW) and the Croatian Science Foundation (RS).

Keywords: hepatitis C virus (HCV) infection, liver transplantation, graft failure, HCV RNA structural analogs, Japanese fulminant hepatitis-1 (JFH-1) HCV

Presentation number: MG 50 Abstract number: ABS-293-ISABS-2011

ANTIOXIDANT N–TERT–BUTYL–α–PHENYLNITRON (PBN) IMPROVES PLACENTAL GROWTH IN FEMALE RATS TREATED BY THE EPIGENETIC DRUG 5-AZACYTIDINE (5-AZAC)

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Our previous results have shown that pretreatment with the antioxidant N–tert–Butyl–α–phenylnitron (PBN) improved some developmental parameters (survival, overall growth, and limb morphogenesis) in fetal rats whose mothers were treated by the epigenetic drug 5-azacytidine during the gestation. The question remained whether the PBN could improve the growth of the placenta in the same model. On the 12th and 13th days of gestation Fisher rats were pretreated by an i.v. injection of PBN (40 mg/kg) and one hour later by an i.p. injection of 5-azacytidine (5mg/kg). On the 15th and 20th days of gestation placentae were isolated. Their weight and immunohistochemically detected and stereologically quantified Proliferating Cell Nuclear Antigen (PCNA) expression was compared to sham controls, treated with PBN and/or 5-azacytidine (5-azaC) and statistically evaluated by Student's t-test. PBN significantly improved overall growth of the placenta when applied before 5azaC. Numerical density of the PCNA signal in samples treated with 5-azaC was significantly lower than in PBN-treated or controls. Pretreatment with PBN seemed not to improve Nv for PCNA. It can be concluded that the nitrone ameliorates teratogenic impact of the epigenetic drug 5azaC upon placenta in a similar way as in fetuses meaning that this DNA demethylating agent acts, at least partially, through activation of the oxidative stress pathways.

Keywords: epigenetic drug, 5-azacytidine, N–tert–Butyl–α–phenylnitron, oxidative stress, placenta

Presentation number: MG 51 Abstract number: ABS-224-ISABS-2011

A NEW KEY TO AN OLD CHEST - ARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION (ACGH) AND RARE DISEASES

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Rare or orphan diseases affect a small number of individuals, have mostly genetic causes and are difficult to diagnose, often requiring an interdisciplinary approach; one of the phenotypes frequently associated with these disorders is intellectual disability (ID). Here, we present four patients clincally diagnosed with non-syndromic ID, where array-based comparative genomic hybridization (aCGH) was decisive in establishing the final diagnostic. Patient no. 1 presented severe ID; severe speech delay; autistic features; severe hyperkinesis; facial dysmorphism; bilateral pyramidal syndrome; cryptorchidism; normal GTG-banded karyotype. aCGH karyotype was arr Xq28(152, 824, 755-153, 262, 507)x3, thus allowing the diagnostic of MECP2 duplication syndrome (DS). To our knowledge, this is the first report of MECP2 DS associating hyperkinesis. Patient no. 2 showed severe ID; severe dyslalia and speech delay; hyperkinesis; facial dysmorphism; camptodactily; normal GTG-banded karyotype. aCGH karyotype was arr 22q11.2(17, 403, 624-17, 713, 104)x1, leading to the diagnostic of DiGeorge syndrome. Patient no. 3 presented moderate ID; dyslalia; facial dysmorphism; hypotonia; fine motor skill disturbances; normal GTG-banded karyotype. aCGH karyotype indicated arr 9p13.1p13.3(33, 745, 489-38, 527, 027)x1. 9p deletions have been reported in association with ID; however, this particular region was not previously described. Patient no. 4 showed severe ID, facial dysmorphism; clinodactily; hearing impairments; cardiac anomalies; genital hypoplasia; GTG-banded karyotype was suggestive of del(3)(p14). aCGH delineated the loss of genetic material as: arr 3p12.3p14.1(65, 317, 864-77, 902, 945)x1, thus indicating a 3p interstitial deletion syndrome. These cases illustrate the use and utility of aCGH in the investigation of the genetic make-up of neuropsychiatric patients and suggest it should become a first-tier approach in non-syndromic ID diagnosis.

Keywords: intellectual disability, aCGH, molecular diagnostic, personalized diagnostic, genomics

Presentation number: MG 52 Abstract number: ABS-225-ISABS-2011

HYPOXIC CELL CULTURE FOR MORE EFFECTIVE CANCER VACCINES

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For some malignancies, vaccines containing allogeneic cancer cells enhanced the overall survival in early clinical trials. Because the cells were cultured at pO_2=20 kPa, it is unclear if they adequately match antigens of tumors in situ where pO_2 is lower. Thus, we postulate that hypoxically grown vaccine cells will better match antigens in tumors in situ. We are testing this hypothesis by studying the effects of hypoxia on prostate cancer (CaP) cells. We analyzed the transcriptome of 100 CaP tissues and 71 adjacent benign tissues and found 24 genes (known as oxygen–regulated) significantly overexpressed in CaP relative to control tissue (p<=0.02). Overexpression of cyclin B1, hyaluronan-mediated motility receptor and disc large (drosophila) homolog-associated protein 5 was associated with high Gleason score and poor prognosis. When CaP cell lines LnCaP, VCaP and DU145 were grown at pO_2=2 kPa, they proliferated faster and secreted more vascular endothelial growth factor than when grown at pO_2=20 kPa (p<=0.05). Interestingly, in hypoxia all three cell lines transcribed more pyruvate dehydrogenase kinase isozyme 1, nuclear prelamin A recognition factor, glucose phosphate isomerase, and glyceraldehyde-3-phosphate dehydrogenase genes than in normoxia (p<=0.05); we found a similar relationship between transcript levels for those genes in CaP cells in situ and nonmalignant control cells (p<=0.005). We continue to characterize the relationship of hypoxically cultured CaP cells and CaP cells in situ to identify and validate TAAs for a more effective therapeutic vaccination. Support: DOD PC094680 (CRG), Minnesota Partnership for Biotechnology and Medical Genomics, and Mayo Clinic Prostate SPORE 5P50CA091956 (FK).

Keywords: cancer vaccines, cell culture, hypoxia, prostate cancer , transcriptome

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THE POTENTIAL DEMOGRAPHIC IMPACT ON THE CHROMOSOME ANOMALIES

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Here are given the results of 10,147 karyotype investigations performed in our laboratory of cytogenetics. The standard clinical contingent which is usually sent for karyotype analysis and genetic counselling was investigated: children with congenital malformations, patients with severe mental retardation, also males and females with disturbances of generative functions. We have also paid special attention to specific contingents in which one could predict low frequencies of chromosome aberrations: unselected newborns (4,032 individuals), the patients suffering from mild mental retardation (2,209 individuals), inmates of schools for blind children (326 individuals), couples who gave birth to babies with malformations or suffered from abortions (612 individuals). Cosidering the size of Lithuanian population, demographic situation, the lifespan of chromosome patients, one can assume that about 15,000 chromosome patients are living in our country. The forecast of chromosomal anomalies in population for the future seems doubtful and complicated because of diverse influences,- either demographic or mutagenic. The situation is clearer only for two types of chromosome changes related to decreasing birthrate: first, for balanced chromosome translocations (reciprocal and Robertsonian type) and, second, for trisomic Down syndrome. At present we are approaching in Lithuanian population the familial model "only one but a healthy baby", and therefore the frequency of balanced chromosome aberrations will accumulate. On the other hand, together with decreasing of birthrate the portion of elderly mothers also lowers, and the frequency of patients with additional 21 chromosome must diminish.

Keywords: karyotype, mutation rate, reciprocal translocation, Robertsonian translocation, demographic

Presentation number: AG 26 Abstract number: ABS-284-ISABS-2011

GENETIC STRUCTURE OF HUMAN POPULATIONS IN BOSNIA AND HERZEGOVINA BASED ON 10 ALU AND 8 BLOOD GROUP POLYMORPHIC DNA LOCI

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Genetic variation based on 18 polymorphic autosomal DNA loci (A25, ACE, APO, B65, D1, FXIIIB, HS2.43, HS4.65, PV92, TPA25, RHC, RHD, RHE, MN, KELL, JK, FY, LU) were investigated among three ethnical groups as well as ten regional subpopulations of Bosnia and Herzegovina (Bosniaks, Bosnian Croats, Bosnian Serbs, Krajina, Posavina, Northeast Bosnia, East Bosnia, Middle Bosnia, Central Bosnia, Sarajevo region, East Herzegovina, Central Herzegovina, West Herzegovina). The sample consisted of 440 nonrelated individuals. DNA was obtained from buccal swabs. Detection of alleles and genotypes was performed by of horizontal gel electrophoresis and The Agilent 2100 Bioanalyser (Agilent Technologies). Parameters of intraethnic genetic diversity were quite the same within all observed ethnic groups. Total gene diversity within all ethnic groups was 0.33. The overall genetic differentiation (Theta) for analyzed loci was 0.34%. Population genetic analysis showed greater interregional than interethnic variation implying that there is no genetic difference between three major ethnic groups in Bosnia and Herzegovina. These results are in agreement with results of previous studies based on other molecular-genetic markers which indicated that three ethnic groups of Bosnia and Herzegovina belong to the same gene pool and have the same origin. Overall genetic differentiation (Theta) was 0.84% and pFST according to that show no statistical genetic differentation among regional subpopulations.

Keywords: human populations, population genetics, genetic differentation, 18 autosomal markers, Bosnia and Herzegovina

ABOUT INVITED SPEAKERS

Christopher Asplen (Gordon Thomas Honeywell Governmental Affairs, Washington, DC and DNA 4 Africa, Chalfont, PA, USA)
Not provided.

Dušica Babović-Vuksanović (College of Medicine, Mayo, Clinic, Rochester, MN, USA)
Dr. Babovic-Vuksanovic is a Professor of Medical Genetics and Pediatrics at Mayo Medical School, a consultant geneticist, and the Chair of the Department of Medical Genetics at Mayo Clinic, Rochester, Minnesota. She completed her M.D. degree University of Sarajevo (Bosnia and Herzegovina) , residency in Pediatrics at Children's Hospital, University of Sarajevo and Mayo Clinic, Rochester, Minnesota. She also completed her fellowship in Medical Genetics at Mayo Clinic. She is board certified in Pediatrics, Medical Genetics, and Clinical Molecular Genetics. She is a member of several national organizations and serves as a Research Advisory Board member of the Children's Tumor Foundation. She is a reviewer for a number of journals and editorial boards. She has been an invited speaker to several national and international conferences. She has expertise and clinical interests for a variety of genetic disorders. She has more than 75 peer-reviewed publications and has authored several book chapters.

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.

Nicolai Bovin (Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation)
Nicolai Bovin is the Head of Carbohydrate Chemistry Laboratory at Shemyakin Institute of Bioorganic Chemistry, Moscow, Russian Federation (IBCh). He received his M.S. degree from the Department of Chemistry Moscow State University, and PhD and Dr.Sci. degrees from IBCh. He has become Professor at 2001. In 1976 he has started working at IBCh at the lab of Carbohydrates and Glycoproteins (Prof. A.Ya. Khorlin) on oligosaccharide synthesis. In 1985-1988 he has been working at the Institute of Biotechnology, Moscow, starting his studies on polymer neoglycoconjugates. In 1988 he has been promoted to the Head of the Laboratory of Carbohydrates at IBCh and now he continues working in this capacity. His current research interests include carbohydrate/protein and carbohydrate/carbohydrate interaction; synthesis of oligosaccharides and neoglycoconjugates; supramolecular chemistry; glycoarrays, natural anti-carbohydrate antibodies; medicinal chemistry: influenza therapy and diagnostics, transplantation, oncodiagnostics and oncotargeting.

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.

Theresa Caragine (Office of Chief Medical Examiner, New York, NY, USA)
Theresa Caragine is a Deputy Director in the Department of Forensic Biology at the Office of Chief Medical Examiner of the City of New York. During her tenure there, Theresa directed the optimization and validation of protocols for Low Template DNA testing. Currently, she manages teams who perform and testify to both Low and High Template DNA testing and conduct forensic DNA research. Recent projects include the development and validation of the Forensic Statistical Tool (FST) software designed to address allelic drop-in and drop-out within a likelihood ratio framework. Theresa received a Bachelor of Science degree in Biology from

Georgetown University and a Ph.D. in Molecular Oncology and Immunology from the Sackler Institute of Graduate Biomedical Sciences at New York University School of Medicine.

Filippo Castiglione (NRC Institute for Computing Applications, Rome, Italy)
FC got a degree in Computer Science (Univ Milan, IT). After one year at ST Microelectronics in Italy, FC started his scientific career doing a doctorate in Scientific Computing at the University of Cologne (Germany). After that, and before initiating his current appointment at the CNR, he was respectively: i) a one-year post-doc at the Department of Computer Science (University "La Sapienza" in Rome), ii) a four-months visiting scholar at Harvard Medical School and iii) a one-year post-doc at the Institute for Medical Bio-Mathematics in Tel Aviv. His research interests range from the study of complex systems in general to the modeling of biological systems, with particular interest in the immune system and related pathologies. He was involved as principal investigator in two EU-funded projects of the ICT for Health of the FP6 and 7. FC published about 70 reviewed research papers among journals, books and conferences proceedings.

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.

Jakov Dulčić (Institute of Oceanography and Fisheries, Split)
Not provided.

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 post-doctoral fellow in the Department of Biology at Princeton and the Department of Medicine at Stanford.

Christopher Evans (Center for Advanced Orthopaedic Studies, Harvard Medical School, Boston, MA, USA)
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 haev 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.

Matthew Ferber (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Dr. Ferber has technical expertise with current capillary electrophoresis based DNA sequencing instrumentation. His current projects focus on transitioning from legacy platforms to the so called "next generation" sequencing (NGS) technologies. Specifically, he is working on developing a more comprehensive hereditary colon cancer screening panel that depends on NGS for implementation.

Dennis Gastineau (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Not provided.

Arezou Ghazani (Harvard Medical School and Massachussetts General Hospital, Boston, MA, USA)
Dr. Ghazani is a nanoengineer (PhD, from University of Toronto) at Massachusetts General Hospital in Boston. She has also accredited training as a Medical Geneticist from the American Board of Medical Genetics (ABMG) at Harvard Medical School. Dr. Ghazani's research includes bridging engineering science and clinical diagnostics and development of novel diagnostic devices using nanotechnology.

Esther Guetta (Danek Gertner Institute of Human Genetics, Sheba Medical Center, Tel-Hashomer, Israel)
I am the director of the Clincal Cytogenetics Unit and the Fetal Cell Research Laboratory at the Genetics Institute, Sheba Medical Center, Israel. I divide my time between these two areas of medical genetics. I am responsible for the activities of the Cytogenetics Unit which carries out prenatal, postnatal and fertility-related karyotype analyses. Upon returning to Israel in 1997, following postdoctoral training at the NIH and Cleveland Clinic, I founded a research laboratory dedicated to developing noninvasive methods for prenatal diagnosis, specifically, enrichment and identification of fetal cells from maternal blood as well as analysis of cell-free DNA in maternal plasma. Our laboratory offers a well-established non-invasive clinical test for fetal sex detection from week 7 of pregnancy and will soon release a noninvasive test for fetal RhD. Our research work on fetal cells in maternal blood is ongoing, and we recently identified novel trophoblast-specific markers.

Karin HaLevi (Institute for Medical BioMathematics, Bene Ataroth, Israel)
I did my B.Sc. in the Faculty of life science in the Tel-Aviv University and M.Sc and Ph.D. degrees in the Weizmann Institute of Science in Israel. I terminated my Ph.D. thesis in 1998 in the field of molecular and cellular biology, focused on the proto-oncogene c-Myc as a transcription factor and inducer of apoptosis and on the control of c-Myc degradation. I worked for more than ten years in biotechnology start-up companies in the fields of drug discovery and drug development. I joined the Institute for Medical BioMathematics (IMBM) in 2008. At IMBM I takes part in two projects: modeling of main intracellular signalling pathways controlling stem cell and modeling of cellular vaccines.

Carol Henderson (Stetson University College of Law, Gulfport, FL, USA)
Not provided.

Thierry Hennet (Institute of Physiology, University of Zürich, Zürich, Switzerland)
Thierry Hennet is a glycobiologist working in the field of congenital disorders of glycosylation. In the last ten years, his team has contributed to the identification of several new diseases and his current interest focuses on the development of possible therapies.

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

Stipan Jonjić (Faculty of Medicine, University of Rijeka, Rijeka, Croatia)
Dominant part of my research deals with viral immunology and pathogenesis of viral infections. We use murine cytomegalovirus (CMV) as a model to study the immunology and pathogenesis of human CMV infection. Among other topics the dominant ones are: viral immunoevasion of NK cells and CD8+T-cells, congenital CMV infections of CNS and the role of NK cells in shaping antiviral CD8 T-cell response. More recently, we became interested in designing CMV vaccines and CMV based vaccine vectors by using recombinant viruses engineered to express cellular ligands for activating NK cell receptors.

Sree Kanthaswamy (California National Primate Research Center, University of California, Davis, CA, USA)
My human and non-human forensic science research is based on the analyses of traces of animal blood, saliva and hair collected at crime scenes or from civil cases for DNA-typing. To further my research interests and provide educational opportunities for students, my lab develops species-specific STRs, SNPs and mtDNA markers for accurate and precise genetic identification and to enhance our population genetics database for each species. I also provide expert witness testimony on animal/veterinary forensic DNA analysis and casework review.

David Kaye (Eberly College of Science, Penn State University, PA, USA)
David H. Kaye is Distinguished Professor of Law at Penn State and Regents' Professor Emeritus at Arizona State University. He has held appointments at the universities of Chicago, Cornell, Duke, Iowa, Utah, and Virginia, as well as universities in England and China. He holds degrees in law (Yale), in astronomy (Harvard), and physics (MIT), and practiced law as a private attorney and federal prosecutor. Professor Kaye's research focuses on the use of science and statistics in litigation, and on forensic genetics. He has served on committees or projects of the American Statistical Association, Federal Judicial Center, National Academy of Sciences, NIH, and NIST. His publications include 11 books and many articles in journals of law, philosophy, psychology, medicine, genetics, and statistics. In addition to these professional activities, he holds the rank of fourth dan in Aikido Kokikai ! and participated in first ascents of mountains in Alaska and China.

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.

Ofer Klein (Rebecca Sieff Medical Center and BioCep, Ltd., Safed, Israel)
Dr. Klein holds a B.Sc. in Agriculture from the Hebrew University in Israel, and a M.Sc. and Ph.D. in Pathology from the Sackler School of Medicine at Tel Aviv University in Israel. Dr. Klein is the chief scientist at BioCep ltd, which has developed the Cell Enrichment Process, a novel method in immuno magnetic cell isolation. Dr. Klein is the director of the research laboratories at the Rebecca Sieff Medical Center in Israel and a lecturer of molecular biology at the Safed college. Dr. Klein was a lecturer of pathology at the Sackler School of Medicine at Tel Aviv University in Israel and the director of the Maccabi HMO hematology laboratories in Jerusalem. Dr. Klein served as a commander in the Israeli Air Force.

Jean-Pierre Kocher (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Dr Kocher joined Mayo Clinic in October 2005 to develop and direct the activities of the newly created Bioinformatics Core. In 2008, he became Chair of the Division of Biomedical Statistics and Informatics (BSI) that regrouped experts in Biostatistics, Bioinformatics, Medical Informatics and Computer Science. The division develops, maintains, and promotes state of the art analytical and informatics methods and expertise provide high-value collaboration and research services for investigators involved in basic science, translational and clinical research. Prior to joining Mayo Clinic, Dr. Kocher served as Executive Officer at Molecular Networks, a chemoinformatics company and Director of Computational Chemistry at Camitro Corp. Dr. Kocher's scientific interest focuses on the development and application of computational methods to advance the understanding of the molecular mechanisms that underlie clinical disorders.

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.

Gunnar Kvalheim (Norwegian Radium Hospital, University of Oslo, Oslo, Norway)
Professor Dr med Gunnar Kvalheim is a specialist in medical oncology and radiotherapy at Oslo University Hospital-Radiumhospitalet. Since 1989 he became the director of the Department of Cellular Therapy at Radiumhospitalet. In October 2009 the Department of Cellular Therapy moved into a new research area and is currently one of the largest academic GMP facility for cellular therapy in Europe. In 1996-2001 he served as international vice-president in ISCT and in 2003-2010 he was co-editor in Cytotherapy. He was also the co-chairman and founder of JACIE. His main scientific activity has been on stem cell biology, bone marrow purging, detection of minimal residual cancer and immunotherapy. He has published 135 articles and book-chapters and supervised 7 PhD students to doctoral thesis. Currently, his main focus is on translational and clinical research in cellular therapy.

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.

Hakon Leffler (Lund University Hopsital Sölvegatan, Lund, Sweden)
MD (1974) and Ph.D. (1981) in medical Biochemistry with emphasis on structure and function of complex carbohydrates (glycosphingolipids). My main research since 1985 has been on galectins, and I was part of discovering and naming this protein family. My research on galectins has focused on defining their carbohydrate binding specificity regarding mechanism and biological roles in cell culture, and since 1998, in collaboration with organic chemist Ulf Nilsson to develop potent galectin inhibitors and fluorescent probes; we have achieved compounds with low nM monovalent affinities, which is among the highest ever observed for a carbohydrate binding protein, thereby challenging the old view that protein-carbohydrates are weak and require multivalency for high affinity. These tools are now used to study the function of galectins and to develop therapeutics against cancer and inflammation. Another recent line of research concerns the interaction of galectins with human serum glycoforms, and its functional consequences.

Henning Madry (Saarland University Hospital and Saarland University Faculty of Medicine, Homburg, Germany)
Henning Madry studied Medicine at the Charité Medical School in Berlin, Germany. Electives in Southampton, Jerusalem, Geneva, Nice and Houston. M.D. thesis "summa cum laude" at the Max-Delbrück-Center for Molecular Medicine, Berlin. Postdoctoral Fellow (supported by the German Academy of Scientists Leopoldina) at the Massachusetts General Hospital, Harvard Medical School, Boston and at the Massachusetts Institute of Technology, Cambridge, MA, USA. In 2000, he founded the Laboratory for Experimental Orthopaedics, Saarland University, Homburg, Germany. Board-certified in Orthopaedic Surgery. Since 10/06 Attending Clinician. From 2008 – 2010 Chairman of the Articular Cartilage Committee of the ESSKA, since 2010 Vice-Secretary General of ESSKA. He currently holds the Endowed Chair of Experimental Orthopaedics and Osteoarthritis Research at the Saarland University.

Damir Marjanović (Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina and Genos, Zagreb, Croatia)
Position: Professor at the University of Sarajevo (B&H), Head of the Laboratory for Forensic Genetics at the Institute for Genetic Engineering and Biotechnology, Sarajevo, Director of the Scientific Department, Genos, Zagreb. More than 100 scientific publications. 37 years old.

Boris Martinac (Victor Chang Cardiac Research Institute, Sydney, NSW, Australia)
Prof. Boris Martinac has co-pioneered characterization of ion channels in microbial cells. Discovery, cloning and structural and functional characterisation of mechanosensitive (MS) ion channels in prokaryotic cells (Bacteria and Archaea) present his major original contribution to the ion channel research field. Prof. Martinac led the study, which eventually resulted in cloning of MscL as the first MS channel identified at the molecular level. The major aim of his research is to

elucidate the role of mechanical force in biophysics of MS ion channels, and to understand the physiology and evolutionary origins of mechanosensensory transduction at the molecular level. Prof. Martinac's recent work expanded into studies of the role MS ion channels may play in neuronal and cardiac diseases.

Marilyn Menotti-Raymond (National Cancer Institute, Frederick, MD, USA)
Dr. Menotti-Raymond's research has focused on development and application of genomic resources in the domestic cat to contribute to understanding of human hereditary disease analogues and genes of general biological interest. The development of comprehensive genetic maps has been a major focus. In the last few years, Dr. Menotti-Raymond's group have mapped and characterized mutations causative of feline spinal muscular atrophy (LIX1) and retinal atrophy (CEP290) (CRX). She is additionally interested in genes responsible for coat color and pattern formation in the cat, and have recently characterized variants at the tyrosinase locus (siamese, burmese), tyrosinase related protein locus (brown and cinnamon coat color), melanophilin (dilute) and long hair (FGF5). The development of an STR genotyping system for genetic individualization of cat specimens was initiated following their first introduction of an animal DNA fingerprint into court.

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.

Giorgio Palù (University of Padova, Padova, Italy)
Director of the Institute of Microbiology, University of Padova, Medical School (1991-1999); Director of the Department of Histology, Microbiology and Medical Biotechnologies,University of Padova, Medical School (1999-2002); Head of the Center of Clinical Microbiology and Virology, Padova University Hospital/Veneto Region (since 1996); Member of the Executive Committee of the Interdipartimental Centre for Innovative Biotechnologies, Padova University (since 1992); the Interuniversity Consortium of Nanotechnologies (since 2003); the Padua start-up company incubator (since 2003); the Biotechnological Association for Oncology Research (since 2003). Coordinator PhD course in Virology (since 1998) and in Biomedicine (since 2007); Pro-Rector, University of Padova (Foreign affairs and international research, since 2002; Dean, Faculty of Medicine and Surgery, University of Padova (since 2004), Director of GMP in Padova (Cell-factory in the Veneto Region – Italy). Italian Society of Virology (president), European Society for Virology (Presidente Vicario); Corresponding Member of the Gesellschaft für Virologie (GfV); Panel scientifico European Center for Diseases Control (ECDC). Author of over 300 international publications on: Molecular and Clinical

Microbiology and Virology; design of viral and non viral vectors for gene transfer; somatic gene therapy and vaccinology; antimicrobial and antitumor therapy and several chapters in international books.

Jef Pinxteren (ReGenesys, Heverlee, Belgium)
He obtained a PhD in Biochemistry at the University of Antwerp in 1994. He obtained a long-term EMBO fellowship to study the role of G-proteins in secretion from granulocytes at UC London. After his fellowship he continued at UCL with further funding. In 1999 he started collaboration with Vytas Bankaitis, first at UAB, later at Chapel Hill. In 2001 he joined the University of Gent for a cord blood stem cell project. He started to isolate MAPC from human bone marrow, and started to collaborate with Catherine Verfaillie in Minneapolis. In 2004 he moved on to the VIB to study proteomics of human MAPC. Finally in 2006, he moved to the newly formed Stem Cell Institute Leuven under Catherine Verfaillie and worked with Athersys (Cleveland, Ohio) to set up the daughter company ReGenesys. In May 2007 he started to work as Head R&D of ReGenesys.

Giulio Pompilio (University of Milan, Milan, Italy)
Dr. Giulio Pompilio (male, MD PhD) is staff surgeon and Head of the Clinical Research Unit for Cardiovascular Regenerative Therapy at Centro Cardiologico Monzino IRCCS, Milan. He is Associate Professor at the University of Milano. He is a cardiac surgeon and a clinician scientist with 15 years experience in cardiovascular clinical and surgical research. His research activities are focused on gene and cell therapy of ischemic cardiomyopathy and peripheral limb ischemia and the standardization of autologous cell therapy products for clinical use. He was a pioneer in Italy as principal investigator of controlled clinical trials in gene and cell therapy for the treatment of refractory ischemia. He serves the European Medicines Agency as external expert for advanced biological products.

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 more than one hundred scientific papers and abstracts in forensic science, population genetics, genetic legacy of Homo sapiens sapiens, clinical medicine and molecular genetics. His papers have been cited more than 1400 times. Dr. Primorac is the co-founder of ISABS and the Center for Forensic Sciences at the University of Split. From 2003 to 2009 he served as Minister of Science, Education and Sports, Republic of Croatia.

Erdmann Rapp (Max Planck Institute for Dynamic of Complex Technical Systems, Magdeburg, Germany)
Dr. rer. nat. Erdmann Rapp (Senior Research Scientist). At the Max Planck Institute for Dynamics of Complex Technical Systems in Magdeburg (Germany), several

research groups are focussing on biological processes and systems. The core facility “Bio-(Process-) Analytics” is headed by Dr. Erdmann Rapp, who is Physico-Chemist and has a strong background in fundamentals of miniaturized separation techniques. The Bio-(Process-) Analytics team is dealing with development, hyphenation (MS, NMR, LIF, UV/Vis, etc.), and application (proteomics and glycomics) of capillary chromatographic and capillary electrokinetic separation methods and techniques and is doing fundamental and applied research in close cooperation with teams working in the fields of biotechnological up- and downstream processing, metabolomics, microbiology, and systems biology. The team has developed HTP methods for preparation and analysis of glycans. The method and system development has resulted in several publications and patent applications and some well-established industrial and academic collaboration.

Pauline Rudd (The National Institute for Bioprocessing Research and Training, Dublin, Ireland)
Professor Rudd obtained a BSc in Chemistry at the University of London and a PhD in Glycobiology at the Open University, UK. She was a Founding Scientist of Wessex Biochemicals (later Sigma London), Visiting Research Associate at The Scripps Research Institute, CA, a Visiting Professor of Biochemistry at Shanghai Medical University PRC, Visiting Scientist at Ben Gurion University of the Negev, Israel and Erskine Visiting Fellow, Canterbury University, Christchurch, New Zealand. She is a Fellow of the Royal Society of Medicine, London and an Adjunct Professor at North Eastern University, Boston. She has more than 190 scientific publications and given over 200 lectures and seminars at international meetings. Before moving her group to NIBRT in Dublin in 2006, Professor Rudd was a member of the Glycobiology Institute for 25 years. When she left she was a Senior Re! search Fellow and a University Reader in Glycobiology at Oxford University.

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.

Eliezer Shalev (Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel)
Prenatal diagnosis, basic reasearch in reproduction.

Stavit A. Shalev (Human Genetic Center, Ha'emek Medical Center, Afula and Technion-Israel Institute of Technology, Haifa, Israel)
Human genetics.

Artemis Simopoulos (The Center for Genetics, Nutrition and Health, Washington, DC, USA)
Dr. Simopoulos graduated from Boston University School of Medicine. She is the President of the Center for Genetics Nutrition and Health, a non-profit organization in Washington, DC. Dr. Simopoulos did her research at the National Institutes of Health on the nutritional and genetic aspects of endocrine disorders. She is the immediate Past President of the International Society of Nutrigenetics Nutrigenomics and has written extensively in the field.

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.

Daniela Steinberger (Institute of human genetics, Justus Liebig University, Giessen and biologis, Frankfurt am Main, Germany)
Not provided.

Mark Stoneking (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany)
I direct the Human Population History Group in the Department of Evolutionary Genetics at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. Our group uses molecular genetic methods to investigate questions related to the origins, dispersals, and relationships of human populations, as well as the role of recent positive selection in human population history.

Ron Tepper (Sapir Medical Center, Kfar Saba, Israel)
I am the chairman of the Israeli Society of Ultrasound in Obstetrics and Gynecology, running the Academic school of Ultrasound of the Israeli Society of US , chairman of Ultrasound unit department of Obstetrics and Gynecology in Meir Medical Center, and chairman Simultech, Medical Simulation Center.

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 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.

Daniel Vanek (Forensic DNA Service, Prague, Czech Republic)
Dr. Vanek leads an independent forensic DNA analysis laboratory which specializes in Forensic DNATyping, Genetic Genealogy, and Ancient DNA Typing. Prior to starting Forensic DNA Service, he lead the Banja Luka laboratory for the International Commission for Missing Persons (ICMT) and was the head of the National DNA Laboratory in the Czech Republic. Dr. Vanek is the founding president of the Czechoslovak Society for Forensic Genetics. He received his PhD in molecular biology from Charles University in Prague.

George Vasmatzis (College of Medicine, Mayo Clinic, Rochester, MN, USA)
Not provided.

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.

Samuel Volchenboum (University of Chicago, Chicago, IL, USA)
I am a pediatric hematologist and oncologist at the University of Chicago. I take care of children with cancer and blood diseases. In addition, I have training in clinical and bioinformatics and am a member of the University of Chicago Computation Institute. My research focuses on using systems biology approaches to elucidate the mechanisms of tumorigenesis in children. In addition, I am developing algorithms to facilitate high-throughput data analysis and to improve dynamic range and sensitivity in mass spectrometry experiments.

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 and regenerative medicine. Together with Professors Moses Shanfield and Dragan Primorac, Prof. Vuk-Pavlović founded ISABS and has contributed to the academic part of organizing its meetings.

Eske Willerslev (University of Copenhagen, Copenhagen, Denmark)
Eske Willerslev is a professor in evolutionary biology and Chief Director, Centre of Excellence in GeoGenetics, Natural History Museum, Copenhagen.

George Wu (University of Connecticut Health Center, Farmington, CT, USA)
Dr. Wu has been a pioneer in the field of targeted delivery of biological substances specifically to liver cells, hepatocytes. He developed the concept of targeted rescue, targeted gene delivery, and most recently an immunocompetent model for HCV infection. He has received numerous awards and prizes including the American Liver Foundation Post-Doctoral Fellowship Award 1980-1, and an American Gastroenterological Association/Industry Research Scholar Award 1985-88, American Liver Foundation Research Prize, 1982, , American Gastroenterological Association-Gastroenterology Research Group Young Scientist Award, 1990, and a Chinese American Medical Society Scientific Award, 1992, was elected a Fellow of the American Gastroenterological Association, 2010, and selected a Fulbright Specialist, 2011. He was elected to the American Society for Clinical Investigation, and the Association of American Physicians Since 2000, he has presented more than 83 lectures nationally, and 31 lectures abroad. He has received 6 patents, and founded two startup companies to commercialize inventions.

Manfred Wuhrer (Leiden University Medical Center, Leiden, The Netherlands)
Dr. Manfred Wuhrer is leading the Glycomics and Glycoproteomics group at the Biomolecular Mass Spectrometry Unit, LUMC, Leiden, The Netherlands. His research is focusing on the development of glycoanalytical techniques and their application in clinical glycomics. Glycosylation aspects of various diseases such as cancer, rheumatoid arthritis, multiple sclerosis, and different infectious diseases are studied. A natural glycan array exhibiting glycans of cancer tissue is used to study anti-glycan autoantibodies as well as their glycan targets in colorectal cancer.

Simcha Yagel (Hadassah Medical Center, Hebrew University of Jerusalem, Jerusalem, Israel)
My work divides into two main areas of interest, OB/Gyn ultrasound, and basic research. In the clinical sphere in recent years my work focuses primarily on 3D/4D ultrasound of the fetal cardiovascular system, including publication of a textbook, Fetal Cardiology. We have published many articles on 3D/4DUS of the fetus, with particular focus on 3D/4D fetal echocardiography and functional evaluation of the fetal heart, neurosonography, as well as other organ systems. In the basic research sphere, our work focuses on placental cell function and gene expression in the development of the placenta, the role of NK cells at the maternal-fetal interface and their role as builders in embryo implantation and placental and fetal development, and studies of the molecular mechanisms involved in the expression of placenta-derived circulating anti-angiogenic factors in the serum of pregnant women and their role in the development of preeclampsia.

Kristin Young (University of Kansas Medical Center, Kansas City, KS, USA)
Dr. Young is an anthropological geneticist by training, whose research focuses on identifying genetic polymorphisms in human populations and investigating the interaction between cultural, environmental, and genetic factors on population structure, health, and disease. As a Research Instructor at the University of Kansas Medical Center in the Department of Family Medicine Research Division, Dr. Young is applying her skills as an anthropological geneticist to the issue of health disparities in minority populations, with a particular emphasis on cancer.

SATELLITE EVENT

Fifth Croatian Human Genetics Conference

SCIENTIFIC PROGRAM

CROATIAN SOCIETY
OF HUMAN GENETICS

All lectures take place in the „Hvar“ conference room.

Monday, June 20, 2011

09.15 Opening ceremony

CYTOGENETICS AND CLINICAL GENETICS:

Chairs: Ingeborg Barišić, Ružica Lasan-Trčić

09.30 **Schinzel A**:
Minor dysmorphic signs: their value for assessment of the etiology of congenital developmental defetcs
10.00 **Ostojić S**, Pereza N, Kapović M, Peterlin B:
Current views on genetic and epigenetic etiology of recurrent spontaneous abortion
10.20 **Pereza N**, Ostojić S, Volk M, Kapović M, Peterlin B:
Functional single nucleotide polymorphisms in promoter regions of matrix metalloproteinase -1,-2,-3 and -9 genes are risk factors for recurrent spontaneous abortion
10.30 **Dumić K**, Krnić N, Škrabć V, Stipančić G, Kušec V, Štingl K, Barišić I:
Classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency in croatia between 1995 and 2010
10.45 **Roganović J**, Barišić N, Jonjić N:
Angiokeratoma in fabry disease: diagnostic but not treatment effectiveness marker
11.00 Šmigovec I, Đapić T, **Kubat O**, Antičević D:
Orthopaedic treatment of metabolic bone diseases
11.15 Discussion

11.30 Coffee Break

Chairs: Sanda Huljev-Frković, Leona Morožin-Pohovski

12.00 **Zuffardi O:**
From genome-wide array to next generation sequencing
12.30 **Morožin- Pohovski L**, Barišić I:
Screening of patients with mental retardation using subtelomeric mlpa assay
12.45 **Božina N:**
Pharmacogenomics and its potential application in personalized medicine
13.00 **Lasan Trčić R**, Rajić L, Femenić R, Bilić E, Dubravčić K, Zadro R, Batinić D, Konja J, Begović D:
Classical and molecular cytogenetic analysis in 324 pediatric patients with acute lymphoblastic leukaemia
13.15 **Odak L**, Barišić I, Morožin-Pohovski L, Sansović I, Dumić K, Jakušić N, Klobučar A, Grgurić J:
Diagnostic algorithm for children with autism
13.30 Discussion

GENETIC EPIDEMIOLOGY, POPULATION GENETICS AND ANTROPOLOGY

Chairs: Nina Smolej Narančić, Marijana Peričić Salihović

15.00 **Peričić Salihović M**, Barešić A, Martinović Klarić I, Cukrov S, Barać Lauc L, Janićijević B:
Traces of migration from India to Europe as written in the mitochondrial DNA of Bayash Roma
15.15 **Barešić A**, Peričić Salihović M:
Mendelian disorders of the Bayash Roma from Croatia
15.30 **Levačić Cvok M**, Musani V, Sušac I, Ozretić P, Sabol M, Car D, Eljuga D, Eljuga L, Levanat S:
BRCA1 and BRCA2 germline mutations in women in Croatia with hereditary predisposition to breast and ovarian cancer
15.45 **Vraneković J**, Babić Božović I, Grubić Z, Wagner J, Pavlinić D, Dahoun S, Čulić V, Brajenović-Milić B:
Down syndrome in Croatia: parental origin, recombination, and maternal age
16.00 Discussion

GENETIC COUNSELLING, PRENATAL DIAGNOSIS

Chairs: Bojana Brajenović- Milić, Vida Čulić

16.45 **Schinzel A**:
Congenital defects in twins: etiology, spectrum, genetic counselling
17.15 **Čulić V**, Mišković S, Zegarac Z, Mijaljica G, Kaštelan T, Pavelić J:
Positive reproductive family history for spontaneous abortion - predictor for recurrent abortions in young couples
17.30 **Roje D**, Kavelj M:
The first five years experience of prenatal diagnosis in Split, Croatia: analysis and evaluation of the results obtained from amniotic fluid samples
17.45 **Wagner J**, Hercog R, Heffer M, Lauc G:
Non-invasive determination of fetal gender and fetal RhD status from maternal blood
18.00 Discussion

Tuesday, June 21, 2011

MOLECULAR AND BIOCHEMICAL BASIS OF MONOGENIC AND COMPLEX DISEASES

Chairs: Ivo Barić, Jadranka Sertić

09.00 **Barić I**, Sarnavka V, Ćuk M, Bilić K, Petković Ramadža D, Martinac I, Zibar K, Muačević D, Merkler M, Reiner Ž, Luetić T, Pažanin L, Žarković K, Fumić K:
Challenges in diagnosis and therapy of inherrited disorders of mitochondrial energy production

09.15 **Borovečki F**:
Genomic approaches to development of new diagnostic paradigms in neurodegenerative diseases

09.30 Harjaček M, **Lamot L**, Frleta M, Gotovac K, Bingula F, Borovečki F:
Diverse gene expression in patients with juvenile spondyloartropathy and claviculas cortical hyperostosis is possibly related to autoinflammatory diseases

09.45 **Sertić J**, Božina T, Lovrić M, Jelaković B, Božina N, Merkler A, Ljubić H, Reiner Ž:
Metabolic syndrome: effects of PPAR γ, APOE, LPL, IL-6, ACE and AT1R gene variants

10.00 Ristić S, **Starčević Čizmarević N**, Seplić J, Živković M, Stanković A, Klupka-Šarić I, Lovrećić L, Peterlin B:
PAI and TPA gene polymorphisms in multiple sclerosis

10.15 **Buretić-Tomljanović A**:
Human genome architecture in schizophrenia and other neuropsychiatric disorders

10.30 Coffee Break

11.00 **Buretić-Tomljanović A**, Vraneković J, Rubeša G, Jonovska S, Tomljanović D, Šendula-Jengić V, Kapović M, Ristić S:
HFE mutations and transferrin C1/C2 polymorphism among Croatian patients with schizophrenia and schizoaffective sidorder
11.15 **Nadalin S**, Jonovska S, Rubeša G, Dević S, Buretić-Tomljanović A**:**
Impact of CPLA2 and COX-2 gene polymorphisms on attenuated niacin skin-flush response in patients with schizophrenia
11.30 **Babić Božović I**, Vraneković J, Starčević Čizmarević N, Mahulja-Stamenković V, Prpić I, Brajenović-Milić B:
MTHFR C677T and A1298C polymorphisms as a risk factor for congenital heart defects in Down syndrome
11.45 **Sansović I**, Barišić I:
Improved detection of meletions/duplications in the DMD gene using the multiplex ligation-dependent probe amplification (MLPA) method
12.00 Bilić K, Zekušić M, **Fumić K:**
The importance of optimizing methods for measuring the activity of alpha-acid glucosidase in the diagnosis of Pompe disease
12.15 Toth O, Mrđenović S, **Heffer M**:
Mouse models for ganglioside deficiency
12.30 Discussion

CANCER GENETICS

Chairs: Jasminka Pavelić, Sanja Mrsić

14.00 **Matijević T**, Pavelić J:
The dual role of toll-like receptor 3 in metastatic cell line
14.15 **Slade N**, Brdar B, Jelaković B, Moriya M, Medverec Z, Tomić K , Karanović S, Vuković I, Wu L,Grollman A:
TP53 mutational signature of aristolochic acid in the upper urinary tract carcinomas
14.30 **Nikuševa Martić T**, Pećina Šlaus N, Zeljko M, Tomas D:
Change of the NF2 gene in neurinomas
14.45 **Rako I**, Čaban D, Jakić-Razumović J, Pleština S, Sertić J:
The role of determinins the status of KRAS gene in colorectal cancer
15.00 **Dević S**, Flego V, Ristić S, Kapović M, Radojčić Badovinac A:
ACE I/D polymorphism in lung malignancy
15.15 Discussion

15.30 Coffee Break

CROATIAN SOCIETY OF HUMAN GENETICS AND CROATIAN SOCIETY OF RARE DISEASE

16.00 Round table - Ivo Barić, Ingeborg Barišić, Ksenija Fumić, Miljenko Kapović, Saša Ostojić, Jelena Roganović, Ana Stavljenić-Rukavina: Rare diseases in Croatia – achievements and prospects

17.00 Acknowledgement to young human geneticist for oral/poster presentation from proffessors Zergollern-Čupak foundation

20.30 Welcome reception, Borak beach restaurant

SATELITE EVENT

Fifth Croatian Human Genetics Conference

CROATIAN SOCIETY
OF HUMAN GENETICS

Abstract number: HDHG-ABS-218-ISABS-2011

MTHFR C677T AND A1298C POLYMORPHISMS AS A RISK FACTOR FOR CONGENITAL HEART DEFECTS IN DOWN SYNDROME

Babić Božović I[1], Vraneković J[1], Starčević Čizmarević N[1], Mahulja-Stamenković V[2], Prpić I[3], Brajenović-Milić B[1]

[1]Department of Biology and Medical Genetics, School of Medicine, University of Rijeka, Rijeka, Croatia; [2]Department of Obstetrics and Gynecology, University Hospital Centre Rijeka, Rijeka, Croatia; [3]Department of Pediatrics, University Hospital Centre Rijeka, Rijeka, Croatia
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The presence of MTHFR C677T and A1298C polymorphisms has been reported as a risk factor for congenital heart defects (CHDs) in Down syndrome (DS). The aims of the present study were to assess the frequency of MTHFR C677T and A1298C in DS in the Croatian population, the relationship between the two parental MTHFR polymorphisms and CHD-affected DS children, and the transmission frequencies of the variant alleles of the two MTHFR polymorphisms in CHD-affected DS. The study population included 124 DS cases and 221 controls. CHDs were present in 50% of the DS cases. Out of 124 DS individuals, 118 mothers and 79 fathers were available for the study. Allele transmission was analyzed in 42 complete parent-offspring triads. The frequencies of the allele, individual, and combined genotypes of MTHFR C677T and A1298C in DS were not statistically different compared to the normal healthy controls. The maternal MTHFR polymorphisms were not found to be a risk factor for DS-related CHDs. The MTHFR 677T allele and the 677TT genotype were significantly more frequent in fathers of CHD-affected DS children than in fathers of DS child without CHD ($P = 0.037$ and $P = 0.036$, respectively). The allele transmission of the two MTHFR polymorphisms showed no deviations from random segregation. Because a great proportion of trisomy 21 cases are lost during pregnancy, paternal, maternal and fetal, not only live-born, MTHFR C677T and A1298C, as well as maternal nutrition and life style during pregnancy, should be analyzed to asses the impact on CHDs in DS.

Keywords: congenital heart defect, Down syndrome, MTHFR C677T polymorphism, MTHFR A1298C polymorphism, allele transmission

Abstract number: HDHG-ABS-289-ISABS-2011

MENDELIAN DISORDERS OF THE BAYASH ROMA FROM CROATIA

Barešić A, Peričić Salihović M

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Numerous small groups of Roma people have been shown to share unique private disease-causing mutations; in this study we investigated four such disease loci to establish the influence of the founder effect on the genetic load of these disorders in the Croatian Bayash Roma populations. We analysed 426 Bayash Roma from two regions in Croatia, Baranja in Eastern and Međimurje in North-western Croatia. PCR-based RFLP assays were used to detect the mutations which were then confirmed by sequencing. Carriers were detected for hereditary motor and sensory neuropathy – type Lom in the Baranja community, while there was no presence for the remaining three diseases noted. Carrier rate for the HMSNL in Baranja region is 1,5%. The genetic load of private disease-causing mutations is evidently decreased in the Croatian Bayash compared to other Vlax Roma groups. This difference could have arisen due to the various factors e.g. high genetic load in other Vlax groups could be a consequence of strict endogamy rules widely practiced after the Bayash left Romania, what would then not be reflected in their Croatian community, or the ancestors of the Croatian Bayash were not in close contact with the high risk groups during the slavery period in Romania.

Keywords: Bayash, Vlax Roma, Mendelian disorders, rare diseases, founder effect

Abstract number: HDHG-ABS-358-ISABS-2011

CHALLENGES IN DIAGNOSIS AND THERAPY OF INHERITED DISORDERS OF MITOCHONDRIAL PRODUCTION

Barić I[1,2], Sarnavka V[1], Ćuk M[1,2], Bilić K[3], Petković-Ramadža D[1], Martinac I[2], Zibar K[2], Muavčević D[2,4], Merkler M[4], Luetić T[2,5], Pažanin L[6], Žarković K[2,6], Fumić K[3], Reiner Ž[2,4]

[1]Department of Pediatrics, University Hospital Centre Zagreb, Zagreb, Croatia; [2]School of Medicine, University of Zagreb, Zagreb, Croatia; [3]Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia; [4]Department of Internal medicine, University Hospital Centre Zagreb, Zagreb, Croatia; [5]Department of Surgery, University Hospital Centre Zagreb, Zagreb, Croatia; [6]Department of Pathology and Cytology, University Hospital Centre Zagreb, Zagreb, Croatia
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Inherited disorders of mitochondrial energy production are clinically a considerably variable group of diseases whose signs and symptoms may appear from intrauterine to late adult age. They may affect any organ but most commonly affect the organs that require most energy: muscles, brain and heart. These disorders are caused by mutations of nuclear and mitochondrial DNA. Biochemical features, none of which must be present in all patients, include increased alanine and lactate concentrations in blood and/or cerebrospinal fluid, and increased urine excretion of Krebs cycle metabolites, 3-methylglutaconic, ethylmalonic and some other organic acids. Microscopic muscle exam may reveal red ragged fibers, altered mitochondria or, immunohistochemically, the deficit of respiratory chain proteins. The diagnostic procedure is often aggravated by limited availability of specific biochemical and genetic analyses used to confirm the diagnosis, and by administrative obstacles. The development of genetic analyses of ever increasing capacity and resolution is, however, encouraging. The possibilities of treatment are limited (ketogenic diet, antioxidants, co-factors/vitamins, coenzyme Q) and are commonly of modest efficacy. Clinical course is not rarely unpredictable and prenatal diagnosis is most often not possible.

Keywords: disorders of mitochondrial energy production, inheritance, diagnosis

Abstract number: HDHG-ABS-362-ISABS-2011

GENOMIC APPROACHES IN DEVELOPMENT OF NOVEL DIAGNOSTIC PARADIGMS FOR NEURODEGENERATIVE DISEASES

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Genomic blood biomarkers hold great promise for development of novel clinical and therapeutic approaches in patients with neurodegenerative diseases. Such biomarkers could prove invaluable in early diagnosis of disease, monitoring of disease progression or assessment of response to therapy. More importantly, they could be helpful in search for disease-modifying new therapies which are very much needed in modern approaches to treatment of neurodegenerative diseases, serving as surrogate endpoints in clinical trials. However, when performing expression profiling experiments aimed at discovery of new biomarker genes, standard operating procedures regarding sample collection, microarray methodology and statistical analysis need to be fully developed and strictly adhered to. We have performed several studies aimed at development of diagnostic biomarkers for neurodegenerative diseases, such as Huntington's disease, spinocerebellar ataxia 17 (SCA-17), or multiple sclerosis and initial results offer promise that such approaches might prove useful in clinical practice. Novel microarray technologies, such as array comparative genomic hybridization (aCGH) also provide new tools for development of innovative diagnostic paradigms in neurological diseases. Crucial for successful application of any genomic biomarker will be confirmation in multiple independent patient cohorts and correlation of the improvement in biomarker endpoint with clinical improvement in longitudinal patient studies. A combination of approaches in biomarker discovery may in the end lead identification of promising candidates at DNA, RNA, protein and small molecule level.

Keywords: genomics, neurodegenerative diseases, array CGH, biomarkers, microarrays

Abstract number: HDHG-ABS-280-ISABS-2011

PHARMACOGENOMICS AND POTENTIAL APPLICATION IN PERSONALIZED MEDICINE

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Pharmacogenomics aims to identify genetic factors underlying variability in drug response, providing solutions to improve drug efficacy and safety. The goal is to develop rational means to optimize drug therapy, with respect to the patient's genotype.There are examples of succsesful translation of pharmacogenetics in clinical practice. Drug-metabolizing enzymes represent a major target of ongoing research in order to identify associations between an individual's drug response and genetic profile. Polymorphisms of the cytochrome P450 enzyme CYP2D6 influence metabolism of codeine, tramadol, hydrocodone, oxycodone, some antidepressants and antypsychotic drugs, beta-blockers and antihypertensives. For CYP2D6 polymorphisms, as predictors of outcome in breast cancer patients treated with tamoxifen, expanded polymorphism coverage is needed to improve risk stratification. Blood concentrations of some NSAIDs depend on CYP2C9 and/or CYP2C8 activity. Genomic variants of these genes associate well with NSAIDs' side effect profile. Well established examples where pharmacogenomic techniques can improve routine treatment include genotyping of TPMT variants for the prediction of thiopurine- induced bone marrow depression, VKORC1 and CYP2C9 analyses for a better control of anticoagulant administration, the SLCO1B1 variant in the context of statin-induced myopathies and DPYD analysis for better prediction of 5-FU toxicity. US FDA has recently changed clopidogrel's prescribing information to highlight the impact of CYP2C19 genotype on clopidogrel pharmacokinetics, pharmacodynamics and clinical response. Potential pharmacogenetic markers for immunosuppressive drugs are CYP3A5 for tacrolimus and sirolimus and UGT1A9 and ABCC2 for mycophenolate. Pharmacogenomics is already recognized and recommended by scientific societies, regulatory agencies and public health organisations.

Keywords: pharmacogenomics, drug-metabolizing enzymes, cytochrome P450, drug transporters, drug safety

Abstract number: HDHG-ABS-187-ISABS-2011

HFE MUTATIONS AND TRANSFERRIN C1/C2 POLYMORPHISM AMONG CROATIAN PATIENTS WITH SCHIZOPHRENIA AND SCHIZOAFFECTIVE DISORDER

BuretićTomljanović A[1], Vraneković J[1], Rubeša G[2], Jonovska S[3], Tomljanović D[4], Šendula-Jengić V[3], Kapović M[1], Ristić S[1]

[1]School of Medicine, University of Rijeka, Croatia; [2]Psychiatry Clinic, Clinical Medical Centre, Rijeka, Croatia; [3]Psychiatric Hospital, Rab, Croatia; [4]Private psychiatric practice, Rijeka, Croatia
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To investigate the possible influence of hemochromatosis gene mutations (HFE-C282Y and H63D) and transferrin gene C2 variant (TF-C2) on susceptibility to schizophrenia and schizoaffective disorder and/or age at first hospital admission. Genotyping was performed in 176 Croatian patients and 171 non-psychiatric Croatian controls using PCR-RFLP analyses. Allele and genotype distributions were not significantly different between two groups. After the age at first admission was analyzed as a continuous variable using the non-parametric Mann-Whitney U-test and Kruskal-Wallis test with negative results, the variable was dichotomized using 40 years as the cutoff. H63D-TFC2 bi-carriers were over-represented among patients in the late disease-onset group [>= 40 years; 7.5% vs. 25%, odds ratio (OR) = 0.243, 95% confidence interval (CI) = 0.068-0.871; P=0.030]. Multiple regression analysis showed no effect of combined H63D-TFC2 genotype to distribution of age at disease-onset in our sample. Investigated HFE mutations and TF-C2 variant are not high-risk genetic variants for schizophrenia/schizoaffective disorder in our population. Also, our data do not support their impact on age at onset of the first psychotic symptoms.

Keywords: schizophrenia, schizoaffective disorder, hemochromatosis gene mutations, transferrin gene variant, age at first hospital admission

Abstract number: HDHG-ABS-254-ISABS-2011

HUMAN GENOME ARCHITECTURE IN SCHIZOPHRENIA AND OTHER NEUROPSYCHIATRIC DISORDERS

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It is hypothesized that rare structural variants in the human genome might contribute to schizophrenia and other neuropsychiatric disorders. Structural variation in the human genome such as deletions, duplications, and inversions, that alter gene dosage, might explain genetic contributions to normal variability of complex traits, but also to developmental anomalies and diseases. Large (>1 Mb) gene copy number variations (CNVs) are often associated with higher penetrance and more severe clinical features. Structural genomic rearrangements in schizophrenia are thought to preferentially disrupt genes that might be involved in nervous system development and function. Several studies have reported higher prevalence of rare CNVs in patients with schizophrenia compared to healthy comparison groups. Although a great majority of CNVs detected in schizophrenia represent rare and unique events, some of them appear recurrently: 22q11.2, 1q21.1, 2p16.3, and 15q13.3 deletions. Recurrent rare CNVs support the hypothesis of the elevated rate of de novo mutations in schizophrenia. The potential role of structural genomic variations in etiology of schizophrenia and other neuropsychiatric disorders needs further attention.

Keywords: schizophrenia, neuropsychiatric disorders, structural genomic rearrangements, low copy repeats, copy number variation

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POSITIVE REPRODUCTIVE FAMILY HISTORY FOR SPONTANEOUS ABORTION AS A PREDICTOR FOR RECURRENT ABORTIONS IN YOUNG COUPLES

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The etiology of miscarriages as well as of recurrent spontaneous abortion (RSA) in chromosomally normal parents is still unexplained. It is also unclear whether some factors related to extended family members (like spontaneous abortions) create predisposition that may end with termination of pregnancy. In general population the incidence is 12-15%. 566 couple were selected for this study in period of 27 years. Average age was 29 for women and 32 for men. There were informations about 1146 spontaneous abortions. Chromosomal analsies of peripheral blood in 90 % had normale chromosomes but some had variants in population (10%). Both males and females had former urinary or/and genital infections and some had evidence of reactivation of latent EBV infection (43%). The most common histopathological findings of the placenta during the first abortion is a degenerative, second are the inflammatory changes (50%). In this study we find positive link between inflammation and hypoxia in the placenta. The purpose of this study was to evaluate the association between RSA and spontaneous abortions (SA) among parents relatives in the first, second and third generation. The percentage of SA among the couple's siblings of first, second and third generation was 14%, 28% (being two times higher in relation to average population), and 15%, respectively. It can be concluded that positive reproductive family history for spontaneous abortion, former urinary or/and genital infections, reactivation of latent EBV infection may be the causal factor for recurrent pregnancy loss and predetermine women that are of greater susceptibility to preterm pregnancy.

Keywords: spontaneous abortions, genetic counselling, siblings, genitourinary infections, latent EBV infection

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ACE I/D POLYMORPHISM IN LUNG MALIGNANCY

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Angiotensin converting enzyme (ACE) plays an important role in cardiovascular homeostasis and inflammation processes. Its plasma levels depend on the insertion/deletion (I/D) polymorphism so that homozygotes for the I allele have the lowest, and homozygotes for the D allele the highest plasma levels. It has been shown that ACE II genotype positively correlates with cell survival thus increasing malignancy. Moreover, it has been found that patients with lung cancer have decreased ACE plasma levels. We investigated whether ACE I/D polymorphism genotype distribution differs between patients with primary lung cancer and patients with lung malignancy as a second primary cancer. 201 patients with primary lung carcinoma as first malignancy and 107 patients with second primary lung carcinoma were included in this study. ACE I/D genotypes were determined by polymerase chain reaction. Genotype distribution and allelic frequencies between patients with primary lung cancer and patients with second primary lung cancer were significantly different (ACE II genotype 16.4% vs. 29.0%, p=0.009; ACE I allele 41.0% vs. 51.9%, p=0.010). Results suggest that the II genotype and the I allele could be risk factors for developing second primary lung carcinoma

Keywords: ACE I/D polymorphism, lung cancer, second primary malignancy, cancer susceptibility, genetics

Abstract number: HDHG-ABS-312-ISABS-2011

CLASSICAL CONGENITAL ADRENAL HYPERPLASIA DUE TO 21-HYDROXYLASE DEFICIENCY IN CROATIA BETWEEN 1995 AND 2010

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder resulting in the loss of activity of one of the enzymes necessary for adrenal steroidogenesis. Aim was to evaluate the incidence, gender, symptoms and age at diagnosis of patients with classical CAH due to 21-hydroxylase deficiency in Croatia. Data were collected retrospectively for all classical CAH patients born between January 1, 1995 and December 31, 2010 and were compared with the data of a previously conducted study evaluating CAH patients discovered between 1964 and 1984. During a 16-year period 51 classical CAH patients were diagnosed. There were 31 salt-wasting (SW; 21 female/10 male) and 19 simple-virilizing (SV; 8 female/11 male) patients. With 694075 live births and 50 CAH patients born over this period in Croatia, the prevalence of classical CAH was estimated at 1:13 881. The lower incidence of SW boys compared to SW girls (10:21) and similar number of SW and SV boys (10:11) indicate that a substantial proportion of SW boys die unrecognized. Owing to better healthcare, the diagnosis was established significantly earlier in SW and SV girls compared to the 1964-1984 period ($p < 0.003$). During 1995-2010 time period, none of the patients died following the diagnosis of CAH and there were no erroneous sex assignments. Despite improvements in healthcare, the diagnosis of CAH in Croatia is still delayed and some of the patients go unrecognized or die. The results of our study support the need of introduction of the newborn screening program for CAH in Croatia.

Keywords: congenital adrenal hyperplasia, CYP21 gene, prevalence, neonatal screening, classical congenital adrenal hyperplasia

Abstract number: HDHG-ABS-359-ISABS-2011

THE IMPORTANCE OF OPTIMIZING METHODS FOR MEASURING THE ACTIVITY OF ACID ALPHA-GLUCOSIDASE IN THE DIAGNOSIS OF POMPE DISEASE

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Pompe disease or glycogenosis type II (OMIM; 232300) is a lysosomal storage disease caused by hereditary deficit of the enzyme α-glucosidase (EC 3.2.1.20). The consequence is progressive glycogen storage, primarily in skeletal and cardiac muscle. Infantile, juvenile and adult type of disease can be distinguished depending on the time of occurrence of first symptoms and severity of clinical picture. Timely diagnosis is a prerequisite for achieving maximum efficacy of the enzyme replacement therapy. After establishing clinical suspicion, an important step in diagnostic procedure is measurement of residual activity of acid α-glucosidase. However, the possibility of false negative results is significantly increased due to presence of several α-glucosidase isoenzymes with the same affinity for the synthetic substrate used in measuring catalytic activity of acid α-glucosidase. In order to maximally increase the specificity of methods for measurement of acid α-glucosidase in dry blood spot samples on filter paper, lymphocytes and cultivated skin fibroblasts, fluorimetric methods were optimized with synthetic substrate 4-methylumbellipheryl-alpha-D-glucoside taking into consideration the levels of proteins, substrates, inhibitors of other isoenzymes, pH buffers and incubation times. Application of thus optimized methods allows reliable screening for Pompe disease from dry blood spot samples on filter paper, confirmation of decreased activity from lymphocyte samples, and estimation of the severity of clinical data from residual enzyme activity in the homogenate of cultured skin fibroblasts. Results of external quality assessment confirmed the diagnostic efficacy of optimized methods.

Keywords: Pompe disease, acid alpha-gucose, diagnostic, efffectiveness, optimizing

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MOUSE MODELS FOR GANGLIOSIDE DEFICIENCY

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Ganglioside storage diseases are well described in some populations, whereas deficiency is extremely rare and postulated as lethal for humans. The mouse models described develop neurological deficit at adult age, which is comprised of impaired peripheral nerve regeneration, dysmyelination and axonal degeneration. We compared B4galnt1 knockout, complete deficiency of complex gangliosides and St8sia1 that lack GD3 synthase and express deficiency in b-series gangliosides. The brain tissue morphology was compared with the use of histological and immunohistochemical methods. Behavioral studies involve activity cage before and after inhalatory anesthesia. Both genotypes show no difference in brain morphology and have no shortening of life expectancy. B4galnt1 knockout develops dysmyelination at adult age accompanied by motor deficiency. Anesthesia induction was significantly prolonged in 3 month old B4galnt1 knockouts, whereas motor behavior in activity cage recovered in shorter time compared with the wild type and St8sia1 animals. The neurological deficit in mouse models of ganglioside deficiency is very mild and is not expressed at the morphological level during juvenile stage. Specific characteristics of the phenotype can be provoked with the use of inhalatory anesthetics, which function at the level of lipid rafts.

Keywords: ganglioside deficiency, mouse models, B4galnt1 knockout, St8sia1 knockout, ganglioside storage diseases

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ORTHOPAEDIC TREATMENT OF METABLIC BONE DISEASE

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Metabolic bone diseases cause a disruption of bone mineralisation and affect the growth plate. These disorders can be congenital like hypophosphatemic rickets and hypophosphatasia, and acquired like vitamin D deficiency rickets and renal osteodystrophy. Treatment by vitamin D and phosphate supplementation is done by pediatricians specialized in metabolic diseases. Orthopedic treatment of metabolic bone diseases implies deformity correction of the lower extremity. Patients with these disorders have altered anatomical and mechanical axes of the long bones, most commonly varus and valgus deformations. Long bone axis deviation larger than 15° usually requires operative treatment which was, until now, mostly done by single or multiple corrective osteotomies followed by external or internal fixation. Recently, new techniques of operative treatment were developed, employing special implants set around the growth plate to enable guided growth. We report on 6 patients (1 male, 5 female) with hypophosphatemic rickets and consequential deformity of the lower extremities treated at our Department. All patients had a varus deformation of the lower extremity (femur and tibia). Surgery was performed on 15 lower extremity segments (10 femora, 5 tibiae). Four patients had corrective osteotomies with internal fixation using plates and screws. Two patients were treated by method of guided growth (Stevens "Eight"–plate). The average age at time of surgery was 12, 5 years (range, 8 – 20). All patients were followed until reaching skeletal maturity, which was defined as the end point of treatment. One patient (one segment) treated with the Stevens "Eight"–plate method developed a hemarthros following a fall, the plate was removed and an arthroscopic was performed. Three patients (three segments) had a reoperation due to deformity relapse caused by remaining growth.

Keywords: hypophosphatemic rickets, surgical treatment, bone deformation, Stevens "Eight"-plate, osteosynthesis

Abstract number: HDHG-ABS-137-ISABS-2011

DIVERSE GENE EXPRESSION IN PATIENTS WITH JUVENILE SPONDYLOARTROPATHY AND CLAVICULAR CORTICAL HYPEROSTOSIS IS POSSIBLY RELATED TO AUTOINFLAMMATORY DISEASES

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Juvenile Spondyloarthropathies (jSpA) and clavicular cortical hyperostosis (CCH – variant of chronic recurrent multifocal osteomyelitis) are characterized by dysregulation of the inflammatory processes. To identify genes with disease-specific expression patterns of patients diagnosed with jSpA, CCH and healthy controls. Peripheral blood samples of 11 new-onset, untreated jSpA patients were analyzed for expression patterns using Human Genome U133 PLUS 2.0 GeneChip, Affymetrix, (54675 probes). Among these patients all were HLA "double positive": 8 B27/B7 (OR 14.82), 2 B27/DRB1 (OR 7.39) and 1 B7/DRB1 (OR 2.61). For comparison, gene expression profiles were obtained from 5 patients with CCH and 4 healthy controls. RT-PCR was used to confirm differential gene expression in patients with obtained gene expression profiles (N=20), and additional 21 jSpA patients, 4 patients with CCH, and 7 healthy controls. Statistical analysis of gene expression patterns in patients with jSpA compared to healthy controls, identified 744 differentially expressed genes at statistical cutoffs fold change 1.5 (p100). Genes differentially expressed in patients with jSpA were compared to the expression profiles of CCH patients, and the analysis showed overlap in 282 genes. jSpA patients exhibit complex patterns of expression in genes related to inflammatory and defense response, MAP kinase and cell cycle, chromatin modulation and transcription, cell death and apoptosis, and interestingly, gene closely linked to autoinflammatory diseases (NRLP3 and TLR-4). Additionally, profiles of patients with jSpA and CCH showed significant concordance in expression of genes linked to autoinflammatory (TLR-4) and autoimmune diseases (CD24).

Keywords: juvenile spondyloarthropathies, jSpA, microarray, clavicular cortical hyperostosis , CCH

Abstract number: HDHG-ABS-306-ISABS-2011

CLASSICAL AND MOLECULAR CYTOGENETIC ANALYSIS IN 324 PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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At the Department of Pediatric Hematology, 324 pediatric cases of acute lymphoblastic leukemia (ALL) were diagnosed. The standard flow cytometric leukemia immunophenotyping, classical and molecular cytogenetic and PCR/RT-PCR techniques were used and providing the framework for a stratified treatment. The expression of T, B and My antigens were assessed by multi-parameter flow cytrometry with routine standard panel of monoclonal antibodies, assessed by multi-parameter flow cytometry. The standard PCR/RT-PCR analyses were carried out for characteristic rearrangements (TEL/AML1, MLL-AF4, BCR/ABL). In the group of 324 cases, 81% showed of B-lineage and 19% of T-lineage. The most common fusion genes were present in 47% overall cases. Cytogenetic analysis was successful in 98% BM samples. Clonal chromosomal abnormalities were present in 71% of patients. In 65% patients we found changes in ploidy. The distribution of ploidy group was: hyperdiploidy with >50 chromosomes in 53%, 20 of them associated with structural chromosomal changes. Hyperdiploidy with 47-50 chromosomes in 32%, psedodiploidy (46, +n, -n) in 3%, and hypodiploidy with 37-45 chromosomes in 11, 5% patients. The most frequently acquired numerical abnormalities were: +21/127 (with 1-6 copy), 9 of them with constitutal trisomy 21, +X/59, +4/56, +6/47, +8/40, +10/32, +14/31 and +17/32. Structural aberrations were found in 35% patients. The most frequent structural abnormalities were: deletion 6q/19, del9p/12, del12p/10, t(8;14)(q24;q32)/7, t(9;22)(q34;q11.2)/5, t(4;11)(q21:q23)/4, t(1;19)(q23;p13)/3, and t(12;21)/18. Rearrangements of chromosome 1p/q, 5q, 14q, and 17p were present in complex karyotype in 27 cases. Results will be discussed and compared with those reported from other groups.

Keywords: pediatric ALL, cytogenetics, FISH, flow cytometric leukemia immunophenotyping, PCR/RT-PCR

Abstract number: HDHG-ABS-275-ISABS-2011

BRCA1 AND BRCA2 GERMLINE MUTATIONS IN WOMEN IN CROATIA WITH HEREDITARY PREDISPOSITION TO BREAST AND OVARIAN CANCER

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Epidemiological data indicates 5-15% of all breast cancer cases are hereditary, and germline mutations in Breast Cancer Gene 1 (BRCA1) and Breast Cancer Gene 2 (BRCA2) account for the majority of hereditary breast and ovarian cancers. The contribution of BRCA1 and BRCA2 mutations to hereditary breast and ovarian cancer in Croatia is unknown. The aim of our study was to estimate the incidence and spectrum of pathogenic mutations in BRCA1 and BRCA2 genes in high risk women in Croatia. The screening was performed by high resolution melting approach, direct sequencing and semi-quantitative multiplex PCR method (Cvok et al. Clin Chem Lab Med 2008;46(10):1376-83). Protocols were certified by EMQN (European Molecular Genetics Quality Network). The complete coding sequences and exon-intron boundaries analyses of both genes were carried out on 142 women with hereditary predisposition to breast and ovarian cancer. Overall, 11 pathogenic mutations were detected, two novel in BRCA1, and three novel in BRCA2. Nineteen BRCA1 and 33 BRCA2 unclassified variants and polymorphisms were also identified, of which two BRCA1 and seven BRCA2 were not previously published. This is the first molecular investigation of the hereditary predisposition to breast and ovarian cancer in Croatia based on BRCA1 and BRCA2 genes. Samples were collected from different regions of the country and the level of pathogenic mutations and distribution of polymorphic variants will contribute to population statistics. This study was funded by The Terry Fox Run 2009 donation and supported by The Terry Fox Foundation and Croatian League Against Cancer.

Keywords: hereditary breast cancer, BRCA1 gene, BRCA2 gene, population genetics, genetic epidemiology

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THE DUAL ROLE OF TOLL-LIKE RECEPTOR 3 IN METASTATIC CELL LINE

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Aim. As the role of TLRs in tumors cells is still not clear, our aim was to investigate the role of TLR3 in primary tumor and metastatic cells (SW480, SW620, FaDu and Detroit 562). Methods. Human primary (FaDu) and metastatic (Detroit 562) pharyngeal tumor cells were analyzed for the expression change in tumor promoting genes by real time PCR and gene array after poly (I:C) treatment. The cell migration potential was screened with Boyden chambers. The effect of TLR3 was ascertained using siRNA technology. The apoptotic cells percentage was determined using a caspase 3/7 activity luminescent assay. The functionality of the TLR3 signalling pathway was analyzed using luciferase assay. Results. We have reported here on the dual role of TLR3 in pharynx metastatic cell line (Detroit 562); on one hand TLR3 activation drove cells to apoptosis while on the other its stimulation contributed to tumor progression by altering the expression of tumor promoting genes (PLAUR, RORB) and enhancing the cell migration potential. Conclusion. Since TLR3 agonists have been used in tumor therapy with the aim to activate immune system, scientific contribution of this work is drawing attention to the importance pro-tumor effect of TLR3, in order to avoid possible side-effects.

Keywords: TLR3, cancer, tumor progression, apoptosis, cell migration

Abstract number: HDHG-ABS-281-ISABS-2011

SCREENING OF PATIENTS WITH MENTAL RETARDATION USING SUBTELOMERIC MLPA ASSAY

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Subtelomeric imbalances are a significant cause of mental retardation/developmental delays (MR/DD) in patients with or without phenotypic abnormalities. Multiplex ligation-dependent probe amplification (MLPA) is relatively low cost, rapid, and high throughput prospective screening tool for copy number quantification. We developed a strategy of screening for chromosomal subtelomere imbalances with two MLPA kits followed by confirmation and delineation of breakpoints with selective telomere follow-up MLPA specific kits. We screened 105 patients with idiopathic MR/DD associated with dysmorphic features and/or malformations and normal karyotype using SALSA P036 and SALSA P070 MLPA kits. Subtelomeric anomalies were found in 10 patients (10/105 – 9.5%). Three were three de novo deletions (4p, 15q and 22q) one deletion and duplication at 19p, three duplications (X/Yp) and three deletions and duplications (dup3p/del18q, dup8p/del18q and del12p/dup22q) resulting from balanced parental reciprocal translocations. Telomere follow-up MLPA specific kits delineated the size of six (6/10 – 60%) subtelomeric abnormalities that ranged from 0, 5 to 6, 1 Mb. These imbalances were not visible on subsequent high resolution karyotyping (600 – 800 bands). High-resolution microscope analysis of involved chromosomes validated and further delineated subtelomeric abnormalities in three patients: del4q16.3dn, der(18)t(3;18)(p26.1;q22.1)mat, der(18)t(8;18)(p23.1;q22.1)pat. Their size ranged between 4, 5 and 13, 8 Mb. Presented diagnostic testing strategy increases significantly diagnostic detection rate of subtelomeric imbalances in patients with idiopathic mental retardation. Our survey confirms the observation that subtelomeric rearrangements are a significant cause of idiopathic MR.

Keywords: idiopathic mental retardation, chromosome, subtelomere, MLPA, karyotype

Abstract number: HDHG-ABS-263-ISABS-2011

IMPACT OF CPLA2 AND COX-2 GENE POLYMORPHISMS ON ATTENUATED NIACIN SKIN-FLUSH RESPONSE IN PATIENTS WITH SCHIZOPHRENIA

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Attenuated niacin skin-flush response potentially marks the cytosolic phospholipase A2/cyclooxygenase-2 (cPLA2/COX-2) cascade abnormalities. Polymorphisms of genes involved in cPLA2/COX-2 pathway and their impact on the niacin skin-flush response were investigated in patients with schizophrenia. Allelic/genotypic frequencies of BanI polymorphism of the cPLA2 gene and A/G variant (rs689466) of the COX-2 gene were determined in 204 Croatian patients and 191 controls by PCR-RFLP analysis. Patches containing 0.1M, 0.01M, 0.001M and 0.0001M solutions of aqueous methyl nicotinate (AMN) were kept for 5 minutes on the forearm skin in 80 patients and 80 controls and visual evaluation of flushing rated from 0 to 3 was done in 5 minute intervals up to 15 minutes by two independent raters. Allelic/genotypic frequencies between patients and controls were not significantly different. Controls showed significantly higher flushing at all concentrations and minute intervals ($P<0.001$). Multiple regression analysis revealed that AMN concentration ($\beta=0.65$), minute interval ($\beta=0.26$), GG genotype of cPLA2 ($\beta=0.16$), and COX-2 polymorphism ($\beta=0.14$) were significant predictors of the flush response. The presence of cPLA2-GG genotype correlated significantly with flushing at higher AMN concentrations (0.1M and 0.01M) and at all time points. Weaker, but statistically significant correlation between flushing and COX-2 genotype was determined at 0.001M concentrations. Regarding flush response, there was no synergy between two polymorphisms. We revealed significant interaction between cPLA2-GG genotype and AMN concentration on niacin response at 10-minute interval. Niacin response was significantly attenuated in patients compared to controls. Both polymorphisms impacted niacin sensitivity in the patient group.

Keywords: niacin skin-flush testing, schizophrenia, cytosolic phospholipase A2/cyclooxigenase-2 cascade, cPLA2 polymorphism, COX-2 polymorphism

Abstract number: HDHG-ABS-228-ISABS-2011

CHANGE OF THE NF2 GENE IN NEURINOMAS

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The purpose of this study was the analysis of changes in the NF2 gene in human neurinomas. Losses of heterozigosity, gross deletions in the NF2 tumour suppressor gene were analyzed using two microsatellite markers: D22S444, whose PCR product is long between 123 and 131 base pairs, and D22S929, who is long 130 base pairs. The main cause for proliferation and transformation of Schwann cells into neurinomas is credited to the inactivation of the Neurofibromin 2 (NF2) gene and his protein product Merlin (Schwannomin). Merlin consists of 595 amino acids and is related to ERM (Ezrin, Radixin, Moesin) protein family. Polymerase chain reaction is used for amplification of DNA fragments isolated from neurinomas and associated blood samples (control samples). Efficiency of the amplification was verified with horizontal gel electrophoresis in agarose gel (2%) with ethidium bromide used for visualisation. The presence of gross deletions was detected using microsatellite markers with Spreadex gels (Elchrom Scientific, Switzerland).

Keywords: NF2 gene, Merlin, loss of heterozigosity, neurinoma, microsatellite markers

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DIAGNOSTIC ALGORITHM FOR CHILDREN WITH AUTISM

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Autistic spectrum disorders (ASD) is a complex neurodevelopmental disorder diagnosed in early childhood. It is characterized by impairment of social interactions, communication, and stereotyped, repetitive behavior. The causes of ASD are mostly genetic, but due to the heterogeneous and often complex nature of disease, molecular basis in many patients remains unexplained. Advances in cytogenetic and molecular techniques provide the possibility to identify the etiology in increasing number of affected individuals. Defining the specific cause of the disorder is especially important because it provides reliable recurrence risk assessment, possibility of prenatal testing and carrier detection. An accurate causal diagnosis is also important for determining the natural course of the disease, potential medical complications and possible therapeutic approaches. Genetic diagnosis therefore should be offered to all affected families. Due to the frequency of autism and increasing number of available diagnostic tests, it is important to find efficient, accurate and rational diagnostic approach that will take into consideration costs and expected diagnostic yield. Here we present diagnostic workup and outcome in 100 patients admitted to our hospital for the evaluation of ASD and propose diagnostic strategy for the clinical genetic evaluation of patients with ASD.

Keywords: autism, genetics, diagnosis, children, chromosome

Abstract number: HDHG-ABS-198-ISABS-2011

CURRENT VIEWS ON GENETIC AND EPIGENETIC ETIOLOGY OF RECURRENT SPONTANEOUS ABORTION

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Recurrent spontaneous abortion (RSA) is defined as three or more consecutive spontaneous pregnancy terminations before 24th week of gestation and fetus weighing less than 500g. It affects 3-5% of couples in their reproductive age and comprises a heterogenous group of nongenetic and genetic disorders. The etiology remains unknown in more than 50% of cases and is referred to as idiopathic RSA. The high percentage of idiopathic RSA cases and the distinct pattern of abortion recurrence in certain couples according to gestation of SA indicates that there are specific genetic factors causing this clinical disorder. Most studies on genetic etiology of RSA include only women, but pregnancy, its successful outcome and even miscarriage are the result of a "genetically determined conflict" involving three genomes (embryo vs mother vs father) at the feto-maternal interface. The presence of both maternal and paternal genomes is essential for normal development of eutherian mammals, due to the phenomenon of genomic imprinting and due to the possible recessive etiology of RSA where the father contributes to fetal genome in an equal manner as the mother. The aim of this presentation is to review the currently known genetic causes of RSA which are classified into chromosomal aberrations (structural and numerical aberrations, recurrent aneuploidy), gene factors (monogenic disorders and single nucleotide polymorphisms) and epigenetic factors (skewed X-chromosome inactivation, genomic imprinting). We will present a summary of our results, including the IGF2, IGF2R, H19 and matrix metalloproteinase genetic polymorphisms in men and women, as possible risk factor for RSA susceptibility.

Keywords: recurrent spontaneous abortion, pregnancy, gene polymorphisms, placenta, genomic imprinting

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FUNCTIONAL SINGLE NUCLEOTIDE POLYMORPHISMS IN PROMOTER REGIONS OF MATRIX METALLOPROTEINASE -1, -2, -3 AND -9 GENES ARE RISK FACTORS FOR RECURRENT SPONTANEOUS ABORTION

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Matrix metalloproteinases (MMP) regulate various processes during human pregnancy, including decidualization, maintenance of corpus lutem, blastocyst implantation and placentation. The aim of this study was to investigate the association of functional promoter polymorphisms in MMP genes with idiopathic recurrent spontaneous abortion (iRSA). 149 couples with iRSA and 149 control couples with at least one live birth and no history of pregnancy loss were included. Polymerase chain reaction and restriction fragment lenght polymorphism analyses were performed to identify the MMP1 -1607 1G/2G, MMP2 -735C/T, MMP2 -1306C/T, MMP3 -1612 5A/6A, and MMP-9 -1562C/T genotypes. There was a statistically significant difference in genotype and allele frequencies of MMP-2 735, MMP-2 -1306 and MMP-9 -1562 between iRSA couples and control couples. The MMP-2 -735T allele (X2=7.53, P=0.006; OR=0.59, P=<0.001), MMP-2 -1306C allele (X2=8.19, P=0.004; OR=1.67, P=0.004) and MMP-9 -1562C allele (X2=7.17, P=0.007; OR=0.63, P=0.008) were more frequent in couples with iRSA. The risk of RSA was related to MMP-2 -735T allele in dominant (ORCT+TTvsCC=1.68; P=0.002) and codominant model (ORCTvsCC=1.70; P=0.003); MMP-2 -1306C allele in recessive (ORCCvsCT+TT=1.75; P=0.005) and codominant model (ORCCvsCT=1.71; P=0.008); and MMP-9 -1562C allele in recessive (ORCCvsCT+TT=1.70; P=005) and codominant model (ORCCvsCT=1.69; P=0.005). Haplotype and genotype combinations of two, three, four and five gene polymorphisms have also revealed statistically significant levels; a 3.31-fold increasd risk of RSA was found in couples with combined MMP-2 -735CC, MMP-2 -1306CC, MMP-1 -16072G2G and MMP-3 -16125A5A (OR=3.31; P=0.012). Our results show that the functional promoter polymorphisms, haplotype and genotype combinations of MMP-1, -2, -3- and -9 could be a genetic determinant for the risk of RSA.

Keywords: pregnancy, extracellular matrix, trophoblast, genetics, single nucleotide polymorphisms

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TRACES OF MIGRATION FROM INDIA TO EUROPE AS WRITTEN IN THE MITOCHONDRIAL DNA OF BAYASH ROMA

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Bayash (Vlax Roma) are one of the founder Roma populations speaking archaic Romanian language and living dispersedly in Central, Eastern and Southeastern Europe. Different Vlax Roma groups are related through shared period of slavery in Romania during the 13th to 19th century. In order to understand in what way this legacy manifested on the genetic heritage of the Vlax Roma compared to other Roma groups, we analyzed mitochondrial DNA of 384 Croatian Vlax Roma from two geographic locations, in the context of 759 European Roma samples, members of different migration categories. Our results show that three haplogroups of different temporal and spatial characteristics, namely X2, U3 and M35, determine the mitochondrial diversity of researched Roma groups. X2 and U3 haplogroups clearly separate the Balkan and Vlax Roma from Roma populations that reached Europe as part of the first migration wave, indicating a possibility of their admixture to Roma populations before arrival to Europe. On the contrary, M35 haplogroup has so far been noted only in the Vlax Roma populations, implying that all Vlax Roma populations descend from one single founder population that might even reach back to the original ancestral Indian population. Founder effects followed by strict endogamy rules, as main factors forming the gene pool of Roma populations, can be traced from India to contemporary small, local communities, as it is the case of two Croatian Vlax Roma populations that show population differentiation despite similar origins and shared demographic history.

Keywords: Bayash, Vlax Roma, mitochondrial DNA, founder effect, endogamy

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THE ROLE OF DETERMINING THE STATUS OF KRAS GENE IN COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the most highly malignant tumors and is among the leading causes of death in developed countries. A very complex and longlasting process, closely related to interaction of external and genetic factors, contributes to development of this malignant disease. Activation of the KRAS oncogene is of importance in colorectal carcinogenesis, and the presence of an activating mutation may be demonstrated in more than 30% of CRC samples. Surgically resected colorectal cancer tissue, embedded in paraffin sections, was used for molecular analysis of the KRAS gene. DNA isolation is performed using QIAamp DNA FFPE Tissue Kit (Qiagen). The status of the KRAS gene is determined by real-time polymerase chain reaction method - Real Time PCR (Roche LightCycler ® 480 Real-Time PCR System) using the procedure of absolute quantification with TheraScreen® KRAS Mutation Kit CE-IVD which is used for detection of seven somatic mutations of the KRAS gene. Out of the total of 67 analyzed colorectal cancer tissue samples, point mutation on codons 12 or 13 was detected in 21 samples (31%). Gly12Val (GGT>GTT) mutation was determined in 13 patients, 4 patients had Gly12Asp (GGT>GAT) mutation, 2 patients had Gly13Asp (GGC>GAC) mutation, and one patient had Gly12Ser (GGT>AGT) and Gly12Cys (GGT>TGT) mutations. The presence of point mutations on codons 12 or 13 of the KRAS gene in colorectal cancer is predictive of the lack of response to therapy with new drugs, and is determined in all patients eligible for immunotherapy. Personalized approach to CRC treatment helps avoid unnecessary side-effects and toxicity, and optimize costs in healthcare system. Combination of target therapy with predictive biomarkers improves efficacy in target patient group and the patients' benefit from applied drugs.

Keywords: KRAS, colorectal cancer, real-time PCR, absolute quantification, mutation

Abstract number: HDHG-ABS-249-ISABS-2011

ANGIOKERATOMA IN FABRY DISEASE: DIAGNOSTIC BUT NOT TREATMENT EFFECTIVENESS MARKER

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Fabry disease (FD) is a rare X-linked recessive disorder caused by mutations in the gene encoding the lysosomal enzyme ?-galactosidase A. The manifestations of FD are progressive and multysistemic, culminating in life-threatening renal, cardiac, and cerebrovascular manifestations. Typical skin lesions are angiokeratomas, that manifest usually at age 5 to 15 years, and could serve as a key diagnostic sign. However, they are not considered as a reliable prognostic marker. Besides, although clinical trials have shown the efficacy of enzyme replacement therapy (ERT), the reports on its effect on skin lesions are scarce and contradictory. We report a 7-year old boy with FD, presented with failure to thrive and marked skin, ocular and renal involvement. Because of the multisystemic nature of FD, ERT with agalsidase beta was commenced, which the boy tolerated well. At 24 months of treatment, he had significantly gained weight and height. His bone age increased from 2 to 4 years. Ocular and renal changes remained stable. Plasma globotriaosylceramide levels, which were elevated at baseline, were significantly reduced. However, angiokeratomas increased in size and number. The boy had not developed any new sign of FD. Although cutaneous signs of FD generally appear during childhood, the diagnosis is often delayed or missed. Our case confirms that vascular skin lesions could serve as a diagnostic sign in FD. However, it does not support angiokeratomas as surrogate marker of the course of the disease or efficacy of ERT.

Keywords: Fabry disease, child, angiokeratoma, diagnosis, treatment

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THE FIRST FIVE YEARS EXPERIENCE OF PRENATAL CYTOGENETIC DIAGNOSIS IN SPLIT, CROATIA; ANALYSIS AND EVALUATION OF THE RESULTS OBTAINED FROM AMNIOTIC FLUID SAMPLES

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Aim: to present the results of invasive prenatal cytogenetic diagnosis in the Department of Obstetrics and Gynecology, Clinical Hospital Center Split. Results: Invasive prenatal cytogenetic diagnosis in the Department is established in April 2005. Until than, a total number of 911 procedures (4.13% of deliveries) have been performed. Cut point age limit was set on 38 years and it presented indication for the amniocentesis in 473 (51.9%) cases. Increased triple test risk (cut point set on 1:250) indicated amniocentesis in 79 (9.0%), family history in 62 (6.8%), personal history in 45 (4.9%), ultrasound diagnosed fetal malformation in 23 (2.5%) and increased nuchal translucency in 9 (0.98%) procedures. Other indications were sporadic. Only five physicians did more than 50 procedures, two of the most involved in the process together 56.4% (514) total, and all the others much less. Valid findings were obtained from 868 (95.5%) samples. The finding that represented an indication for abortion was found in 25 (2.7%) samples: Down syndrome in 19 (2.08%), Edwards syndrome in 4 (0.4%) and Patau syndrome in 2 (0.2%) of them. In three pregnant women a miscarriage followed as a complication of surgery (0.3%); in two of them few days, and in one three weeks after procedure. Conclusion: First experiences of invasive prenatal cytogenetic diagnosis in Split are behind us. Experience of genetic counseling, the development of cytogenetic laboratories, introduction of first and second trimester screening tests and the enthusiasm of staff in this segment of the profession guarantee the further progress.

Keywords: prenatal diagnosis, amniocentesis, cytogenetics, Down syndrome, pregnancy

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IMPROVED DETECTION OF DELETIONS/DUPLICATIONS IN THE DMD GENE USING THE MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) METHOD

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Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD), the most common forms of dystrophinopathies, are X-linked disorders caused by mutations of the DMD gene. DMD gene spans 2.4 Mb on Xp21 and contains 79 exons. Until recently, the majority of the DMD deletions have been traditionally identified in affected males by using a multiplex polymerase chain reaction (PCR) approach that simultaneously amplifies ~19 exons. However, by that approach duplications and female carriers could not be identified with certainty. Here we report the use of Multiplex Ligation-dependent Probe Amplification (MLPA) assay in simultaneously screening of all 79 DMD exons for deletions and duplications in DMD/BMD patients and female carriers. We validated the MLPA assay by testing 19 unrelated male patients already screened by multiplex PCR. The MLPA results were in concordance with the results of multiplex PCR in 9 cases. In six DMD/BMD patients we could more precisely determine the extent of the deletions which could be of prognostic value for the patients. There were differences in the deletion breakpoints determined by the two methods in two patients, probably due to point mutation or small insertion/deletion in the MLPA probe hybridization site. In addition, we detected two duplications that had been missed by multiplex PCR. The assay reliably identified 4 female heterozygotes. The MLPA assay outperforms the Beggs and Chamberlain multiplex PCR test and should be the method of choice for the detection of DMD rearrangements in DMD/BMD patients, as well as in female carriers.

Keywords: Duchenne/Becker muscular dystrophy, DMD gene, MLPA, multiplex PCR, deletion/duplication screening

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MINOR DYSMORPHIC SIGNS: THEIR VALUE FOR ASSESSMENT OF THE ETIOLOGY OF CONGENITAL DEVELOPMENTAL DEFETCS

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The most frequent indications for chromosome examinations are congenital developmental anomalies and/or developmental delay. However, besides chromosome aberrations there are many other causes for congenital developmental defects, and it would therefore be desirable to restrict the examinations to those patients in whom non-chromosomal causes are less likely based on the analysis of the clinical findings, especially what is called minor anomalies or dysmorphic signs. Another major issue at determination of the cause of mental deficiency is: was it acquired due to unfavourable circumstances before, at or soon after birth or was the basis laid early during intra-uterine life? Careful clinical examination and the knowledge of the pathogenetic mechanisms leading to minor developmental anomalies (dysmorphic signs) is often very helpful with respect to distinction between these two possible aetiologies. Many minor developmental defects have their origin early in pregnancy, and it is often possible to trace them back to an adverse influence, be it genetic or environmental, at a given stage of pregnancy, specifically when the structure in question is formed. Examples include transverse palmar creases, clinodactyly of little fingers, the “high-arched” palate which mostly is prominent lateral palatine ridges, prominent ears, coxa valga, abnormal scalp hair patterning and many others. Although such determinations would not allow to distinct between genetic and in utero acquired, they allow the conclusion that minor anomalies in a newborn have their origin during early intra-uterine development which makes it less likely that an associated mental deficiency had been acquired due to unfavourable conditions at birth and thus is independent of the dysmorphic features.

Keywords: dysmorphic features, congenital developmental defects

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TWINING AND CONGENITAL DEVELOPMENTAL DEFECTS

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Several studies have shown that the incidence of congenital developmental defects (CDD) is increased in twin versus singleton newborns. The increase is mainly due to a higher incidence of malformations and disruptions in monozygotic (MZ) twins. Following a classification of CDD in malformations, deformations, disruptions, dysplasias, the following can be observed: (1) Deformations are increased in incidence in MZ and DZ twins. (2) Disruptions of the vascular type are more frequent in MZ twins with a single placenta. The pathogenesis includes postmortal tissue breakdown and embolization of thrombotic or necrotic tissue via placental anastomoses into the circulation of the surviving twin. Due to anastomoses in the fetal circulation (ductus Arantii and ductus Botalli), the embolus can bypass liver and lungs and arrive at the periphery, most often brain arteries, mesenterial branches or extremity arteries. Consequences can be porencephalic cysts up to hydranencephaly, intestinal atresia, amputation of one limb, but also e.g. cysts in liver or kidneys. (3) The largest group of CDD in twins are congenital malformations and malformation complexes of early onset. The later twin separation occurs, the more frequent is it combined with CDD: monochorionic monoamniotic twins have the highest incidence. In spontaneously aborted fetuses the incidence of MZ twins is at least 2x higher than in singleton abortions, and concordance is high while for CDD t'is mostly around 50% in MZ newborns. In concordantly affected twins, the severity of the defect(s) is mostly quite different.

Keywords: twins, congenital developmental malformations, deformations, disruptions

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METABOLIC SYNDROME: EFFECTS OF PPARGAMMA, APOE, LPL, IL-6, ACE AND AT1R GENE VARIANTS

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The role of genetic factors in the development of metabolic syndrome (MS) has been widely recognized, but the contribution of genes has not yet been fully clarified. We investigated the possible role of gene polymorphisms of PPARgamma (Pro12Ala), ApoE (ε2, ε3, ε4), LPL (P+/-), IL-6 (-174G>C), ACE (I/D) and AT1R (1166A>C) in MS. Genotyping of PPARγ, LPL, IL-6, AT1R and ACE was performed by PCR-RFLP, and of APOE by real-time PCR in a group of 263 patients and 180 controls. We found association of LPL variants with waist circumference (p=0.02), BMI, LDL and HDL (p=0.05); APOE and ACE significantly corelated to cholesterol (p=0.01), D and E2 allele contributed to its increased level while APOE2 corelated to lower HDL (p=0.03); ACE and AT1R variants correlated with LDL cholesterol (p=0.04); ACE D predisposed to increased glucose level (p=0.02). Borderline associations were found between blood pressure and combination of variant alleles of PPAR and APOE (p=0.06), APOE and ACE (p=0.06); BMI and APOE (p=0.06). Gene variants of APOE, LPL, ACE, AT1R and PPARγ could be susceptibility factors of obesity, lipid status, and glucose intolerance contributing to development of MS.

Keywords: metabolic syndrome, obesity, lipid status, glucose intolerance, genetic factors

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TP53 MUTATIONAL SIGNATURE OF ARISTOLOCHIC ACID IN THE UPPER URINARY TRACT CARCINOMAS

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Endemic (Balkan) nephropathy (EN), a chronic renal disease affecting residents of rural villages situated near tributaries of Danube River, is strongly associated with transitional cell (urothelial) carcinoma of the upper urinary tract (UUC). Aristolochic acid (AA), a powerful nephrotoxin and human carcinogen, was shown recently to be the causative agent in EN. In EN, exposure occurs through ingestion of bread prepared from flour contaminated with AA. After metabolic activation AA forms covalent DNA adducts in renal cortex and urothelial tissues. Aristolactam-DNA adducts generate unique mutational spectra in p53 tumor suppressor gene, which together with the presence of DNA adducts in the renal cortex serve as biomarkers for aristolochic acid nephropathy and associated urothelial carcinomas. TP53 mutation spectrum was dominated by A:T->T:A transversions located almost exclusively on the non-transcribed DNA strand with unique „hot spots“ at several splice sites and at codons 131 and 209. TP53 gene mutations at this position have not previously been reported. The mechanism underlying the observed strand bias appears to be a selective failure to excise AL-DNA adducts by global genomic nucleotide excision repair. This factor also may account for the remarkable persistence of these adducts in human tissues (in some cases more than 50 years). In summary, aristolochic acid joins vinyl chloride and aflatoxin as human carcinogens with a definitive mutational signature. This important information, coupled with the use of AL-DNA adducts as a biomarker, should prove useful in establishing the role of AA ingestion in countries with a high prevalence of UUC.

Keywords: p53, aristolochic acid, signature mutation, urothelial cancer, emdemic (Balkan) nephopathy

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CTLA-4 +49 A/G GENE POLYMORPHISM IN CROATIAN AND SLOVENIAN MULTIPLE SCLEROSIS PATIENTS

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Polymorphisms in the CTLA-4 gene are known to be important in several autoimmune diseases, including multiple sclerosis (MS). Previous studies on the impact of CTLA-4 +49 A/G gene polymorphism have given contradictory results. We investigated the possible influence of this polymorphism on MS susceptibility and disease behavior in Croatian and Slovenian populations. Genotyping was performed in 367 MS patients and 480 control subjects using PCR-RFLP method. The G allele was present in 216 (58.9%) MS patients vs. 282 (58.7%) healthy controls (p = 0.975, OR = 1.01, 95% CI = 0.76-1.32). No significant differences were observed in CTLA-4 +49 A or G allele distribution between patients and controls, indicating that this polymorphism does not influence susceptibility to MS in the surveyed populations. No correlation was observed between G allele carrier status and age at disease onset, disease course, or severity.

Keywords: multiple sclerosis, cytotoxic T lymphocyte antigen 4, gene , polymorphism, association studies

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PAI AND TPA GENE POLYMORPHISMS IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a complex inflammatory demyelinating disease of the central nervous system with both genetic and environmental contributing factors to onset and progression of the disease. Previously published data showed impaired fibrinolysis in MS. Fibrinolysis is regulated by a balance between the key fibrinolytic enzyme tissue-type plasminogen activator (t-PA) and its inhibitor (PAI-1). In the present study, an association of the TPA Alu I/D and PAI-1 4G/5G genetic polymorphisms with MS were analysed. The study was conducted within the framework of the Central and Southern-East European Multiple Sclerosis Genetics Consortium (CSEEMSGC) which include four populations (Croatian, Slovenian, Serbian and Bosnian and Herzegovian) that share the same geographic location and has a similar ethnic background of Slavic origin. In total 885 patients and 656 ethnically matched healthy blood donors with no history of MS in their families were genotyped using PCR-RFLP method. TPA DD homozygosity was shown as protective (OR=0.79, 95% CI 0.63-0.99, P=0.037) and PAI 5G5G as risk factor (OR=1.30, 95% CI 1.01-1.66, p=0.038) for MS. The significant effect of genotype/carrier combination was detected in 5G5G/I carriers (both of these separately carry significant risk for MS) with higher OR (OR=1.39 95%CI 1.06-1.82, p=0.017) for MS than in separate analysis, suggesting a gene–gene interaction.

Keywords: multiple sclerosis, PAI 4G/5G, TPA Alu I/D, gene polymorphism, susceptibility gene

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DOWN SYNDROME IN CROATIA: PARENTAL ORIGIN, RECOMBINATION, AND MATERNAL AGE

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To asses (i) the parental origin of trisomy 21 and the stage that nondisjunction occurs and (ii) the relationship between altered genetic recombination and maternal age as risk factors for trisomy 21. The study included 102 Down syndrome cases and their parents from the Croatian population. Genotyping analyses were performed by PCR using 11 STR markers along chromosome 21q. The vast majority of trisomy 21 was of maternal origin (93%), followed by paternal (5%), and mitotic origin (2%). The frequencies of maternal meiotic I and meiotic II errors were 86% and 14%, respectively. The highest proportion of cases with zero recombination was observed among those with maternal MI derived trisomy 21. A higher proportion of telomeric exchanges were presented in cases with maternal MI errors and cases with young mothers, although these findings were not statistically significant. The present study is the first report examining parental origin and altered genetic recombination as a risk factor for trisomy 21 in a Croatian population. The results support that trisomy 21 has a universal genetic etiology across different human populations.

Keywords: Down syndrome, genetic recombination, maternal age, meiotic nondisjunction, STR markers

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NON-INVASIVE DETERMINATION OF FETAL GENDER AND FETAL RHD STATUS FROM MATERNAL BLOOD

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Approximately 15% of Caucasian pregnancies are potentially at risk for severe hemolytic disease of the fetus and newborn due to Rhesus D incompatibility. Information about prenatal fetal gender has also gained in importance due to the growing number of X-linked disorders (over 150). The diagnosis of these two fetal genetic traits in our prenatal diagnostic laboratories still relies on invasive procedures, which are associated with a risk of fetal loss, and which may actually lead to a boosting of the maternal anti-Rhesus D titer. For this reason, we decided to develop non-invasive alternative by analyzing cell free fetal DNA from maternal blood. 170 maternal blood samples from different gestational weeks (8-40) were collected. After the separation of maternal plasma and extraction of cell free DNA, two different real time PCR assays were performed: presence of exons 5 and 7 of the RhD gene and non-coding region of the SRY gene were investigated. Fetal gender and RhD status of each sample was confirmed phenotypically at birth. Fetal gender was correctly determined in all of 170 cases (100%). Out of 70 samples from RhD negative women, fetal RhD status was correctly identified in 68 of them (97%), while 2 samples were falsely negative. Our results show that cell free fetal DNA analysis from maternal blood is reliable from the early gestation and that it has potential for routine diagnostic practice, so that invasive procedures in a case of X-linked diseases could be avoided and the unnecessary use of expensive prophylactic treatments prevented.

Keywords: non-invasive prenatal diagnosis, fetal gender, fetal RhD status, cell free fetal DNA, maternal blood

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FROM GENOME-WIDE ARRAY TO NEXT GENERATION SEQUENCING

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There is a growing awareness among health professionals of the role of genetics in causing or giving susceptibility to almost all branches of pathology with the exception of the trauma field. DNA sequencing has opened the way for new technologies that have clarified the genomic basis of a number of diseases whose causes could not be clarified by conventional linkage approaches. Through the application of genome-wide array techniques to cohorts of patients with mental retardation, autism, epilepsy, and schizophrenia several genomic regions have been identified that, deleted or duplicated, underlie these disorders and have even identified new syndromes previously embedded in the large category of idiopathic mental retardation. These methods have also allowed us to associate individual genes to specific diseases. The latest next-generation sequencing techniques are now showing how genetic testing has become an indispensable aid in medical practice and not just investigations to be required by a narrow category of diseases.

Keywords: not provided

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JOUBERT SYNDROM AN INTERESTING COLLABORATION

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Group of disorders characterized by hypoplasia of the cerebellar vermis with the characteristic neuroradiologic 'molar tooth sign', and accompanying neurologic symptoms, including dysregulation of breathingpattern and developmental delay. Other variable features includeretinal dystrophy and renal anomalies. We presented a young family from Syria with two boys from consanguinous marriage. One had DandyWalker anomaly abnormal, jerky eye movements and neurodevelopmental delay, other had nystagmus cerebellar atrophy Dandy Walker anomalyhypotonic syndrome and both had caractheristic 'molar tooth sign' on MRI. Gene analysis is realysed from Split to USA for research purpose, and with mutation we will be able to give genetic counselling to this young family.

Keywords: Joubert syndrom, mental retardation, genetics deasese, rare syndroms, CNS dissease

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WORLDWIDE SPREAD OF THE TP53 GENE POLYMORPHISMS ARG72PRO AND INTRON 3 (+16BP)

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To compare the allele frequencies of two polymorphisms in Croatian population with data for other populations worldwide. The TP53 gene is called “genome keeper” due to its activation in case of DNA damage causing the cell cycle arrest enabling thus the DNA repair, or activating the mechanisms of apoptosis. The polymorphisms Arg72Pro and intron 3 (+16bp) studied here have been suggested to be associated with various diseases being therefore interesting in the population context as well. Frequencies of studied alleles were compared using the population differentiation test. The data were obtained by searching the Medline. The frequency of the Arg allele in Croatian population was 75.9%, while the frequency of the A1 allele was 85.7%. The frequency of the Arg allele was similar to the frequencies of Portuguese (77.2%), Hungarians (77.7%), Russians (75.4%) and Yakuts (75.6%). The biggest difference in Arg frequencies from the Croatian population was obtained for Nigerians (37%) and Australian Aborigines (45%). The frequency of the A1 allele in Croatian population was similar to frequencies in other European populations (83-88%), Indian (83%) and Turkish population (86%) while the most pronounced difference emerged with the South African population. The worldwide frequency gradient of the Pro allele on codon 72 as well as of the A2 allele in intron 3 of the TP53 gene was found to spread from north to south. The allele frequencies in Croatian population were in concordance with the available data for other European populations.

Keywords: p53, polymorphism, Arg72Pro, world gradient, Croatia

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EFFECTS OF ABCB1 DRUG TRANSPORTER GENOTYPES ON ANTIEPILEPTIC DRUG DISPOSITION

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25-30 percent of epileptic patients remain resistant to different antiepileptics. ABC transporters which are responsible for drug transport across intestinal, renal and hepatic epithelial membranes and blood-brain barrier represent significant factors of variability in bioavailability of different drugs. The aim of the study was to evaluate the impact of ABCB1 (coding for P-glycoprotein) variants C1236T, G2677T/A, C3435T on antiepileptic drug disposition. We therefore correlated plasma levels of lamotrigine in mono- and polytherapy (carbamazepine, oxcarbazepine, levetiracetame, phenytoin, phenobarbital, topiramate, valproate) with gene variants. A total of 222 epileptic patients, aged 16-76 years, were stratified into lamotrigine monotherapy group (n=58), a group receiving lamotrigine plus inductors (n=98), and those receiving inhibitors (n=29) or both (n=37). ABCB1 genotyping (C1236T, G2677T/A), C3435T, was performed by Real-time PCR and PCR-RFLP. Therapeutic drug monitoring was performed by HPLC with diode array detector and immunoassay. A statistically significant correlation was confirmed between lamotrigine concentration and additional drugs ($p<0.001$), type of epilepsy, GGT, ALT, age and weight. Statistical analysis showed correlation between lamotrigine concentrations and C1236T ($p=0.01$) and G2677T/A ($p=0.5$) variants of the ABCB1. Lamotrigine concentrations in subjects who were T allele carriers were significantly decreased. Haplotype analysis showed that 1236T-2677T-3435C carriers had the lowest dose-corrected lamotrigine level ($p<0.001$), followed by 1236T-2677T-3435C haplotype ($p<0.04$). The obtained results show that ABCB1 polymorphisms exert an influence on antiepileptic drug pharmacokinetics and may serve as a pharmacogenetic marker of lamotrigine bioavailability.

Keywords: antiepileptics, lamotrigine, pharmacogenetics, ABCB1, transporter

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MICROSATELLITE LOCI ON CHROMOSOME X AND THEIR APPLICATION FOR THE RAPID PRENATAL DETECTION OF CHROMOSOME X NUMERIC ABNORMALITIES

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Chromosome X short-tandem repeat (X-STR) analysis was conducted in order to determine its role in prenatal and postnatal diagnostics of genetic disorders caused by the chromosome X numeric abnormalities. We investigated genetic variability of 5 X-markers (DXS9895, DXS6810, DXS6803, GATA172D05 i HPRTB) in a sample of 183 healthy Croatian individuals (90 males and 93 females) and explore the possibility of its usage in the prenatal screening for chromosome X numeric abnormalities. According to the population screening results, Croatian population shows no significant differences comparing to other European populations regarding allelic frequencies of the investigated X-markers. A set of 5 X-STR markers was shown to be sufficiently informative for the successful determination of the chromosome X numeric abnormalities in the Croatian population. Among 13 blood samples from patients with X chromosome disorders (Turner Syndrome - 6 cases; Klinefelter Syndrome - 5 cases and Triplo X Syndrome - 2 cases) have been tested using five X-STR loci. Since no false positive or negative results were observed in the patient's group, the present results confirm the diagnostic value of investigated X-STR loci for prenatal detection of chromosome X numerical disorders. The results of this study represents an important step that may lead to improved prenatal diagnostics in Croatia.

Keywords: short tandem repeats, X-chromosome, aneuploidy, prenatal diagnosis, Croatians

Presentation number: HDHG 5 Abstract number: HDHG-ABS-162-ISABS-2011

MOLECULAR DIAGNOSTICS OF SPINOCEREBELLAR ATAXIA TYPES 1, 2, 3, 6, 7

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Spinocerebellar ataxias (SCA) are clinically and genetically heterogeneous group of autosomal dominant neurodegenerative diseases with the frequency of 3/100.000. The most frequent types are SCA1, SCA2, SCA3, SCA6 and SCA7, and they are the consequence of the CAG triplet elongation in the coding region of some genes: ataxin 1, ataxin 2, ataxin 3, CACNA1A, and ataxin 7 genes. The aim was to investigate individuals suspected for SCA selected among patients with clinical symptoms of the disease and/or positive family history. SCA1, SCA2, SCA3, SCA6 and SCA7 were determined using multiplex-PCR (M-PCR) and capillary electrophoresis in 131 subjects with suspicion of ataxia, and 3 EMQN controls. Analysis of 134 DNA samples revealed the following ranges of triplet (CAG)n for five SCA types. SCA1: 19-35 (CAG)n, intermediary alleles were identified in 4 samples (2 samples with 36 and two samples with 37 (CAG)n); SCA2: 17-30 (CAG)n, an allele with full 36 (CAG)n mutation in one sample; SCA3: 14-31 (CAG)n, alleles were identified with full mutation 68 (CAG)n and 69 (CAG)n; SCA6: 6-14 (CAG)n, an allele with 21 (CAG)n was identified in one sample; SCA7: 7-17 (CAG)n, no mutation was identified in any of the samples. In comparison to the previously applied method of PCR/PAG-electrophoresis, the advantages of the five types of SCA (SCA1, SCA2, SCA3, SCA6 and SCA7) analysis using M-PCR/capillary electrophoresis are increased accuracy and precision and considerable saving in time. Analysis of all SCA types could significantly promote clinical practice.

Keywords: spinocerebellar ataxia, ataxin gene, CAG triplet, SCA1, 2, 3, 6, 7, M-PCR/capillary electrophoresis

Presentation number: HDHG 6 Abstract number: HDHG-ABS-152-ISABS-2011

ECTODERMAL DYSPLASIA- A FAMILY REPORT

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Ectodermal dysplasias (ED) are a heterogeneous group of genetically and heterogenous conditions that are characterized by abnormal development of ectodermal structures. The most affected structures are teeth, skin and its derivatives (hair, sweat glands) along with other ectodermal structures. The purpose of this work was to present the family with 3 boys affected with ED. We described the clinical report of male infant (sick twin) aged two months and his brothers with absence of eyebrows and eyelashes, saddle nose, dry flaky skin, rare thin blond hair, and large number of dental anomalies with pathohistological-skin positive analysis for ectodermal dysplasia. Oligodontia in the primary dentition in the case of two older brothers, as well as severe oligodontia in the permanent dentition. Only a few abnormally formed teeth erupted (microdontia and conical teeth), and at the later then average age. Radiographic examination confirmed previous clinical findings and determined taurodontism of the molar teeth. They presented pseudoprognathisam of the mandible due to micrognathisam of the maxilla. Based on the positive familly history (two older brothers), clinical picture and pathohistological findings of the skin we concluded that the child was also affected with ED. We are still not able to do a gene test for this family, we have grown skin fibroblasts and isolated and frozen samples for DNA analysis for EDA gene, EDAR gene EDARADD form of ectodermal dysplasia, but also NEMO gene.

Keywords: ectodermal dysplasia, genetics, dentistry, DNA analysis, clinics

Presentation number: HDHG 7 Abstract number: HDHG-ABS-310-ISABS-2011

A PATIENT WITH ATYPICAL PROGERIA SYNDROME DUE TO HETEROZYGOUS E159K MUTATION IN LMNA GENE

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Atypical progeria syndrome (APS) is a rare premature ageing syndrome mostly caused by mutations in lamin A/C (LMNA) gene. Until today 16 patients with this rare condition have been reported and genotype-phenotype correlations are just beginning to emerge. We present a 14 year old boy with APS, a child of healthy unrelated parents. He was born at term, BW/BL 3650g/53cm. At birth he had facial asymmetry and hypotonia, syndactyly on both feet, webbing on both hands and bilateral hip dysplasia. He had allegedly normal growth until the age of 4 years, when significant failure to thrive became evident. He presented to us for the first time at the age of 14 years due to asymmetric short stature (height 127.5 cm (-4, 5 SD), upper/lower segment ratio 0.57), striking hypotrophy, absence of fat tissue (weight 17.4 kg (-5 SD), BMI 10, 54 kg /m2) and severe kyphoscoliosis. Physical examination revealed facial asymmetry with progeroid appearance (prominent eyes, beaked nose, and small mandible, high forehead with prominent vein pattern and thin, scant hair and eyebrows). He had right torticollis due to contraction of m. trapesius, markedly deformed torso as well as severe cervical kyphoscoliosis and thoracic lordosis. Skin was very thin with prominent veins and generalized lipoatrophy. Auscultation revealed a grade 2 systolic murmur due to ASD type II. Wrist X- rays showed significant osteoporosis and hypertrophy of residual trabecular architecture, shortened, flattened and unshapely contoured distal phalange. LMNA genotyping showed E159K mutation, already described in one patient.

Keywords: atypical, progeria, LMNA, mutation, phenotype

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CLINICAL REPORT: NEWBORN WITH PARTIAL TRISOMY 21Q21 AND PARTIAL MONOSOMY 5P15.3 DUE TO BALANCED RECIPROCAL TRANSLOCATION 5;21 IN THE FATHER

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This study describes an extremely interesting case of simultaneous occurence of two syndromes (Down and Cri du Chat) in a male newborn, resulting form paternal balanced reciprocal translocation. To our knowledge, this case has not yet been described in the literature. We performed routine cytogenetics and fluorescence in situ hybridization (FISH). Probands karyotype was designated as 46, XY, der(5)t(5;21)(p15.3;q21)pat. A reciprocal balanced translocation was demonstrated in the father, between chromosome 5p and 21q with 46, XY, t(5;21)(p15.3;q21) karyotype. Maternal karyotype was normal 46, XX. Results of routine cytogenetics and FISH were in cohesion with our clinical suspitions, and confirmed that clinical pictures of Down syndrome and Cri du Chat syndrome are correlated with trisomy for the 21q22.3 band, and deletion of 5p15 band, respectivelly.

Keywords: Down syndrome, Cri du Chat syndrome, partial trisomy 21, partial monosomy 5, balanced translocation

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CHROMOSOME ABERRATIONS IN ACUTE VIRAL INFECTION

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Viruses can cause nonspecific damage and chromosome aberrations to the mitotic apparatus. Investigations show that the expression of early genes of some viruses in host cells induces chromosomal damage inhibiting DNA synthesis and cell division. The most common aberrations were chromosome fragmentation, translocations, centromeric and chromosomal/cromatide breakage and fractures whose formation is considered to be random with no specific sites on chromosomes. We present a case of chromosomal aberrations and translocations in a patient with acute herpes zoster virus infection. The indication for karyotyping was sterile marriage. For patient and his wife we used short culture of peripheral blood lymphocite with standard GTG banding method. Chromosome analysis showed aberration in three methaphase: (1) centromeric breakage of chromosome 9, (2) complete deletion of short arme of chromosome 9 del(9p), (3) centromeric breakage of chromosome 1 and deletion of long arm chromosome 9 del (9q31) with translocation between chromosome 5 and 11: t(5;11). To exclude problems during harwesting we repeated karyotiping. Two month later we had normale male karyotipe (46, XY) in all analysed cells. We were informed that during first analysis the patient had acute infection of herpes zoster virus.

Keywords: chromosome, mitosis, virus, herpes zoster virus, chromosome damage

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PRENATAL DIAGNOSIS IN SPLIT

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Laboratory for Human Genetics, Department for Medical Genetics, Pediatrics Clinic, CHC Split was founded in the year 1983. According to data from the archives 12th of December1983. was made first karyotype from a peripheral blood lymphocytes; 1987. first genetic counseling, and from 1989. we started with analysis of the bone marrow. The chromosome analysis (long culture) of amniotic fluid and spontaneous abortion material started on 10th of July 2007.year from then till now 694 amniotic fluid (29 pathological) and 130 spontaneous abortions were analyzed. Variations in the population of mostly chromosome 9 as 9qh + and inv (9) we found in 5 females and 7 males fetuses and two balanced translocation. Trisomy of a regular type was found among fetuses as followed: 21, 18 and 13, one had an unbalanced translocation Robertson type 21; 21. Among spontaneous abortions there were 3 triploidy (69, XXX or 69XXY), four monosomy X, and trisomy of chromosomes 2#, 3#, 5#, 6#, 7#, 9#, 10#, 12#, 13#, 15#, 16#, 20# and 22#. We present our data from this short period of our experience of long chromosome culture with two interesting cases one 46, XX, del(12p)(11.2-pter) and one familial translocation with four spontaneous abortions and one child with triple X syndrome.

Keywords: prenatal diagnosis, amniocentisis, cytogenetics, spontaneous abortions, genetic counselling

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REPETITIVE ANEUPLOIDY OF DIFFERENT CHROMOSOME IN CONSECUTIVE PREGNANCIES

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There are numerous reports of trisomy in consecutive pregnancies, including same or different chromosomes. There is no clear explanation of cell mechanism of repetitive aneuploidy, but this could be explained by maternal age, gonadal mosaicism, mosaicism of sex chromosomes, gene distribution and genetic predisposition of chromosomes nondisjunction. We present 4 couples with repeated trisomy of different chromosomes (18, 13 and 21) in successive pregnancies in perod of 4 years. Analised couples have normale karyotypes and family history data of spontaneous abortions. One couple could'nt have healthy offspring. The first child was born with Edwards syndrome, and quickly died. After four years a couple got a child with Patau syndrome /47, XX, +13, rob (13; 13) (q10, q10)/. The other three couples had a healthy child followed by more subsequent pregnancies with trisomy of chromosome 13 and 21/18 and 13/13 and 21. Trisomies were detected with chromosome analysis of amniotic fluid, stillborn child (trisomy18) or after a elective abortion (trisomy 21-by PCR).

Keywords: chromosomes, aneuploidy, trisomies, elective abortion, consecutive pregnancy

Presentation number: HDHG 12 Abstract number: HDHG-ABS-234-ISABS-2011

THE EFFICACY OF INTERPHASE FISH DIAGNOSTICS IN PEDIATRIC ACUTE LEUKEMIAS OVER THE PERIOD OF TWO YEARS AT THE CLINICAL CENTER OF THE UNIVERSITY OF SARAJEVO

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Interphase FISH (fluorescent in situ hybridization) for acute leukamias has been established two years ago at the Clinical Center of the University of Sarajevo, Bosnia and Herzegovina. The aim of this study is to retrospectively analyze the efficacy and use of FISH for the diagnosis and prognosis of the pediatric acute leukemias. Methods: During the 2009-2010, there were 40 pediatric acute leukemias at the Clinical Center of the University of Sarajevo (34 acute lymphoblastic and 6 acute myeloid leukemias). Thirty peripheral blood samples were sent to the cytogenetics laboratory from 29 patients. The samples were harvested using standard cytogenetics protocols, either as direct culture or overnight culture (if karyotype was requested). If the diagnosis was acute lymphoblastic leukemia, the interphase FISH panel included t(9;22), 11q23 deletion, t(12;21), and occasionally t(8;14). If the diagnosis was acute myeloid leukemia (AML), the FISH panel was specific for 5q deletion, 7q deletion, centromere of chromsome 8, and specific AML probes: t(8;21), t(15;17), inv(16), and 11q23 deletion. All probes were purchased from Abbott Laboratories, Illinois, USA. Results: Six out of 29 samples (21%) had cytogenetic abnormalities, including rare abnormalities like ABL amplification. Conclusion: The use of interphase FISH in diagnostics of pediatric acute leukemias is worthwhile because it gives the valuable diagnostics and prognostic information; for example, one patient had t(12;21), which is associated with good prognosis, while patient with ABL amplification had poor survival.

Keywords: interphase FISH, pediatrics acute leukemia, molecular cytogenetics, acute lymphoblastic leukemia, acute myeloid leukemia

Presentation number: HDHG 13 Abstract number: HDHG-ABS-195-ISABS-2011

PURE TRISOMY 10P RESULTING FROM AN EXTRA STABLE TELOCENTRIC CHROMOSOME

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This is a presentation of a child with very rare trisomy of the chromosome 10 short arm (10p), due to centric fission of maternal chromosome 10. Conventional cytogenetics and fluorescence in situ hybridization (FISH) showed a child's karyotype to be 47, XX, +fis(10)(p10)mat, whereas the healthy maternal karyotype was 47, XX, -10, +fis(10)(p10), +fis(10)(q10). This is the first clinical report of a child with a pure trisomy 10p as a result of consequence of familial unequal transmission of telocentric chromosome with a fully functional centromere. In cases reported so far the trisomy 10p is accompanied with additional chromosome imbalances and that causes some of phenotypic characteristics. However, the clinical features described in the current case are caused by pure trisomy 10p and, thus, delineate the 10p trisomy syndrome phenotype such as growth retardation, development delay, craniofacial dysmorphism, foot abnormalities and heart defect.

Keywords: trisomy 10p, chromosome, child, simple fission, phenotype

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ATP7B GENE MUTATIONS ASSOCIATED WITH INCIDENCE OF WILSON DISEASE IN CROATIAN POPULATION

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Wilson disease (WD) is an autosomal recessive disorder of copper metabolism resulting from the absence or dysfunction of copper transporting P-type ATPase (ATP7B). More than 400 disease causing mutations of the ATP7B gene have been identified to date. Aim of ATP7B gene sequencing analysis is to confirm WD diagnosis in patients with atypical clinical presentation or equivocal copper studies and to investigate mutations present in croatian population. Genomic DNA was used to amplify 21 exons of the ATP7B gene. Sequencing analysis was performed by PCR and capillary electrophoresis with BigDye Terminator v3.1 kit on AB Genetic analyzer 3130xl. Here we report results of sequencing analysis of the ATP7B gene. We have analyzed coding region of the ATP7B gene of clinically diagnosed WD patients from Croatia, already screened for the most common His1069Gln mutation. It accounts for 54, 4% of Wilson disease alleles in croatian population. Out of the total number of 71 tested patients with WD, molecular analysis has confirmed the clinical diagnosis in 44 patients (61, 9%) so far. 17 (23, 9%) patients are homozygous for the most common His1069Gln mutation. In 13 patients (18, 4%) only one mutation has been identified. Mutations in croatian population are mostly distributed in exons 5, 8, 13, 14, 15, 18, 20 and 21 of the ATP7B gene. Sequencing analysis of the ATP7B gene is the best method to establish frequency of mutations in specific population so the screening test panel for most common mutations can be developed for this population.

Keywords: ATP7B, Wilson disease, sequencing analysis, copper, mutation frequency

Presentation number: HDHG 15 Abstract number: HDHG-ABS-161-ISABS-2011

MOLECULAR DIAGNOSTICS OF SPINAL MUSCULAR ATROPHY (SMA) AND SMA CARRIERS BY DETERMININGTHE NUMBER OF SMN1 AND SMN2 GENE COPIES

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SMA is the second most frequent fatal autosomal recessive disease, with incidence 1/10,000 live-borns and 1/40-1/60 carrier frequency. 95% SMA patients are homozygotes for exon 7 deficiency, and 3.6% are compound heterozygotes (with point mutations on the second allele). SMA carriers are heterozygotes, without disease symptoms, with one copy of exon 7 SMN1. The aim was to determine SMA patients and carriers by analyzing DNA of individuals with SMA in their medical history or with disease symptoms. We determined the number of SMN1 and SMN2 gene copies using MLPA tests and capillary electrophoresis in 14 patients with SMA suspicion and 4 EMQN control subjects. DNA analysis of 18 samples determined two SMA carriers with one copy of exon 7 SMN1 (one carrier: 2 copies of exon 8 SMN1 and 0 copies of SMN2; another carrier: 1 copy of exon 8 SMN1 and 2 copies of SMN2); 6 SMA patients with 0 SMN1 copies (two: 2 SMN2 copies; two: 3 copies SMN2, and two pts: 4 SMN2 copies); 10 healthy subjects with 2 SMN1 copies (five: 2 SMN2 copies; four: 1 SMN2 copy; and one sub: 0 SMN2 copy). In relation to previously used method, RFLP/gel electrophoresis, advantage of DNA analysis performed by using MLPA tests/capillary electrophoresis is the possibility to diagnose SMA patients and carriers on the basis of the copy number. This method cannot be applied to determine other point mutations or the location of other SMN1 copies on the same chromosome.

Keywords: spinal muscular atrophy, carriers, gene copies, SMN1, SMN2

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FAMILIAL TRANSLOCATION WITHOUT PRENATAL DIAGNOSIS

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Pregnancies with chromosome aberration entail numerous moral and ethical implications for physicians, married couples and even society. The chromosome diagnosis is the major cause of elective abortions and increasing worldwide. The couples are provided with necessary information to make an informed decision and supported regardless of whether decide to terminate or continue the pregnancy. In some countries if couple decides to give a birth to mentally retarded child after positive prenatal diagnosis, society refuse to help them with social security, school etc. We present a family where grandfather and father are healthy carriers of balanced chromosomal translocation 16; 20 and one brother and son with mental retardation, vesicoureteral reflux and cleft palate. The couple had two miscarriages and than two children with unbalance karyotype from this familial translocation. Both children are involved in the treatment of neuropediatrician, cardiologists, and physiologists. After the first birth the couple was at genetic counseling and they were informed of the existence of a familial translocation and the possibility of birth a children with disabilities, but the couple rules out the possibility of prenatal diagnosis, preimplantation diagnosis or abortion with medical indication.

Keywords: familial translocation, t(16, 20), FISH, genetic counselling, prenatal diagnosis

Presentation number: HDHG 17 Abstract number: HDHG-ABS-272-ISABS-2011

BECKWITH WIEDEMANN SYNDROME: CLINICAL AND EPIDEMIOLOGICAL STUDY OF A LARGE SERIES OF PATIENTS IN EUROPE

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Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder characterized by macroglossia, high birth weight, omphalocele, visceromegaly, hypoglycaemia, tumour predisposition, and congenital malformations. To determine relevant epidemiological and clinical characteristics in a large series of patients with BWS in Europe. We present clinical and epidemiological characteristics of 197 patients with BWS that were identified by retrospective analysis among 13 546 771 pregnancies that were monitored in EUROCAT network of population based registries in 1980-2007period. The clinical manifestations of BWS patients reflect the variable presentation of the syndrome. The infants had a high birth weight for their gestational age. Prematurity was present in 74/166 (44.57%) cases. The most frequent features were omphalocele (54.72%; 81/148) and macroglossia (52.7%; 78/148). Associated anomalies were present in 68 (46%) patients and included mainly cardiovascular (30/148; 20.28 %), urinary (26/148; 17.56%) and limb defects (14/148; 9.15%). Of the 70 cases detected prenatally, 12 (17.1%) were terminated due to severe anomalies. The overall recorded number of late foetal deaths/stillbirths with BWS was 8, and of deaths in the first week of life 7, resulting in a total perinatal mortality rate associated with BWS of 76.14 per 1000 births. The minimal estimated prevalence rate of BWS in Europe is 1 in 69930 births. All cases were sporadic. Beckwith Wiedemann syndrome is a rare disorder. Presence of various associated anomalies decreases overall first week survival rate in this patients to 86.14% (170/197).

Keywords: Beckwith Wiedemann, syndrome, genetics, epidemiology, congenital

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A NOVEL DE NOVO DIR DUP (16) (Q12.1-Q21) IN A GIRL WITH BEHAVIORAL DISORDER, MILD COGNITIVE IMPAIRMENT, SPEECH DELAY, AND DYSMORPHIC FEATURES: CASE REPORT AND REVIEW OF THE LITERATURE

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We report here on the 10-year follow up and clinical, cytogenetic and molecular investigation of a girl admitted for evaluation because of speech delay, learning difficulties, aggressive behavior and dysmorphic facial features that included high forehead, round face, epicanthic folds, low-set dysplastic ears, flat nasal bridge, long flat philtrum, thin upper lip, small mouth, and short neck. The analysis of high-resolution GTG- and CTG-banding chromosomes suggested a de novo direct duplication of 16q12-q21 region and FISH analysis with WCP-16 probe confirmed that the duplicated genetic material originates from chromosome 16. Subsequently, array-based comparative genomic hybridization (aCGH) analysis with a 75 kb resolution showed a 9.92 Mb gain on the long arm of chromosome 16 at bands q12.1 through q21. To the best of our knowledge, this is the first case of duplication 16q12.1q21 described in literature. Several genes within the duplicated region are of interest for possible correlation with clinical features present in our patient. Clinical and cytogenetic findings are compared with the small number of reported patients with pure duplications 16q partially overlapping the one seen in our patient. Clinical phenotype seem to be distinctive between the proximal-intermediate and intermediate-distal regions of the long arm of chromosome 16. In particular, we have observed a set of dysmorphic features that could present a characteristic dup 16q11.2-q13 phenotype. The present work illustrates the advantages of an integrative approach using both conventional and molecular techniques for the precise characterization and genotype-phenotype correlation in patients with dysmorphism, behavioral problems and learning difficulties.

Keywords: interstitial duplication, chromosome 16, oligonucleotide, microarray, speech delay

Presentation number: HDHG 19 Abstract number: HDHG-ABS-163-ISABS-2011

LIFETIME OF CHILDREN WITH CHROMOSOMAL ANOMALIES

Pavić TŠ, Čulić V, Mišković S, Lozić B, Mijaljica G, Dragišić-Ivulić S

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90% or more of children with trisomy 18- Edwards' syndrome die within the first year of life. Trisomy 13 - Patau syndrome, is the third most prevalent chromosomal anomaly 1:8000-12000, 28% of children die in the first week of life, 44% in the first month, 86% from the first year; 13% have congenital heart defects, cardiopulmonary failure, 69% and 4% of pneumonia. The median survival for age is 2.5 days. A small number of cases survive until puberty. In Croatia 58, 8 % of children with trisomy 21 die in first 4 years of live and 30, 6% older than 30 years of life. We presented a girl at the age of 7 years with trisomy 13. In addition to medical problems that are unique to this chromosomal syndrome, these patients present complex medical problems common to all persons with chromosomal anomalies. The primary and tertiary care consultants who are able to provide knowledge and sensitive supportive care to children with trisomy 13, 18 and 21 to their parents are performing a service of significant benefit, no matter how brief the life span of the child may be.

Keywords: chromosome aberrations, trisomies, lifetime, ethics, Patau syndrome

Presentation number: HDHG 20 Abstract number: HDHG-ABS-122-ISABS-2011

A RARE CASE OF 8Q23.3-Q24.13 MICRODELETION WITH LANGER-GIEDION SYNDROME PHENOTYPE WITHOUT TRPS1 GENE DELETION

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Langer-Giedion syndrome (LGS) is a contiguous gene syndrome caused by a hemizygous deletion on chromosome 8q23.3-q24.11 involving TRPS1 and EXT1 genes. We report on a girl with a microdeletion at 8q23.3-q24.13 and clinical features of LGS. In addition to the classical LGS phenotype, the patient also has premature adrenarche. The patient is a 4-year-old girl with delayed psychomotor development and craniofacial dysmorphic features consisting of large, laterally protruding ears, bulbous nose, broad nasal bridge, elongated upper lip, thin vermilion border, and sparse scalp hair. Radiographic examination of both hands revealed delayed bone age, brachyphalangia, brachymetacarpia and cone-shaped epiphyses. Multiple cartilaginous exostoses were detected on long and short tubular bones. Her pubertal development was classified as Tanner stage 3 premature pubarche. Hormonal analysis revealed elevated DHEAS and androstendion indicating premature adrenarche. Molecular genetic analysis was performed to confirm the diagnosis of LGS. Array-comparative genomic hybridization revealed a 7.5Mb interstitial deletion at 8q23.3-q24.13 (Chr8:116.921.245bp-124.442.990bp) leaving the TRPS1 gene intact. Although the deletion of TRPS1 and EXT1 genes is responsible for craniofacial and skeletal features of LGS, there have been previous reports of patients with features of LGS and 8q24 deletions leaving the TRPS1 gene intact. To our knowledge, this is the third such case. These three patients have similar proximal breakpoints suggesting a functional disturbance of TRPS1 gene and deletion of potential TRPS1 regulatory sequences. Also, the combination of LGS with premature adrenarche has not yet been described, however this combination in our patient is likely by chance.

Keywords: genetics, dysmorphology, malformation syndrome, multiple exostoses, microdeletion

Presentation number: HDHG 21 Abstract number: HDHG-ABS-226-ISABS-2011

PROLIFERATION OF DECIDUAL CELLS IN RAT EMBRYOS IS GREATER BEFORE THAN AFTER GASTRULATION

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During pregnancy, endometrial cells undergo series of morphological and physiological alterations leading to transformation into decidual cells. This is essential for the successful implatantion and placentation of the early embryo and its proper development. As in humans, similar alterations occur in rodents; thus, rat can be considered an appropriate experimental model to study decidual development in humans. The purpose of this study was to compare the proliferation of rat decidual cells before and after embryo gastrulation stage. Pregnant rats were euthanized on the 8th day (before gastrulation) and on the 11th day (after gastrulation) of gestation. Uteruses were isolated under binocular magnifying scope and excised deciduomas fixed in 4% formaldehyde. One part was stained with H&E for classical histological analysis whereas the other part (from 6 randomly selected deciduomas) was used for PCNA (proliferating cell nuclear antigen; a marker of cell proliferation) immunohistochemistry. The PCNA-positive cells were counted using stereological method for numerical density (Nv) on Weibel's multipurpose test grids with 42 points and analyzed with Ellipse 3D program. We found that there were significantly more proliferating decidual cells before gastrulation (Nv = 39151±4354; 8th day of gestation) than after gastrulation stage (Nv = 13301± 3196; 11th day of gestation) ($p<0.01$, Student's t test). In conclusion, our result shows that during gestation, decidua is characterized by cell proliferation that predominates in early pregnancy but decreases later on, after gastrulation is completed, as decidua assumes other functions of protecting the developing embryo.

Keywords: decidua, trophoblast, proliferation, gastrulation, placenta

Presentation number: HDHG 22 Abstract number: HDHG-ABS-126-ISABS-2011

THE EVIDENCE OF PROINFLAMMATORY AND PROTHROMBOTIC ROLE OF LEPTIN RECEPTOR GENE

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In this study we investigated association of the common polymorphic variants in the LEPR gene with obesity, serum lipids and inflammatory markers C-reactive protein (CRP) and fibrinogen. The leptin receptor, encoded by the LEPR gene, is a single transmembrane protein that exerts the function of leptin, the protein that is known to regulate food intake, energy homeostasis, reproductive and immune function. We selected 32 single nucleotide polymorphisms (SNP) in/near LEPR gene from genome-wide association study data (Human Hap300 Illumina platform) from Vis cohort (N = 986), including two commonly investigated SNPs: Arg109Lys and Arg223Gln. Association of polymorphisms with anthropometric measures of obesity, concentration of serum lipids, CRP and fibrinogen was tested using analysis of variance and logistic regression analysis. Several SNPs in intron 2 region were clearly associated with serum lipids and only marginally with anthropometric measures of obesity, while SNPs near the 3' region of the gene correlated significantly with fibrinogen and HDL. Analysed SNPs of LEPR gene were generally not found to be significant predictors of obesity in contrast to their marked involvement in lipid metabolism. Moreover, our results suggest an important proinflammatory and prothrombotic role of leptin signaling system.

Keywords: LEPR, obesity, inflammation, fibrinogen, Vis cohort

Presentation number: HDHG 23 Abstract number: HDHG-ABS-217-ISABS-2011

THREE CASES OF TRIPLOIDY IN SECOND TRIMESTER OF PREGNANCY

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Triploidy refers to a complete extra set of haploid chromosomes in the embryo. It occurs approximately in 2% of conceptuses, but most abort spontaneously during the first trimester. The extra haploid set can be derived from maternal or paternal origin (digyny or diandry). This condition is a sporadic event and not associated with a mother's age. The aim of the study is to compare three different triploid karyotypes using sonographic, fetal echocardiographic and post mortem examination of fetal findings in second trimester of pregnancy. All ultrasound cases showed fetal markers which indicate amniocentesis. Triploid fetuses showed a wide variety of congenital anomalies such as intrauterine growth restriction, complex fetal heart disease, holoprosencephaly, oligohydramnios, hypoplastic kidneys, syndactyly, cleft lip and palate and fetal ascites. To identify any possible chromosomal abnormality, cytogenetic investigation was carried out on the amniotic fluid samples. Cytogenetic analysis was performed using the GTG banding technique. The fetal karyotypes showed triploidy with 69, XXX, 69, XXY, or 69, XXX/46, XX chromosome complements. After termination of the pregnancies, fetal autopsy confirmed the ultrasound findings indicative of triploidy. A long survival to reach the second trimester of pregnancy and no evidence of partial hydatidiform molar changes in the placenta suggests that the triploidy was of maternal origin. It is very important to recognize as early as possible a sonografic findings indicative for triploidy to enable early fetal karyotyping to reduce possible maternal complications.

Keywords: triploidy , prenatal karyotype, fetal ultrasound, fetal echocardiography, post mortem examination

Presentation number: HDHG 24 Abstract number: HDHG-ABS-301-ISABS-2011

MINOR PHYSICAL ANOMALIES IN CHILDREN WITH MENTAL RETARDATION, HEARING AND VISION IMPAIRMENT

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Background: Minor physical anomalies (MPA) can serve as a strong tool in the delineation of many major malformations, syndromes, metabolic and psychiatric disorders. Aim: To asses the frequency and weight of MPA in developmentally disturbed (DD) children: mentally retarded (MR), children with hearing impairment (HI), and vision impairment (VI). Methods: The study was carried out on a sample of 776 children aged 5 to 20 years; 473 DD children, and 303 healthy subjects. The patient sample comprised 268 MR children (157 boys and 111 girls); 121 HI children (82 boys and 39 girls) and 84 VI children (37 boys and 47 girls). Children with recognizable genetic syndromes were excluded from the study. Waldrop physical anomaly score was used to asses MPA in all groups. Results: The average number of MPA per individual (W1) was significantly higher in MR group (M = 4.09), HI group (M = 4.32), VI group (M = 3.27) than in healthy controls (M = 1.89; $p<0.001$). Means of Waldrop weighted scores (W2) were also significantly higher in MR group (M = 3.81), HI group (M = 3.97), VI group (M = 3.24) than in control group (M = 1.70; $p<0.001$). There were no significant sex differences in W1 and W2 in different groups. Conclusions: The significantly higher number of minor anomalies per person and their high weighted score in all three groups of children with DD children suggests that they are associated with common underlying factors leading to disturbed prenatal child development.

Keywords: minor physical anomalies, mental retardation, hearing impairment, vision impairment, developmental disturbances

Presentation number: HDHG 25 Abstract number: HDHG-ABS-269-ISABS-2011

CHARACTERIZATION OF A FAMILIAL CASE WITH COMPLEX CHROMOSOME REARRANGEMENT INVOLVING CHROMOSOMES 1, 10, 11, 13 AND 18

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Complex chromosome rearrangements (CCR) are rare structural chromosome aberrations that can be found in patients with phenotypic abnormalities or in phenotypically normal patients presenting recurrent miscarriages and infertility. Here we present clinical and cytogenetic findings in an 8-year old boy who was referred due to the psychomotor retardation, facial dysmorphy and hyperactivity. GTG banding revealed CCR involving chromosomes 1, 10, 11, 18 and 19. Additional investigation of family members' chromosomes revealed that the father has normal karyotype, but patient's mother and sister have similar CCR. Besides, our patient has de novo reciprocal translocation between chromosomes 18 and 19. Karyotypes of mother's parents were normal. Further clarification and breakpoint determination was done after the application of the various FISH probes: MCB, WCP, subtelomeric and centromeric. The patient's karyotype was characterized as molecular cytogenetically unbalanced, while mother's and sister's karyotypes were molecular cytogenetically balanced. This case of CCR is very complex with six breakpoints. The 11q translocated segment had split, with 11q23-q25 going to the der(1) and 11q25-qter to the der(10). In mother der(1) is consisted of three chromosomal segments: 1, 10 and 11. Surprisingly, daughter has double two way exchange, t(13;18) originated from mother, and t(1;11) which is not present in this form in mother. That could be explained by presence of at least two different CCR cell lines in gonads. To the best of our knowledge, this is the first report of such a familial CCR, especially regarding the additional de novo translocation and a possibility of maternal gonadal mosaicism.

Keywords: structural chromosome abberation, complex chromosome rearrangements, gonadal mosaicism, psychomotor retardation, facial dysmorphy

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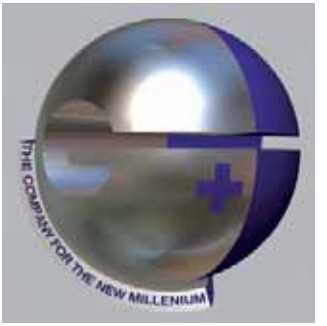

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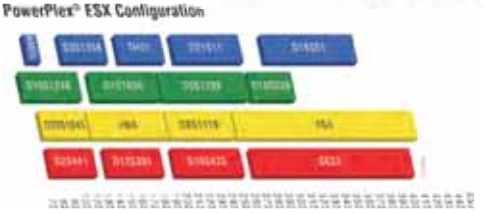

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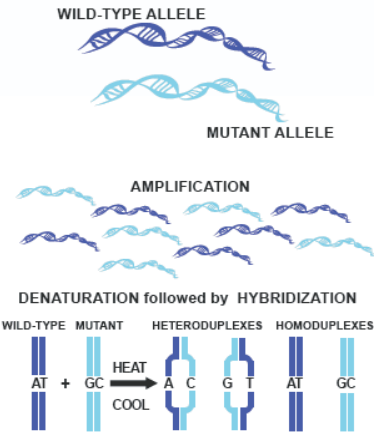

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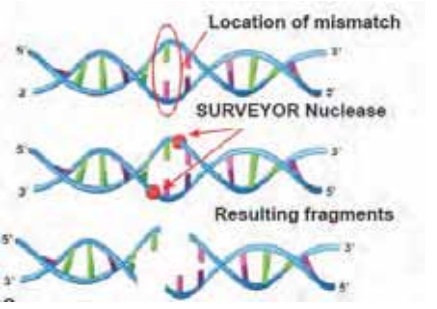

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