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EXTO PRODUKCIJA

Zagreb, September 2005

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Dear Colleagues

We welcome you to the Fourth European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine in Dubrovnik, Croatia, September 5-9, 2005. The event will feature the latest advances in forensic and clinical genetics and related areas. Just as importantly, it will be a forum for exchange of pertinent information, ideas and technical developments in the form of lectures, a technical hands-on workshop, round-table discussions and poster presentations.

The previous meetings in Split (1997), Dubrovnik (2001) and Zagreb (2003) were highly successful. With participants from more than 60 countries these events were truly international. The registration for this meeting attests to the continuing international interest in this program.

This meeting in Dubrovnik offers cutting-edge scientific contents enriched by the ethereal beauty of the historic town on the pristine Adriatic. Dubrovnik is the heir of an extraordinary tradition that begot major achievements in science, medicine, literature, music and visual arts. We are certain that you will be inspired by the city also known as the Pearl of the Adriatic.

The programs in forensic medicine and molecular and cellular medicine will run in parallel with the inaugural, methods and closing sessions held jointly. In keeping with tradition, this year the Program Committee bestowed four Young Investigator Awards. The winners will present their work at the special plenary session on the last day of the meeting.

In addition, on September 5 we will hold the first assembly of the newly founded International Society for Applied Biological Sciences (ISABS; www.isabs.hr) to discuss and adopt the bylaws and elect officers. The goal of ISABS is to promote development of disciplines combining clinical medicine, molecular genetics and medicine, forensic sciences and biotechnology. We hope you will find it worthwhile to join us in this endeavor.

As in previous years, the Croatian Medical Journal is the official journal of the meeting. The issue for August 2005 (Vol. 6, No. 4) is dedicated to our meeting and contains many pertinent papers. We believe that the proceedings of this conference will be accepted as well as have the proceedings of our past events. These have been widely accepted as documented by more than 70,000 hits at the CMJ web page (www.cmj.hr) and the high citation rate of the papers published therein.

We look forward to your participation and your scientific contribution.

Sincerely,

Moses Schanfield, Ph.D.
Dragan Primorac, M.D., Ph.D.
Stanimir Vuk-Pavlović, Ph.D.

Conference/Program Directors

CONFERENCE ORGANIZER

Program Directors

Moses Schanfield, George Washington University, Washington, DC, USA
Dragan Primorac, University of Split, Split and Josip Juraj Strossmayer University, Osijek, Croatia
Stanimir Vuk-Pavlović, Mayo Clinic College of Medicine, Rochester, MN, USA (Executive director for this conference)

Organizers

International Society of Applied Biological Sciences
Mayo Clinic College of Medicine and University of Zagreb International Program in Advanced Medical Education

Standing Committee of the Mayo Clinic College of Medicine—University of Zagreb International Program in Advanced Medical Education

Stanimir Vuk-Pavlović, Ph.D., Chair
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John La Forgia, M.B.A.
Nijaz Hadžić, M.D. Ph.D.
Thomas McDonald, M.D.
Dragan Primorac, M.D., Ph.D.
Mark Wilhelm, M.D.

Program Committee

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Frederick Bieber (Harvard Medical School, Boston, Massachussets)
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Doron Lancet (Weizmann Institute of Science, Rehovot, Israel)
Henry Lee (University of New Haven, West Haven, Connecticut)
Timothy Palmbach (University of New Haven, West Haven, Connecticut)
Jeffrey Platt (Mayo Clinic College of Medicine, Rochester, Minnesota), Chair
Gregory Poland (Mayo Clinic College of Medicine, Rochester, Minnesota)
Dragan Primorac (University of Split, Split, Croatia and University of Osijek, Osijek, Croatia)
Moses Schanfield (George Washington University, Washington, DC)
David I. Smith (Mayo Clinic College of Medicine, Rochester, Minnesota)
Stanimir Vuk-Pavlović (Mayo Clinic College of Medicine, Rochester, Minnesota)

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Congress Service

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Kardinala Stepinca 21
20 000 Dubrovnik
Croatia

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Fax: +385 20 437 289
e-mail: als@als.hr
http://www.als.hr

Scientific Program Information.

Certificate of Attendance

Certificates of attendance will be available at the registration desk.

Young Investigator Awards

Members of the Program Committee selected abstracts among entries submitted by investigators under the age of 40. Author(s) of each selected abstract will receive the Young Investigator Award Certificate and EUR500 and will present their work from the podium at the special plenary session on September 9.

Credits

The 4th European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine has been approved for 20 credit points (participants) or 25 credit points (lecturers) by the Croatian Medical Chamber.

Sponsor Exhibition opening hours

Set up: Sunday, September 4, 2005 18:00 to 20:00
Monday, September 5, 2005 noon to 18:00
Tuesday, September 6, 2005 08:30 to 20:00
Thursday, September 8, 2005 08:30 to 20:00
Dismantling: Friday, September 9, 2005 18:00 to 20:00

Language

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

Poster Setup

Tuesday, September 6, 2005, 07:30 to 08.30
Poster board numbers can be found in the author's index. The staff at the poster Presenter's Desk will help you in finding both the number and location of the board.

Poster Exhibits

Tuesday, September 6, 2005 08:30 to 20:00
Thursday, September 8 2005 08:30 to 19:00

Poster Discussion with vine and cheese reception

Tuesday, September 6, 2005 19:00 to 20:00
Thursday, September 8 2005 18:00 to 19:00
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.

Poster Removal

Thursday, September 8 2005 19:30 to 20:30

Program Changes

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

Registration Desk Opening Hours

Sunday, September 4, 2005 16:00 to 20:00
Monday, September 5, 2005 07:30 to 20:00
Tuesday, September 6, 2005 07:30 to 16:00
Thursday, September 8, 2005 07:30 to 16:00

Slide and PowerPoint Preview Room

A slide and PowerPoint preview room will be available to all speakers. We encourage all lecturers to present data as a PowerPoint presentation.

Recipients of the 2001 Young Investigator Awards

Forensic IdentityTesting: Frontiers in Molecular and Cellular Medicine:

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

Recipients of the 2003 Young Investigator Awards

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

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)

General Information

Badges
Name badge is required to participate in scientific sessions, exhibitions and poster viewing. Badges will be provided to participants, accompanying persons and exhibitors at registration. Please wear you badge at all events at the conference venue. Handling fee for replacement badges is €10.

Bank Services
The official currency in Croatia is the Croatian kuna (HRK). The official exchange rates:
1 EUR = 7.37 HRK* (August 24, 2005)
1 USD = 6.02 HRK* (August 24, 2005)
* Please note that these exchange rates are variable

Busines hours for post offices and banks
Post offices and banks are generally open for business from 8:00 a.m. to 7:00 p.m., Monday through Friday.

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

Electricity Supply
220-240 V, 50 Hz

Insurance
Participants are encouraged to make their own arrangements pertinent to health and travel. By registering for the 4th European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine, Dubrovnik, Croatia, September 5–9, 2005, participants accept that neither the organizers and its agents nor the sponsors and exhibitors nor the Adriatic Luxury Services assume any liability whatsoever.

Message Center
A Message Center is available at the registration desk.

Official Carrier
Croatian Airlines is the official carrier of the Conference. Croatia Airlines is offering a 50% discount on international flights on individual published IATA airfares and a 25% discount on domestic flights on C, Y and B airfares to Dubrovnik for the event participants and accompanying persons. This discount is applicable for Croatia Airlines flights only, on tickets purchased at the Croatia Airlines offices and agents. The discount pertains to travel from 29 Aug to 16 Sep 2005. To obtain the discount, Croatia Airlines discount voucher and a confirmed registration form or other written proof of participation must be presented at the time of purchase. If you would like to take advantage of this special offer, please contact your nearest Croatia Airlines office. The list of Croatia Airlines offices, as well as the discount voucher and all information concerning granted discount can be found at www.croatiaairlines.com – at your service – official carrier.

Public Transportation in Dubrovnik
Buses are the main means of public transportation in Dubrovnik. Almost all hotels have easy access to buses.

Restaurants
Most restaurants in Dubrovnik are opened from 8:00 a.m. to 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.

Service Center
Photocopying, typing, production of overhead transparencies and computer print-outs are available at the Dubrovnik Palace Hotel Service Center for a fee.

Shopping
Store hours in Dubrovnik are usually 8:00 a.m. – 9:00 p.m. Monday to Saturday. Some are open on Sundays. Most shops accept all major credit cards.

Smoking Policy
The 4th European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine is a non-smoking event. Smoking is prohibited everywhere on conference grounds and at all functions associated with the conference.

Special requirements
Registrants with special requirements for physical communication and dietary requirements should contact Adriatic Luxury Services, official congress service agency in advance.

Information
Conference staff will be pleased to help you with any conference and travel information you may need.

Taxi
Numerous taxi stands are located throughout Dubrovnik city centre and in front of the hotels. Please address questions regarding taxi transportation to the concierge at your hotel.

Hotel Information
Dubrovnik Palace Hotel resides on the beautiful Lapad peninsula, a short drive from the historic city walls of Dubrovnik. Surrounded by lush green pine trees, the Hotel enjoys a spectacular coastal position overlooking the sea. This five star luxury hotel is isolated from the urban turbulence and is ideally located to meet guests that seek privacy and relaxation. Facilities in standard and superior rooms include: en-suite bathroom with guest bathrobes and slippers, balcony with sea view, flat screen satellite TV with internet, air-conditioning, mini bar, direct dial telephone, work desk and safe deposit box.

Services: indoor swimming pool, 2 indoor jacuzzi pools, fitness club, relaxation terrace, beauty treatment rooms.

Access to the walls of the Old City of Dubrovnik from the hotel is a matter of a few minutes by car, bus or taxi. In the summer days you can take the boat directly from the hotel's own quay to take you to the old port.

Distances:
-from Dubrovnik int. airport: 25 km (cca 25 minutes)
-from Old Town 4,5 km (few minutes by car, bus, taxi or boat)
-from the sea: few meters
-from the beach: has its own beach
-from the bus station: 4 km
-from the harbour: 4 km

Transportation to/from Hotel-Airport
-Time by taxi: approx. 25-30 minutes
-Cost by taxi: approx. 250,00- 280,00 kn (one way)
-Cost by hotel limousine: approx. 270,00 kn (one way)
-Time by bus: approx. 45 minutes
-Cost by bus: approx. 25,00 kn

For additional information about hotel please check: http://www.dubrovnikpalace.hr

Congress tour

Wednesday, September 7, 2005

A guided boat trip to Elaphite Islands
Price per person: EUR 40 (Transfer, guide, local drink aboard, lunch and entrance fees)
Menu: fish, meet, vegetables, vine and juice.
Duration: half day

The Elaphite Islands can be seen from Dubrovnik. They are the uninhabitated Daksa, Sv. Andrija, Ruda, Jakljan, Tajan and Olipa and the inhabitated Koločep, Lopud and Šipan. A welcome drink will be served en route to Koločep where we will make a short sightseeing stop. Buffet lunch will be served while sailing to Lopud where we will visit the 15th-century church of St. Mary and enjoy some leisure time before returning to Dubrovnik in preparation for the evening academic program and conference dinner.

SPEAKERS

Advanced Forensic Genetics in Fighting Crime and Biodefense

- **Antonio Alonso** *(National Institute of Toxicology and Forensic Sciences, Madrid. Spain)*
- **Christopher Asplen** *(Smith Alling Lane, LLC, Washington, DC, USA)*
- ***Frederick Bieber** *(Harvard Medical School, Boston, Massachusetts, USA)*
- ***Zoran Budimlija** *(Office of Chief Medical Examiner, New York, New York, USA)*
- ***Theresa Caragine** *(Office of Chief Medical Examiner, New York, New York, USA)*
- **Heather Miller Coyle** *(Connecticut State Police, Meriden, Connecticut, USA)*
- ***Cecelia Crouse** *(Palm Beach Sheriff's Office, Palm Beach, Florida, USA)*
- ***Dora Gransden** *(Promega, Madison, Wisconsin, USA)*
- **Joy Halverson** *(QuestGen Forensics, Davis, California, USA)*
- **Jürgen Henke** *(Institut für Blutgruppenforschung, Cologne, Germany)*
- **Mitchell Holland** *(Forensic DNA Consultants, Manassas, Virginia, USA)*
- **Edwin Huffine** *(Bode Technology Group, Springfield, Virginia, USA)*
- **Susan Jones** *(US Armed Forces Institute of Pathology, Washington, DC, USA)*
- **Demris Lee** *(Armed Forces DNA Identification Laboratory, Rockville, Maryland, USA)*
- ***Henry Lee** *(University of New Haven, West Haven, Connecticut, USA)*
- ***José Lorente** *(University of Granada, Granada, Spain)*
- ***Stephen Morse** *(Center for Disease Control and Prevention, Atlanta, Georgia, USA)*
- **Garth Nicolson** *(Institute for Molecular Medicine, Huntington Beach, California, USA)*
- ***Nicola Oldroyd** *(Applied Biosystems, Warrington, United Kingdom)*
- ***Vladimir Parpura** *(University of California, Riverside, California, USA)*
- **Thomas Parsons** *(Armed Forces DNA Identification Laboratory, Rockville, Mary land, USA)*
- ***Gregory Poland** *(Mayo Clinic College of Medicine, Rochester, Minnesota, USA)*
- **Dragan Primorac** *(University of Split, Split, Croatia and University of Osijek, Osijek, Croatia)*
- **Ronald Reinstein** *(Supervising Criminal Court, Maricopa County, Arizona, USA)*
- **Moses Schanfield** *(George Washington University, Washington, DC, USA)*
- **Debbie Smith** *(Williamsburg, Virginia, USA)*
- **Paula Hoffman Wulff** *(American Prosecutors' Research Institute, Alexandria, Virginia, USA)*

- ***Joint session speaker**

Advances in Molecular and Cellular Medicine

- **Charles Auffray** *(CNRS, Villejuif, France)*
- **Allan Dietz** *(Mayo Clinic College of Medicine, Rochester, Minnesota, USA)*
- **Henry Erlich** *(Roche Molecular Systems, Alameda, California, USA)*
- **Michael Frank** *(Duke University, Durham, North Carolina, USA)*
- **Eli Gilboa** *(Duke University, Durham, North Carolina, USA)*
- ***Michael Hammer** *(University of Arizona, Tucson, Arizona, USA)*
- **David James** *(Mayo Clinic College of Medicine, Rochester, Minnesota, USA)*
- ***Robert Huber** *(Nobel Laureate 1988, Max Planck Institute for Biochemistry, Martinsried, Germany, USA)*
- **Doron Lancet** *(Weizmann Institute of Science, Rehovot, Israel)*
- **Hans Lehrach** *(Max Planck Institute for Molecular Genetics, Berlin, Germany)*
- ***Alemka Markotić** *(Institute of Immunology, Zagreb, Croatia)*
- **Brenda Ogle** *(Mayo Clinic College of Medicine, Rochester, Minnesota, USA)*
- **Jeffrey Platt** *(Mayo Clinic College of Medicine, Rochester, Minnesota, USA)*
- **Yair Reisner** *(Weizmann Institute of Science, Rehovot, Israel)*
- ***Pavao Rudan** *(University of Zagreb, Zagreb, Croatia)*
- **David I. Smith** *(Mayo Clinic College of Medicine, Rochester, Minnesota, USA)*
- ***Davor Solter** *(Max Planck Institute for Developmental Biology, Freiburg, Ger many)*
- **Igor Štagljar** *(University of Toronto, Toronto, Ontario, Canada)*
- ***Mark Stoneking** *(Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany)*
- **Raimo Tanzi** *(Applied Biosystems, Monza, Italy)*
- **Andre Terzic** *(Mayo Clinic College of Medicine, Rochester, Minnesota, USA)*
- **Stanimir Vuk-Pavlović** *(Mayo Clinic College of Medicine, Rochester, Minnesota, USA)*

***Joint session speaker**

THE 4TH EUROPEAN-AMERICAN SCHOOL
IN FORENSIC GENETICS AND
MAYO CLINIC COURSE IN ADVANCED
MOLECULAR AND CELLULAR MEDICINE

Dubrovnik Palace Hotel
Dubrovnik
Croatia
September 5-9, 2005

SCIENTIFIC PROGRAM

The 4th European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine

(Designations F and M pertain to the concurrent forensic and molecular medicine programs, respectively)

Sunday, September 4, 2005

Arrival and Registration at (Top Floor, Dubrovnik Palace Hotel)

Monday, September 5, 2005

Location: Mare Conference Room, Top Floor

Introductory Plenary Session (D. Primorac, M. Schanfield, S. Vuk-Pavlović, Conveners)

9:00 Inauguration of the Fourth European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine

9:30 DNA and modern human origins
Michael Hammer, University of Arizona, Tucson, Arizona, USA

10:15 DNA evidence in modern criminal investigation
Henry Lee, University of New Haven, West Haven, Connecticut, USA

11:00 Coffee break

11:15 Keynote Address
- **Introduction of the speaker**
  Molecular machines for protein degradation
  Robert Hube, Nobel Laureate 1988; Max Planck Institute for Biochemistry, Martinsried, German

Location: Mare Section I, Top Floor

12:00 Opening of sponsor exhibits

Location: Mare Conference Room, Top Floor

Applied Anthropolgy Plenary Session (D. Primorac, M. Schanfield, S. Vuk-Pavlović, Conveners)

15:00 Introduction from the chair

15:05 Humans – the next genetically modified organisms?
Davor Solter, Max Planck Institute for Developmental Biology, Freiburg, Germany

15:50 Reading signatures of population histories – From language to DNA
Pavao Rudan, University of Zagreb, Zagreb, Croatia

16:35 Coffee break

16:50 Enhancement methods for locating biological evidence at crime scenes
Aiding crime scene reconstruction through DNA analysis
Collection and preservation of DNA evidence in complex crime scenes
DNA evidence in high–profile case investigation: The cases of Kobe Bryant, John F. Kennedy, Vincent Foster and Bill Clinton
Henry Lee, University of New Haven, West Haven, Connecticut, USA

18:00 Adjourn

Location: Mare Conference Room, Top Floor

19:00 Meeting of the International Society for Applied Biological Sciences (ISABS) with elec tion of officers

20:30 Welcome Reception at Revelin Fortress, Dubrovnik Old City (please check for time of bus departure from your hotel)

Tuesday, September 6, 2005

Location: Mare Sections I and II

7:30 (F and M) Poster set-up and exhibition

Location: Mare Conference Room, Top Floor

(F) Interpreting and Reporting Forensic Evidence (M. Holland, Convener)

8:30 Introduction from the chair

8:35 High throughput approaches to casework analysis: Laboratory prac tices and procedures
Mitchell Holland, Forensic DNA Consultants, Manassas, Virginia, USA

9:15 Application of DNA analysis to a worldwide mission of identifying hu man remains
Edwin Huffine, The Bode Technology Group, Springfield, Virginia, USA)

9:55 Coffee break

10:15 Erroneous refutation of the Romanov DNA testing results
Thomas Parsons, Armed Forces DNA Identification Laboratory, Rockville, Maryland, USA

10:55 AFDIL's challenges and responses to Operation Iraqi Freedom and beyond
Demris Lee, Armed Forces DNA Identification Laboratory, Rockville, Mary land, USA

11:35 Interpretation of complex STR mixtures
Mitchell Holland

12:00 Discussion

Location: Dubrava Conference Room, Reception Floor

(M) Molecular Genetics in Identification, Characterization and Diagnosis of Disease (D. Smith, Convener)

8:30 Introduction from the chair

8:35 Positional cloning for identifying disease-related genes
David I. Smith, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

9:15 HLA population genetics and susceptibility to disease
Henry Erlich, Roche Molecular Systems, Alameda, California, USA

10:00 Coffee break

10:20 Genetics based therapy targeting: the example of glioblastoma
C. David James, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

11:05 Transcriptional profiling: a powerful tool to study disease
David I. Smith

11:50 Discussion

Location: Mare Conference Room, Top Floor

(F) Forensic Evidence in the Court of Law (Ch. Asplen, Convener)

15:00 Introduction from the chair

15:05 DNA: Chains to freedom
Debbie Smith, Williamsburg, Virginia, USA

15:45 Prosecutorial challenges in DNA application
Paula Hoffman Wulff, American Prosecutors Research Institute, Alexan dria, Virginia, USA

16:25 Cofee Break

16:40 Judicial perspective on courtroom applications of DNA
Judge Ronald Reinstein, Supervising Criminal Court, Maricopa County, Phoenix, Arizona, USA

17:20 Perspectives on DNA in the appellate court
Christopher Asplen, Smith Alling Lane, LLC, London, England

17:50 Presentation of the Training CD–ROM and discussion

Location: Dubrava Conference Room, Reception Floor

(M) Advances in Genome Methods (D. Lancet, Convener)

15:00 Introduction from the chair

15:05 Transcriptomics and systems biology: Thirty years and 39 steps in expression profiling with microarrays
Charles Auffray, CNRS/Université Pierre et Marie Curie–Paris 6, Villejuif, France

15:40 Removing bottlenecks in gene expression analysis from genome to single gene
Raimo Tanzi, Applied Biosystems, Monza, Italy

16:25 Interactive proteomics of membrane proteins: the first five years and beyond
Igor Štagljar, University of Toronto, Toronto, Ontario, Canada

17:00 Coffee break

17:20 SNPs, genetic variability and pharmacogenetics
Doron Lancet, Weizmann Institute of Science, Rehovot, Israel

17:55 From functional genomics to systems biology
Hans Lehrach, Max Planck Institute for Molecular Genetics, Berlin, Ger many

18:30 Discussion

Location: Mare Sections I and II

19:00 (F and M) Poster presentation and discussion with wine and cheese reception

21:00 Dubrovnik Palace Hotel, poolside program

Wednesday. September 7, 2005

8:30 Half day boat trip to Elaphite Islands for participants and accompany ing persons (please check for time of bus departure from your hotel)

Location: Mare Conference Room, Top Floor

Special evening addresses and conference dinner

18:00 Social mixer

18:30 Human origins through molecular anthropology
Mark Stoneking, Max Planck Institute for Evolutionary Anthropology, Leip zig, Germany

19:00 Dinner, first course

19:30 Turning the bones of Christopher Columbus: A DNA analysis of the remains of the discoverer of the New World
José Lorente, University of Granada, Granada, Spain

20:00 Dinner, main course followed by desert and entertainment

Thursday, September 8, 2005

Location: Mare Conference Room, Top Floor

Methods Plenary Session: New Technologies in Medical and Forensic Genetics (F. Bieber, Convener)

8:30 Introduction from the chair

8:35 Microarray technology
Frederick Bieber, Harvard Medical School. Boston, Massachusetts, USA

9:10 Laser capture microscopy
Zoran Budimlija, Office of Chief Medical Examiner, New York, New York, USA

9:45 Microfabricated capillary array electrophoresis
Cecelia Crouse, Palm Beach Sheriff's Office, Palm Beach, Florida, USA

10:20 Coffee break

10:35 Low-copy STR typing
Theresa Caragine, Office of Chief Medical Examiner, New York, New York, USA

11:10 Evolution of technology for human identification analysis
Nicola Oldroyd, Applied Biosystems, Warrington, United Kingdom

11:45 Development of human and human male DNA quantitation systems using a novel fluorescent two-primer real-time PCR method
Dora Gransden, Promega, Madison, Wisconsin, USA

12:00 Adjourn

Location: Levanat Room, Ruža vjetrova Conference Center, Third Floor

12:00 Workshop: New Technologies in Clinical and Forensic Genetics (F. Bieber, Director)
Including presentation: ChargeSwitch® technology—a novel highly sensi tive DNA purification technol ogy, optimized for forensic applications
Richard Watts, Invitrogen, Paisley, United Kingdom

Location: Mare Conference Room, Top Floor

(F) Forensic Evidence from Non-Human DNA (M. Schanfield, Convener)

15:00 Introduction from the chair

15:05 Identification of canine and feline DNA in forensic investigations
Joy Halverson, QuestGen Forensics, Davis, California, USA

15:45 Botanical DNA evidence in criminal and civil cases
Heather Miller Coyle, Connecticut State Police, Meriden, Connecticut, USA

16:30 Coffee break

16:50 DNA assays for microorganism detection, identification and Individu alization
Susan Jones, US Armed Forces Institute of Pathology, Washington, DC, USA

17:30 Future of forensic testing of non-human DNA
Moses Schanfield, George Washington University, Washington, DC, USA

18:10 Discussion

Location: Dubrava Conference Room, Reception Floor

(M) Novel Immunotherapy (J. Platt, Convener)

15:00 Introduction from the chair

15:05 Toll like receptors and balance of resistance and injury
Jeffrey Platt, Mayo Clinic College of Medicine, Rochester, Minnesota

15:35 Rebuilding the T cell compartment
Brenda Ogle, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

16:05 A multi-pronged approach to cancer immunotherapy
Eli Gilboa, Duke University, Durham, North Carolina, USA

16:40 Coffee break

17:00 Complement as a determinant of host defense
Michael M. Frank, Duke University, Durham, North Carolina, USA

17:30 Immune tolerance–pros and cons
Jeffrey Platt, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

18:00 Boosting immunity: A round table discussion

Location: Mare Sections I and II, Top Floor

18:00 (F and M) Poster presentation and discussion with wine and cheese reception

Friday, September 9, 2005

Location: Mare Conference Room, Top Floor

(F) DNA in Homeland Security and Armed Forces (D. Primorac, Convener)

8:30 Introduction from the chair

8:35 DNA analysis in identification of war victims
Dragan Primorac, University of Split, Split, Croatia and University of Osijek, Osijek, Croatia

9:10 State-of-the-art public health tools for forensic microbiology
Stephen Morse, Center for Disease Control and Prevention, Atlanta, Georgia, USA

9:50 Identification of chronic infections in Gulf War veterans and their im mediate family by PCR
Garth Nicolson, Institute for Molecular Medicine, Huntington Beach, California, USA

10:30 Coffee break

10:50 Which STR polymorphisms are required for identification? Lessons from complicated kinship cases
Jürgen Henke, Institut für Blutgruppenforschung, Cologne, Germany

11:30 Challenges of DNA profiling in mass disaster investigations
Antonio Alonso, National Institute of Toxicology and Forensic Sciences, Madrid. Spain

12:10 Discussion

Location: Dubrava Conference Room, Reception Floor

(M) Advances in Graft Engineering and Cell Therapy (S. Vuk-Pavlović, Convener)

8:30 Introduction from the chair

8:35 Methods and problems in advanced cellular graft engineering
Stanimir Vuk-Pavlović, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

9:00 Critical concepts in haploidentical bone marrow transplantation
Yair Reisner, Weizmann Institute of Science, Rehovot, Israel

9:40 Development of a cellular therapeutic product; the example of my eloid dendritic cells
Allan Dietz, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

10:20 Coffee Break

10:40 Embryonic stem cells in cardiogenesis and tissue repair
Andre Terzic, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

11:20 Porcine embryonic tissue precursors for organ transplantation
Yair Reisner

12:00 Discussion

Location: Mare Conference Room, Top Floor

Concluding Plenary Session
13:30 Young Investigator Award Ceremony and Oral Presentations

14:30 Biological Weapons and Biodefense (G. Poland, Convener)
14:30 Introduction from the chair

14:35 Bioterrorism–history and current reality
Gregory Poland, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

15:15 Designing micromechano-sensors for detection of biological warfare agents: the example of type B Botulinum toxin
Vladimir Parpura, University of California, Riverside, California, USA

15:45 Innate immunity to biothreat agents as a basis for therapy development
Alemka Markotić, Institute of Immunology, Zagreb, Croatia

16:15 Coffee break

16:30 Laboratory Response Network: Critical component of preparedness for bioterrorism
Stephen Morse, Center for Disease Control and Prevention, Atlanta, Georgia, USA

17:00 Discussion

Closing of the conference

ABSTRACTS OF PLENARY LECTURES

CHALLENGES OF DNA PROFILING IN MASS DISASTER INVESTIGATIONS

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In mass disaster there is often a need for managing, analyzing, and comparing large numbers of biological samples and DNA profiles. This requires the use of laboratory information management systems for large-scale sample logging and tracking coupled with bioinformatics tools for DNA database searching according to different matching algorithms, and for the evaluation of the significance of each match by likelihood ratio calculations. There are many different interrelated factors and circumstances involved in each specific mass disaster scenario that may challenge the final DNA identification goal, such as: the number of victims, the mechanisms of body destruction, the extent of body fragmentation, the rate of DNA degradation, body accessibility for sample collection, or the type of DNA reference samples available. Here we examine the different steps in DNA identification (DNA sampling, DNA analysis and technology, DNA database searching, and concordance and kinship analysis) reviewing the “lessons learned” and the scientific progress made in some mass disaster cases described in the scientific literature. Especially we emphasise the valuable scientific feedback that genetic forensic community has received from the collaborative efforts of several public and private USA forensic laboratories in assisting with the more critical areas of the World Trade Center (WTC) mass fatality of September 11, 2001. The main challenges in identifying the victims of the recent South Asian Tsunami disaster, which has produced the steepest death count rise in history, will also be considered. We also present data from two recent mass fatality cases that involved Spanish victims: the Madrid terrorist attack of March 11, 2004, and the Yakolev-42 aircraft accident in Trabzon, Turkey, of May 26, 2003.

PERSPECTIVES ON DNA IN APPELLATE COURT

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As the legal admissibility of DNA becomes settled in most countries, the issues that arise in the appellate process regarding DNA technology are fewer in number. The establishment of the scientific validity and legal admissibility of DNA has left defense attorneys with fewer claims. Even as newer technologies are applied to the investigative process, the legal challenges fail to be as vigorous as when DNA was first introduced as a basic crime fighting technology. However, post-conviction DNA testing of evidence in cases when convictions had already been established is one area in which new jurisprudential challenges lay. This presentation will address concerns that come up in the context of this relatively new appellate application of DNA. Included will be examples of cases in the United States, United Kingdom and the Philippines.

TRANSCRIPTOMICS AND SYSTEMS BIOLOGY: 30 YEARS AND 39 STEPS IN EXPRESSION PROFILING WITH MICROARRAYS

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Expression profiling with arrays has a long history, since the introduction of cDNA cloning and colony-grid hybridization in 1975. The technology has developed steadily during the next two decades until novel chemistry, robotics and computation tools stimulated its explosive growth in the context of the Human Genome Project. Microarrays are now widely used to monitor transcriptomes in a variety of organisms, physiological and pathological conditions, generating a large flow of data, which find their way to public data repositories. Yet, microarray technology is still in a relatively immature stage compared to sequencing, and important limitations remain in the production of the accurate and extensive data required for integrative systems biology approaches to the complexity of living systems. To realize the full potential of microarray technology, and provide meaningful insights into the behavior of the biological systems investigated, it is essential to develop and implement standards at each and all steps of the process. These include careful study design, controlled annotation of resources and extensive quality control of experiments with associated quality metrics, the use of robust statistics including power analysis for hypothesis testing, and data description, registration and storage in public repositories using ontologies and controlled vocabularies. We will review some of the 39 key steps that we found essential to establish a generic quality assurance pipeline for gene expression profiling, using cancer expression profiling as a case study. We will thus highlight the potential of microarrays to uncover molecular signatures of diagnostic value, to provide a rich source of working hypotheses on underlying functional and regulatory networks, and to help identify targets for therapeutic intervention.

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DNA MICROARRAYS IN HUMAN GENETICS: APPLICATIONS TO GENOMICS, DIAGNOSTICS AND FORENSIC MEDICINE

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DNA microarray and related technologies are useful in research, laboratory diagnostics and for study of heritable genomic variation. DNA microarrays offer novel possibilities for gene expression studies across the genome through monitoring the gene expression of hundreds of genes simultaneously. When combined with other biological and clinical information, microarray data also provide chances to identify genes associated with disease. This presentation will provide an overview of array and related technologies, including expression arrays, cGH microarrays as well as alternatives such as multiplex ligation PCR amplification (MLPA). Issues of reliability and informatics will be discussed, as will applications such as SNP genotyping, bioinformatics, toxicological research and drug discovery and applications to forensics.

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FORENSIC APPLICATIONS OF LASER CAPTURE MICRODISSECTION: USE IN DNA-BASED PARENTAGE TESTING AND PLATFORM VALIDATION

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Laser capture microdissection (LCM) refers to the integration of existing light microscopic instrumentation and newer technology utilizing pulsed laser beams. Pulsed laser allows targeting of specific regions of tissues to be separated and placed into snap-cap tubes for DNA extraction and analysis. Thus, LCM can be used to separate specific cell types within whole organs or tissue sections without altering sample chemistry or morphology. Separations of cells and tissues are necessary in a wide array of research investigations and LCM has rapidly been incorporated into the daily work of research pathologists. In contrast to the relatively crude conventional microdissection performed using a narrow pipette, needle, or scalpel blade, LCM allows cell- or tissue-specific isolation of placental chorionic villi from archival paraffin-embedded tissue sections, leaving the maternal tissue intact. We have ongoing expertise in use of LCM as a tool for isolation of human chorionic villi from admixed maternal tissue. Recovery of POC material after alleged sexual assaults was followed by formalin fixation of POC tissue, followed by embedding in paraffin. Multiple 5 µm unstained tissue sections are mounted on glass slides. To specifically extract the fetal chorionic villi from the maternal decidual tissue, the PixCell II (Arcturus, Carlsbad, CA, USA) LCM system was applied according to the manufacturer's specifications. In brief, the tissue sample mounted on a glass slide was first studied under a light microscope. Once the cells of interest (i.e., placental chorionic villi) were identified, a LCM cap is placed over the target area and focused infrared diode laser beam was activated. Pulsing of the laser (90 mW of pulse power, 15 µm laser spot size) through the cap has caused the thermoplastic film to form a thin protrusion that bridged the gap between the cap and the placental tissue and adhered to the cells. Now attached to the cap, the targeted chorionic villi were simply removed from the rest of the placental tissue by lifting of the cap. After tissue separation (maternal decidua from fetal placental villi) in cases of alleged sexual assault of female victims, DNA typing results were consistent with paternity of the named assailant. Polymerase chain reaction (PCR) amplification of villi after LCM of 9-15 STR loci was accomplished; as the quantity and quality of DNA yielded from fetal cells isolated by LCM was sufficient. We also report on beginning validation of the LCM instrument platform, using archival formalin-fixed human fetal products of conception (POC). We demonstrated the reliability and utility of LCM for forensic applications when high specificity of a particular analyzed cell population or tissue is required. Care must be taken during routine pathology procedures to avoid admixture of tissues (and DNA) with unwanted extraneous biological tissues. The use of LCM in forensics is not yet widely appreciated and promises to answer an unmet need for a technology for efficient separation of cells or tissues in forensic mixtures.

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DEVELOPMENT OF HUMAN AND HUMAN MALE DNA QUANTITATION SYSTEMS USING A NOVEL, FLUORESCENT, TWO–PRIMER REAL–TIME PCR METHOD

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Heavily multiplexed short tandem repeat (STR) analysis has become the dominant technology in DNA-based human identification. Although highly informative, these assays require a defined range of template quantity to produce optimal results. Additionally, resources can be conserved with accurate assessment of DNA quality and assessment of minimum quantity. Currently, many practitioners observe either high levels of false negative results (due to lack of sensitivity) or subjective conclusions (due to visual comparison of band intensities) based on common hybridization-based methodologies. Amplification-based methods for quantitation provide a high level of sensitivity while real-time methods can deliver a dynamic range that often exceeds end point assays. A numerical output also increases the objectivity of the data interpretation. A real-time PCR method has been developed for the quantitation of total human and human male DNA in purified samples using the specificity of interaction between two modified nucleotides to achieve quantitative PCR analysis. One of the PCR primers includes a modified nucleotide (iso-dC) adjacent to a fluorescent label on the 5´ end. The second PCR primer is unlabeled. The reaction mix includes deoxynucleotides and iso-dGTP, which has been modified to include dabcyl quencher. The only nucleotide incorporated at the position complimentary to iso-dCTP is dabcyl iso-dGTP. The incorporation of the dabcyl iso-dGTP adjacent to the fluorescent dye results in a reduction in signal that allows quantitation during amplification. Associated analysis software has been developed to visualize amplification data from various instrument platforms, plot standard curves and calculate DNA concentrations of unknowns. Relative to other real-time approaches, this methodology provides specificity through the use of fluorescently labeled primers compared to DNA binding dyes and simplicity compared to probe-based quantitative PCR approaches. Data will be presented demonstrating the performance of assays using human autosomal (total human) and human Y-chromosome (male human) targets for quantitation.

A HIGH SENSITIVITY DNA TESTING METHODOLOGY: PROBLEMS AND PROPOSED SOLUTIONS

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DNA recovered from fingerprints and touched objects can produce database eligible profiles. In order to maximize DNA recovery and profile generation from less than 100 picograms of DNA, extraction, quantitation, amplification, and capillary electrophoresis protocols were optimized to minimize DNA loss and maximize sensitivity. Moreover, guidelines regarding the interpretation of the resultant data were modified to accommodate the inherent variability with profiles from low copy number (LCN) DNA profiles. Significant adjustments to these parameters will be presented and the potential problems and proposed solutions discussed. Simple extraction procedures with fewer steps recovered more DNA than protocols with many manipulations. From our extraction studies, we recommend a thirty-minute digestion with 0.01% SDS and proteinase K at 56 ˚C, followed by incubation at 100 ˚C for 10 minutes, and sample concentration as well as purification with a Microcon® 100 (Millipore) membrane pretreated with Poly A RNA to prevent sample loss. Enhancements to amplification with Identfiler™ (Applied Biosystems) reagents included additional cycles, an elongated annealing time, and a reduced reaction volume. Adding magnesium to the reaction proved beneficial in preliminary studies for degraded samples. Regarding electrophoresis, increasing the injection voltage and time, as well as the sample input, improved allelic detection. Alternatively, post PCR purification for samples below 20 picograms yielded additional alleles without these electrophoresis adjustments. Due to stochastic effects with LCN DNA samples, only alleles that occurred in two of three amplifications were assigned. Pooling the three amplifications products for electrophoresis mediated some of these effects. Nevertheless, specific rules were formulated for mixture deductions and assignment of homozygotes even for non-mixtures. Interpretation of amplification results in such a manner generated full profiles from 25 picograms of DNA and partial profiles from as little as 6.25 pg. Application of our methodologies to simulated low copy number evidentiary samples generated at least a 30% success rate. Therefore, implementation of these processes, due to the numerous potential sources of LCN DNA samples, will enhance the recovery of biological evidence from crime scenes and may be a significant source of database profiles.

FORENSIC BOTANY: USING PLANT EVIDENCE TO AID IN FORENSIC DEATH INVESTIGATION

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Forensic botany is still underutilized as a resource in forensic casework although it has been used on occasion. It is an area of specialty science that could include traditional botanical classification of species, DNA, or materials evidence (trace and transfer evidence), crime mapping or geo-sourcing, all dependent on the specific case application under consideration. Critical to the evaluation of plant evidence is careful collection, documentation and preservation for later scientific analysis. This presentation will review proper procedures for evidence collection and storage, options and the scientific rationale for scientific testing (microscopy, DNA, AFLP, etc.) and summarize some recent cases where botanical evidence played a role in establishing either manner or time of death. Plant evidence can be useful for determining if a death was due to an accident, suicide or homicide or what time of year burial may have taken place. In addition, plant evidence can be used to determine if a crime scene is primary or secondary, to locate missing bodies and to add to historical investigations. Many additional forensic botany cases are described in the references below.

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IMPROVING EFFICIENCY OF A SMALL FORENSIC DNA LABORATORY: VALIDATION OF ROBOTIC ASSAYS AND EVALUATION OF A MICROCAPILLARY ARRAY DEVICE

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The PBSO Serology/DNA Section has conducted DNA analysis for over twelve years and has experienced the pressures associated with increased casework load. Since 2000, there has been a precipitous gap between the number of cases submitted and the number analyzed (46%). In order to address the issues of a burdened DNA program, the section identified and eliminated ineffective, laborious and time-consuming tasks while preparing for future technologies. Improvement in efficiency over the past few years includes searching for more automated DNA technologies. Organic DNA extraction has been replaced with the Promega DNA IQ Extraction System using the magnetic bead method in conjunction with the BioMek2000 DNA robotic workstation. This has more than quadrupled the number of DNA casework samples that can be extracted in a week. Quantification of DNA samples has been semi-automated by implementation of a real-time quantification PCR method (qPCR). The PBSO validated the Applied Biosystems Sequence Detection System 7000 instrument and the Quantifiler-Human and Quantifiler-Y Quantitation Kits demonstrating a fast and time-saving method to routinely use qPCR to quantify casework evidentiary samples although the Y-Quantification system was not as reliable when compared to the human quantification system. The qPCR system dramatically reduced the number of negative DNA samples that used to be carried on to amplification thus saving time and money. Using PowerPlex 16™ single amplification system has reduced time necessary for the electrophoresis of amplified product and data interpretation. In addition, half the amount of template DNA is used in a single multiplex reaction thereby conserving evidence. The Serology/DNA section is also looking towards the future. The PBSO is currently engaged in a collaborative effort with the University of California at Berkeley and the Virginia Division of Forensic Science (VDFS) to increase DNA casework throughput by the evaluation of a microfluidic device for the rapid separation of STR fragments using a microcapillary array electrophoresis (uCAE) technology. In approximately 20 minutes, 96 samples may be electrophoresed. The advantage of using a µCAE instrument over current multicapillary STR detection platforms includes the ability to utilize small sample volumes thus conserving evidence, increased sensitivity and significantly greater throughput capabilities.

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DEVELOPMENT OF A CELLULAR THERAPEUTIC PRODUCT: THE EXAMPLE OF MYELOID DENDRITIC CELLS

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A long-standing goal of cancer treatment has been the induction of anti-tumor immunity. Critical to the induction of an anti-tumor immune response is the appropriate activation of naïve and memory T cells. The cells most potent in activating these and other immune effector cells are dendritic cells (DCs). Because it is difficult to control DC activation and delivery of tumor–associated epitopes in vivo, most pertinent clinical trials have used ex vivo cultured DCs. To date, over one thousand patients have been treated with ex vivo cultured autologous dendritic cells with rather few low–grade adverse events. However, the lack of standardization in clinical trial design, evaluation and execution has limited the progress in clinical studies of DC vaccines. Monocytes are the most common sources for ex vivo DC preparation providing ample supply of DCs that are a rather rare cell type. Advances in DC biology demonstrated that different DC activation states result in different biological responses and that only fully differentiated (mature) DC have the capacity to stimulate an anti-tumor response. Consequently, an optimal protocol for the generation of clinical DC would rely on monocytes differentiated into a uniform population of mature DC. An optimal protocol must also take into account the impact of regulations that restrict, or in some cases disallow, tissue culture components that are otherwise used in research–grade dendritic cell culture. Finally, DC preparation protocol must be simple, reliable, and meet the proposed standardization criteria set for DC vaccines. Here we review the field of DC vaccine protocols and describe our experience in developing a simple yet rigorous method for patient–specific manufacture of large numbers of uniformly mature DC.

HLA POPULATION GENETICS AND DISEASE SUSCEPTIBILITY

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The HLA class I and class II loci are the most polymorphic genes in the human genome, with some loci displaying more than 400 alleles. Specific HLA alleles have been associated with many different diseases, including autoimmune disorders (e.g., Type 1 diabetes), infectious diseases (e.g., Hepatitis C virus), and some cancers (e.g., cervical cancer). The strong linkage disequilibrium (LD) that characterizes the loci within the HLA region make it hard to identify the allele responsible for the observed disease association. For example, multiple alleles at multiple HLA loci are strongly associated with Type 1 diabetes, notably alleles at the DRB1 and DQB1 loci. Different human populations can differ significantly in allele frequency distribution and patterns of linkage disequilibrium. Population genetic studies of the global distribution of allelic diversity can reveal patterns of selection and identify phylogenetic relationships among human populations. Disease association studies in populations with differing patterns of LD can help identify specific disease–associated alleles and specific combinations of alleles. Analysis of HLA class I and class II allele and haplotype frequency data generated from a variety of populations reveals that selection is balanced. Phylogenetic analyses of these data allow the testing of specific anthropological hypotheses, e.g., colonization of the Pacific and the Americas. The patterns of HLA disease association with type 1 diabetes and multiple sclerosis in different populations will be discussed.

COMPLEMENT AS A DETERMINANT OF HOST DEFENSE

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The serum complement system, discovered over a century ago, was among the first elements of innate immunity to be identified. Complement, comprised of over thirty plasma and cell bound proteins, has three major functions. 1) The control of infection by direct lysis of susceptible organisms. 2) Opsonization, the binding of complement peptides to an organism that interact with specific receptors on phagocytes cells to promote phagocytosis. 3) The generation of inflammatory peptides, fragments of complement proteins that are formed during activation that mediate aspects of the inflammatory response. Complement is present in very primitive organisms. It functions by the sequential activation of a series of plasma proteins with the generation of specific enzymes on the proteins or protein complexes. The cell membrane bound components of the complement system usually are regulatory molecules designed to control the degree of complement activation and the extent of damage. Three pathways of complement activation are recognized. Two are phylogenetically quite primitive and do not require antibody. These include the lectin pathway, activated by the binding of various lectins such as mannan binding lectin to specific sugars on microorganisms, and the alternative pathway activated when spontaneous hydrolysis of C3 engages alternative pathway proteins. All come together at the later steps in the complement cascade. With the appearance of adaptive immunity we see the appearance the third complement pathway, the classical pathway, activated by antibody. Complement functions to augment the immune response by focusing antigens on the surface of antigen presenting cells via complement receptors and complement deficient animals have a variety of immune defects. Regulatory proteins exist to control complement activation. Partial deficiency of the C1 inhibitor is associated with hereditary angioedema. Polymorphisms of another complement control protein, factor H, are associated with the development of hereditary hemolytic uremic syndrome and macular degeneration in the elderly. The absence of CD59 on cells, an acquired defect in the bone marrow, leads to the development of paroxysmal nocturnal hemoglobinuria. Currently in development are agents that down regulate complement action but none are currently available for clinical use.

A MULTI PRONGED APPROACH TO IMMUNOLOGICAL CONTROL OF CANCER

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The challenge of cancer immunotherapy is to stimulate a powerful and sustained immune response in patients. To stimulate $CD8^+$ cytotoxic T cells (CTL), cancer patients are vaccinated with ex vivo generated autologous dendritic cells (DC) transfected with mRNA encoding tumor antigens. Preclinical studies have demonstrated the potency of this approach in murine and human models. A striking feature of the early clinical experience in patients with prostate and renal cancer, led by Dr. Johannes Vieweg and colleagues, was that the majority of the patients responded immunologically to the vaccine. A three-pronged approach is currently developed to stimulate potent $CD4^+$ T cell immunity: mRNA encoded products are redirected into the endosomal class II presentation pathway by appending appropriate targeting signal sequences, invariant chain expression is transiently inhibited using antisense oligonucleotides or siRNA to facilitate the loading of nascent MHC class II molecules with endogenously derived peptides, and DC are co-transfected with mRNA encoding OX40 ligand to enhance the persistence of the activated T cells. Countering negative costimulatory signals and immune suppressive circuits that function to attenuate tumor immunity is used to enhance the persistence of the vaccine–induced immune response. Aptamer-based inhibitors are used to block the function of the negative costimulatory molecules CTLA-4, PD-1 and B7H1. Aptamers, a new platform technology for drug discovery, offer important advantages over protein-based biologicals for human therapy. The well documented genetic instability of tumor cells is responsible for the propensity of tumor cells to evade the immune system and could limit, if not defeat, much of the promise of this treatment modality. Since stromal cells are diploid, genetically stable and show limited proliferative capacity, targeting the stroma, namely immunizing against products which are preferentially, not necessarily exclusively, expressed by stromal cells, could substantially reduce the incidence of immune evasion. We have shown that immunization of mice against angiogenesis-associated products such as VEGR-2, Tie-2 or VEGF, inhibits tumor growth in the absence of significant autoimmune pathology. Additional stromal targets are currently evaluated in murine tumor models and human preclinical systems to set the stage for clinical trials in cancer patients

FORENSIC DNA IDENTIFICATION OF ANIMAL–DERIVED TRACE EVIDENCE: THE TOOLS LINKING VICTIMS AND SUSPECTS

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Animal hairs from household pets are common findings at crime scenes. DNA identifications of individual animals from hairs and other sources have linked victims and suspects in criminal investigations in the United States, Canada, and Great Britain. Evidence from such investigations has been admitted in trial proceedings in all three countries. DNA profiles generated from the amplification of canine microsatellite markers, coupled with databases developed for pedigree verification by purebred dog registries, provide investigators with match probabilities and/or likelihood ratios similar to those from human microsatellite analysis.[1,2] These databases have been scrutinized for population substructure (expected in non-randomly breeding populations) and a population substructure value of $\theta = 0.11$ has been recommended for calculating the significance of DNA matches. In addition to microsatellites, feline and canine mitochondrial typing methods have been developed for those samples, such as the majority of shed hairs, with insufficient nuclear DNA. Databases for these species indicate that there is less population diversity then has been recognized in human mitochondrial typing.[3] As in humans, mitochondrial typing cannot distinguish maternal relatives. Likelihood ratios from canine mitochondrial matches in cases to date ranged from 9 to 100, but rare types are seen in approximately 25 percent of dogs so that higher likelihood ratios can be expected in a similar proportion of casework. Despite these limitations, mitochondrial DNA matches of animal hairs collected from crime scenes have contributed to a number of criminal investigations and have been admitted in four trials in the USA.

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DNA AND MODERN HUMAN ORIGINS

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Fossil evidence links human ancestry with populations that evolved modern gracile morphology in Africa some 130,000 to 160,000 years ago. Yet fossils alone do not provide clear answers to the question of whether the ancestors of all modern Homo sapiens comprised a single African population or an amalgamation of distinct archaic populations. While mtDNA data support a recent origin of anatomically modern humans (AMH) from a single, small African population (Recent African Replacement model), nuclear loci present a more complicated picture. Some loci are consistent with a recent African replacement, while others suggest a more complex history of larger effective population size and/or ancient subdivision. Two major problems associated with interpreting the heterogeneous patterns of variation observed at multiple nuclear loci are that sampling procedures vary across studies, and experimental designs generally lack statistical power to detect archaic admixture. We are undertaking a systematic survey of DNA sequence variation at 90 unlinked nuclear loci and developing a statistical framework to explicitly test the hypothesis of no admixture between modern and archaic forms, and for estimating the admixture ratio (if the null hypothesis is rejected). Preliminary data from two loci that show evidence of ancient admixture will be discussed. A gene tree constructed from sequence data at a pseudogene locus on the X chromosome roots in East Asia and has a most recent common ancestor some two million years ago. The pattern of nucleotide variation at an independent non-coding locus on the X chromosome locus reveals two major lineages that have not undergone recombination for over one million years, and statistically rejects the null hypothesis of panmixia during the early ancestry of anatomically modern humans. Genetic patterns at this locus support an “African multiregional” model of the origin of AMH, with gene flow between modern and/or archaic forms in Africa.

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WHICH SHORT TANDEM REPEAT POLYMORPHISMS ARE REQUIRED FOR IDENTIFICATION? LESSONS FROM COMPLICATED KINSHIP CASES

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We discuss five examples of complicated deficient parentage cases, which could sufficiently be resolved by extensive DNA typing using short tandem repeat (STR) and restriction fragment length polymorphisms (RFLPs). The latter have greatly contributed to the solution of deficiency cases, although their application is only feasible, if high molecular weight DNA and time are in abundance. This apart, RFLP technique is available in a few laboratories only, and its extinction can be expected in medium term. This development will pose a problem unless more highly polymorphic short tandem repeat (STR) systems are at the service of forensic genetic laboratories. The required “new”, additional STR polymorphisms must be able to fully replace RFLPs in terms of their respective information content. STR loci of this quality are e.g. ACTBP2 (SE33), D5S2360 and gonosomal loci. Moreover, the newly introduced STR kit “Human Type Chimera” is considered valuable from this point of view.

MOLECULAR MACHINES FOR PROTEIN DEGRADATION

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Within cells or subcellular compartments misfolded and/or short-lived regulatory proteins are degraded by protease machines, cage-forming multi-subunit assemblages. Their proteolytic active sites are sequestered within the particles and located on the inner walls. Access of protein substrates is regulated by protein subcomplexes or protein domains, which may assist in substrate unfolding dependent of ATP. Five protease machines will be described displaying different subunit structures, oligomeric states, enzymatic mechanisms, and regulatory properties.

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APPLICATION OF DNA ANALYSIS TO A WORLDWIDE MISSION OF IDENTIFYING HUMAN REMAINS

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Obtaining DNA profiles from skeletal remains is among the most challenging types of DNA testing. DNA laboratories inexperienced in such testing may encounter significant difficulties when developing their methodologies. While low level DNA testing on skeletal remains began more than a decade ago, the development of large–scale DNA assisted identification systems for the World Trade Center and the former Yugoslavia earlier this decade greatly extend the capabilities of such testing. DNA testing systems will likely be created in various nations around the world to assist in the identification of the missing. However, DNA testing is multidimensional and systems created for the humanitarian identification of the missing can also serve to support civil society and the rule of law. To fully realize this multidimensional potential of DNA testing and to reduce the chances of errors, poor quality work, or external influences, it is important that DNA laboratories adhere to internationally recognized standards of excellence and undergo independent external reviews. This measure of accountability will instill a confidence in the integrity and quality of results from the contributing laboratory; therefore, each DNA laboratory involved in the identification of the missing should become accredited by an internationally recognized accrediting body.

GENETICS BASED THERAPY TARGETING: THE EXAMPLE OF HUMAN GLIOBLASTOMA

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In spite of the frequent occurrence of epidermal growth factor receptor (EGFR) gene amplification in glioblastoma (GBM), the most common and most malignant CNS tumor, there are no EGFR-amplified GBM cell lines for use in testing EGFR–targeted therapeutics in vivo. Over the past five years, my laboratory has developed 23 GBM xenografts maintained through serial passaging in the flanks of nude mice. These xenograft lines established renewable GBM resources for studying EGFR amplification-associated tumor biology and allowed studying the effect of EGFR amplification on GBM response to EGFR–targeted therapeutics. This interest is spurred by the fact that GBM is the only human cancer with frequent EGFR amplification and by the association between EGFR amplification status and EGFR expression (i.e., tumors with amplified EGFR gene express extremely high levels of EGFR protein). Because of these associations, GBM should provide an ideal model for assessing the importance of EGFR amplification status to tumor sensitivity to EGFR-directed therapies. One can predict that patients with amplified EGFR in GBM would be excellent candidates for EGFR-targeted therapies, similarly to patients with HER2-NEU amplified in breast carcinomas who respond to erb-B2–directed antibody therapy. Nine of our 23 GBM cell lines (39 percent) display amplified EGFR, an amplification frequency in keeping with observations in patient populations. The presentation will focus on the use of this xenograft panel in assessing GBM response to therapy as a function of tumor EGFR amplification status.

DNA ASSAYS FOR THE DETECTION, IDENTIFICATION AND INDIVIDUALIZATION OF SELECT AGENT MICROORGANISMS

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We will review DNA assays used for the detection, identification and individualization of Bacillus anthracis, Yersinia pestis, Francisella tularensis, Burkholderia mallei and Brucella abortus. These select agent microorganisms are historically significant having been experimented with, or used as a bioweapon or as a terrorist agent, and are the subject of intense research in the biodefense and bioforensic area. If the presence of a bioterror agent is suspected, sensitive and specific assays for rapid detection and identification are necessary, however, DNA methods for microbial individualization of the sample may also be applied in order to individualize the strain and potentially determine the source of the microorganism. Methods utilized at the Armed Forces Institute of Pathology (AFIP) for select agent microbial DNA analyses include DNA extraction, DNA quantitation, real-time polymerase chain reaction of genetic targets unique to the select agent microorganism, microbial 16S ribosomal gene DNA sequencing, amplified fragment length polymorphism (AFLP) PCR and more recently repetitive-element polymerase chain reaction (REP-PCR) DNA fingerprinting. The methodologies of 16S ribosomal gene sequencing and DNA fingerprinting of microorganisms are well established within the field of diagnostic microbiology for microbial DNA identification, as well as DNA typing for epidemiological and genetic relatedness studies. 16S–Ribosomal DNA sequencing and AFLP DNA fingerprinting have been validated at the AFIP laboratory for identification and as a possible strain-typing tool for Bacillus anthracis, Yersinia pestis, Francisella tularensis, as well as Brucella and Burkholderia species. The continued development and implementation of new DNA based methods with increased sensitivity and defined specificity will be particularly useful for the detection of residual microbial DNA signature in situations where the microorganism has been rendered unviable by decontamination procedures or not able to be cultured on microbiological media.

SNPS, GENETIC VARIABILITY AND PHARMACOGENETICS

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As the worldwide genome project has progressed, a new phase in genetic research has dawned – studies of DNA sequence variations among individuals. In humans, single nucleotide polymorphisms (SNPs) amount to as much as 1/1000 to 1/300 along the entire genome. SNPs are amply documented in databases, including our own GeneCards.1 At the Weizmann Institute we have implemented the Sequenom mass spectrometry system for high throughput SNP scoring, and have launched numerous studies of DNA variations. Among polygenic diseases, SNP combinations are believed to underlie the occurrence and severity of numerous widespread diseases. In one study, we have focused on fine mapping of a schizophrenia susceptibility locus within a 23-Mb genomic region at chromosome 6q23. Ten candidate genes were selected based on functionality and expression. SNP-based genotype–phenotype correlation has led to the identification of a very strong association signal, currently under further investigation. We are also studying olfactory receptors (ORs), which comprise the largest gene superfamily; in humans, more than 50 percent are recently "pseudogenized". We have discovered that a significant fraction of ORs segregate between an intact form and a pseudogene form. Every individual has a unique combination of intact/disrupted ORs, a pronounced case of functional diversity.2 The segregating pseudogenes are now investigated as causative in odor blindness (specific anosmia). Finally, in a pharmacogenetics study, we have recently identified the markers that predict response to Copaxone (glatiramer acetate, GA) immunotherapy for relapsing multiple sclerosis.3 Fractional cohorts of two clinical trials (174 patients) were genotyped for 63 SNPs within 27 candidate GA response genes. Seven genes showed significant associations with GA response, with the most significant associations detected with three genes that play central roles in multiple sclerosis immugenicity, antigen processing and presentation. Our results indicate that the response to GA may be genetically controlled by a limited number of genes. Thus, pharmacogenetics based personalized treatment for multiple sclerosis seems within reach, and sets an example for the pharmacogenetics of other complex diseases.

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THE ARMED FORCES DNA IDENTIFICATION LABORATORY'S CHALLENGES AND RESPONSES TO OPERATION IRAQI FREEDOM AND BEYOND

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Operation Iraqi Freedom (OIF), Operation Enduring Freedom (OEF) and the continued war in Iraq have constituted the first major prolonged conflict AFDIL has faced since its establishment in 1990. We present the challenges faced in processing a large volume of samples including the unpredictable requirement to process samples in less than 48 hours, identify U.S. and foreign civilians in addition to military service members and manage continuous long–term operations. Prior to the war against Iraq, there was the threat that U.S. service members may encounter weapons of mass destruction such as Bacillus anthracis spores. Consequently, our laboratory had to develop a decontamination plan so DNA analysis could be used to identify any fallen service members while ensuring the protection of all people coming in contact with the biologically contaminated remains. One of the methods evaluated involved storing human remains in 10 percent formalin for various periods of time. Ten percent formalin is known to destroy anthrax, but also exerts detrimental effects on DNA. We also investigated ^{60}Co γ-photon irradiation as a method for decontaminating human remains. An average γ-photon radiation dose of 51.7 kGy was used to sufficiently kill all B. anthracis spores. Both methods successfully destroyed B. anthracis surrogate spores and yielded short tandem repeat (STR) profiles. The Armed Forces DNA Identification Laboratory (AFDIL) supports the Armed Forces Medical Examiner System (AFMES) by providing nuclear DNA analysis on human remains for identification and re-association. Causes of death range from single gunshot wounds to massive explosions resulting in fully intact remains to severely fragmented remains in various stages of decomposition. DNA samples are taken at autopsy and compared to DNA profiles obtained from military bloodstain reference cards housed at the Armed Forces Repository of Specimen Samples for the Identification of Remains (AFRSSIR). Not all of the victims are military personnel nor are they all U. S. citizens. This requires coordination of family references and personal effects. Despite the declared end of combat operations in Iraq hostilities have continued. As a result, AFDIL has experienced an increase in workload greater than 300 percent relative to prewar casework statistics. However, through the combined efforts of the AFMES, AFDIL and AFRSSIR casualties have been identified expeditiously. Over 1000 identifications have been made using a combination of dental, fingerprint and DNA comparison. Currently DNA analysis has been performed on more than 3200 unknown specimens with a 99 percent success rate and no U.S. service member has gone unidentified. The opinions and assertions expressed herein are solely those of the authors and are not to be construed as official or as the views of the U.S. Department of Defense or the U.S. Department of the Army.

GENETIC PROFILING IN FORENSIC CASEWORK

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The improvements of DNA typing techniques and the expansion of DNA databases have changed the fundamental concepts in criminal investigations. We have conducted DNA analysis from a variety of physical evidence pertinent to recent homicide and rape investigations. The results of DNA typing from some of these cases often produced interesting genetic and interpretation issues such as mixed sample interpretation, identical twins genetic profiles, time and sequence of sample deposits, DNA typing of relatives and the legal issue of near match. Different ways of applications of DNA typing and DNA database searching in forensic casework have been reported in the United States and around the globe. Some of the reported cases raise serious genetic, forensic and legal issues. I will use nationally and internationally known homicide, rape and child abuse cases to demonstrate these points. I will discuss numerous cases including the Florida case of the convicted sex offenders who used numerous aliases and the North Carolina case when a brother's DNA was used to solve a murder case. In Louisiana a genetic profile was used to solve a serial killer case and proved that psychological profile was wrong. Finally, I will present the lessons drawn from the notorious double murder case of O.J. Simpson, rape cases of William Kennedy Smith and Kobe Bryant, Louisiana serial murder case and Willard Brown case.

FROM FUNCTIONAL GENOMICS TO SYSTEMS BIOLOGY

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The phenotype of any organism is determined by three components: genome, environment, and random effects. Thus, life can be considered a computational process dealing with information from the genome and the environment. Therefore, systems biology–the quantitative modelling of biological processes–is an essential next step in the understanding of biology. In our view, this global approach represents the only chance for a real 'understanding' of many complex biological phenomena. To reach this higher level of "understanding", we try to establish computer models to duplicate the complexity of biology. The models must reproduce the complex networks of life. Diseases manifest themselves as disturbances in these networks. Consequently, we will have to elucidate these networks to understand, diagnose and possibly cure diseases. Basically, the information from all genes, their pattern of expression at the mRNA and protein level, structure and function of their protein products, their effects on metabolism, and ultimately structure and function of the cell, and its many subcomponents have to flow into these models. It is, therefore, essential to apply the available high throughput techniques and develop new strategies and methods to produce the huge number of data needed to reproduce life by computer models. Such models might not only be the best form to store and use the enormous amounts of information generated by genome projects on many organisms, but could also be a key step towards generating quantitative predictions, e.g., allowing quantitative predictions of the response of individual patients to specific drugs, the focus of pharmacogenomics. In establishing these models we must rely on functional genomics, the systematic analysis of the function of all genes and gene products from many organisms. Unlike traditional biology that has examined single genes or proteins in isolation, systems biology has, as its goal, the ability to model and quantitatively predict the complex interaction of many levels of biological information. The integration of the data coming from the various genomic technologies to generate information that describes biological systems is the straight continuation of the genome project.

TURNING THE BONES OF CHRISTOPHER COLUMBUS: A DNA ANALYSIS OF THE REMAINS OF THE DISCOVERER OF THE NEW WORLD

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Almost 500 years after his death on May 20, 1506, many mysteries remain unsolved about the location of the remains of Christopher Columbus, the legendary sailor and discoverer–"The Admiral". After his death in Valladolid his bones were moved to Seville in 1509. In 1544, following Columbus' own wishes specified in his last will, his remains were relocated to the Cathedral in Santo Domingo, Hispaniola (today Dominican Republic), his favourite place in America. In 1795, the Spanish ceded the island to the French and the remains were moved to Havana, Cuba, the closest Spanish colony at that time. In 1898, after Cuba's independence, Admiral's bones were finally sent back to Seville and were laid to rest in the Cathedral of this Andalusian city. Controversy started in 1877 when Dominicans were rebuilding the Cathedral in Santo Domingo. They found a wooden ossuary inscribed with "Admiral Christopher Columbus" in the crypt; inside they found some bones, but not the complete skeleton. Since at the time the remains should have been in Havana, Dominicans claimed that the Spanish did not remove Admiral's bones, but those of a relative buried nearby. Since then, Columbus has had two gravesites, in Seville and Santo Domingo. We are now trying to solve the mystery of the real tomb by DNA analysis. The DNA from Seville, designated "Q", is being compared to DNA of Christopher's son Hernando and brother Diego, both interred in Seville. By comparing Q's and Diego's mtDNA, we found identical mitochondrial DNA sequences supporting the notion that the remains in Seville are Columbus'. These results are awaiting the complete analysis of HV1 and HV2 regions that is currently underway. Meanwhile, we are still trying to get the permission to exhume the remains in the Dominican Republic. Another aspect of this project pertains to the many theories about Columbus' origins. The best known and the most widely accepted theory claims that Columbus was an Italian from Genoa who later came to Spain. Other theories (such as the one based on linguistic analysis of Admiral's letters that reveals a Catalan influence) are less widely accepted. To contribute to the question of Columbus' origins, we are trying to extract the Y–chromosome DNA from the poorly preserved putative Admiral's remains or from Hernando's. We're working with a set of Orchid's "Y" and autosomal SNPs, and with "Y" STRs. In addition to information regarding Admiral's origins, these data could shed light on his relations by comparison with the putative relatives that are now identified, such as Prince Charles of Viana. In this quest for evidence we are proudly independent; we are not trying to prove any theory about Columbus' origins and the location of his remains. The international "Columbus Identification DNA Team", that I just lead, includes the groups of Mark Stoneking (Leipzig, Germany), Olga Rickards (Rome, Italy), Bruce Budowle (Quantico, Virginia), Jeanine Baisch (Dallas, Texas), Angel Carracedo (Santiago, Spain), and Daniel Turbon (Barcelona, Spain). I gratefully acknowledge the support and technical help of Orchid Cellmark, Elchrom, ABI and Promega Corporation.

INNATE IMMUNITY TO BIOTHREAT AGENTS AS A BASIS FOR THERAPY DEVELOPMENT

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An attack with biothreat agents against people could be used to cause not only illness, death, but also fear, societal disruption and economic damage. To diminish such effects there is a need for the development of intensive therapy against biothreat agents. The innate immune system is the first and effective system that immediately overcomes nearly all potential infectious agents. The critical role of this system is in the initial recognition and response to pathogens, as well in regulating the adaptive immune response. To distinguish pathogens from self-components, the innate immune system uses a wide variety of relatively invariable receptors, named pathogen-associated molecular patterns (PAMPs). Their role was better understood after the identification of pattern-recognition receptors (PRRs). Toll-like receptors (TLRs) are crucial in the innate immune response to pathogens, they recognize and respond to PAMPs. Vaccinia virus (VV), the vaccine used to eradicate smallpox, encodes many immunomodulatory proteins to evade the host immune response. Viral targeting of TLR signaling molecules, and a novel mechanism used by VV to modulate the host immune response were recently demonstrated. It was also suggested by in vivo experiments in mice that TLR4 does not contribute to resistance to airborne type A Francisella tularensis infection. Dendritic cells (DCs) are vital in the defense against pathogens, including biothreat agents. To sense pathogens, DCs express pathogen recognition receptors such as TLRs and C-type lectins. Recent studies demonstrate that DC-SIGN is a more universal pathogen receptor that also recognizes Ebola virus. So far there is evidence that several viruses which cause viral hemorrhagic fevers (Ebola, Marburg, Lassa and Argentine) may infect DCs. Identification of biothreat agents and their components that induce DC maturation, combined with methods of delivering antigens of interest to DC would provide useful solution for the future DC vaccines. The ongoing research projects examine the ability of some synthetic oligonucleotides to trigger the innate immune system and improve the host ability to survive infection by biowarfare agents. To develop effective vaccines and therapeutics against biothreat agents, it is critical to gain a better understanding of both innate and adaptive immune response.

THE LABORATORY RESPONSE NETWORK: A CRITICAL COMPONENT OF PREPAREDNESS FOR BIOTERRORISM

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An effective response to bioterrorism will have to be rapid because of the small window of opportunity during which prophylaxis or other control measures can be implemented. The Laboratory Response Network (LRN) was created to facilitate the rapid identification of threat agents and is a critical component of CDC's public health mission to enhance readiness to detect and respond to bioterrorism at the local, state and federal levels. LRN was established because the existing national laboratory infrastructure competent to deal with biological (or chemical) terrorism was extremely limited. LRN was developed by the CDC in concert with the Association of Public Health Laboratories and in collaboration with the Federal Bureau of Investigation and the United States Army Medical Research Institute of Infectious Diseases. LRN builds on the existing interaction of nationwide public health laboratories, which participate in routine disease surveillance activities, and has a dual function in detection and response to agents released by a bioterrorist and to those that occur naturally. LRN members operate as sentinel laboratories or reference laboratories. Sentinel laboratories are, for the most part, hospital laboratories and other community clinical laboratories. This is because in the aftermath of a covert bioterrorism attack patients will seek care at the many widely dispersed hospitals where these laboratories exist. Sentinel laboratories participate in the LRN by using simple algorithms to rule out or refer the critical agents that they encounter in their routine work to nearby LRN reference laboratories. Reference laboratories provide confirmatory testing using standard protocols and reagents for rapid identification and confirmation of threat agents. There are currently 151 LRN reference laboratories in the U.S., Canada, United Kingdom, Australia, Germany, and South Korea. There are also federal laboratories with biosafety level 4 facilities that can handle agents such as Ebola and Variola major, for which other laboratories have insufficient safety facilities or unvaccinated staff. These laboratories also identify agents in specimens that have been referred by LRN reference laboratories and identify recombinant organisms (e.g., chimeras) that may not be recognizable by conventional methods. Reference laboratories also maintain extensive culture collections of critical agents against which the isolate(s) from an outbreak may be compared using molecular methods to determine its likely source.

NUCLEIC ACID–BASED ASSAYS IN PUBLIC HEALTH RESPONSE TO BIOTERRORISM AND EMERGING INFECTIOUS DISEASES

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Deliberate dissemination of a biological agent via a number of different routes, including air, water, food, or infected vectors presents the latest challenge to the global public health. The deliberate nature of such dissemination will often be obvious, as in the case of multiple mailed letters containing highly refined anthrax spores. However, some forms of bioterrorism may be more covert and result in an “outbreak” or “epidemic” of either a common or uncommon disease. In the context of bioterrorism, it is important to differentiate between a naturally occurring disease and one that is due to the intentional release of a biologic agent. To make matters more difficult, some of the diseases of concern, including many uncommon diseases, are either endemic or can occur naturally in the United States. A set of epidemiological clues based on distinctive epidemiology and laboratory criteria of varying specificity have been proposed to evaluate whether an outbreak may be of deliberate origin. The clues focus on aberrations in the typical characterization of an outbreak by person, place, and time in addition to consideration of the microorganism. Some of the clues, such as a community-acquired case of smallpox, are quite specific for bioterrorism whereas others, such as similar genetic typing of an organism may simply denote a natural outbreak. A combination of clues, especially those that suggest suspicious point source outbreaks, will increase the probability that the event is due to bioterrorism. The microbiology laboratory, through the development and application of nucleic acid-based assays and molecular typing methods, has made significant contributions to the response and epidemiologic investigation of bioterrorism and other emerging disease threats. Genotypic methods have been developed to “fingerprint” pathogenic microorganisms. This information is most useful when collected, analyzed, and integrated into the results of an epidemiological investigation. An example of how nucleic acid¬–based techniques have enhanced epidemiologic investigations is PulseNet, an international molecular subtyping network for food borne disease surveillance, which will also help identify outbreaks due to the intentional contamination of food.

DIAGNOSIS AND TREATMENT OF MULTIPLE, CHRONIC BACTERIAL AND VIRAL INFECTIONS IN GULF WAR ILLNESSES, CHRONIC FATIGUE SYNDROME AND FIBROMYALGIA SYNDROME

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Gulf War illnesses (GWI), Chronic Fatigue Syndrome (CFS) and Fibromyalgia Syndrome (FMS) have overlapping signs and symptoms,1 and chronic viral and bacterial infections are a major source of morbidity.2-4 We examined 200 CFS and 200 GWI patients and their immediate symptomatic family members. GWI patients (above some 42 percent) had mycoplasmal infections inside blood leukocytes, but not blood plasma or serum. The most common species (about 80 percent) was Mycoplasma fermentans, which was also found in more than 80 percent of family members with similar signs/symptoms.5 In contrast, in nondeployed, healthy adults the incidence of this species was approximately 2 percent.6,7 In more than 50 percent of civilian CFS/FMS patients we found a variety of mycoplasma species (M. fermentans, M. pneumoniae, M. hominis, M. penetrans, M. genitalium).8,9 The most common species in North Americans was M. pneumoniae8 and in Europeans M. hominis.9 Rheumatoid arthritis patients also exhibited high frequencies of mycoplasmal infections (above 45 percent) of various species.10 We examined CFS patients for infection by Chlamydia pneumoniae (7 percent positive) and HHV-6 virus (28 percent positive). Among micoplasma–positive and HHV-6–positive patients, 7.2 percent and 7 percent, respectively, were also Ch. pneumoniae–positive. Similarly, 27 percent of the micoplasma–positive patients were also HHV-6–positive, indicating there was no preference for or clusters of infections.11,12 Mycoplasma–positive and Ch. pneumoniae–positive patients have been successfully treated with multiple six–week cycles of antibiotics with nutritional support,2-4 and HHV-6–positive patients were treated with immune enhancement (IE). Patients on antibiotic/IE relapsed within weeks after the first cycle of therapy, but after up to six cycles most of them recovered. Patients who recovered no longer tested positive.4,7 We conclude that subsets of GWI, CFS, FMS and RA patients have transmittable chronic bacterial and viral infections, and treatment of these chronic patients with appropriate therapy can result in slow recovery.

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REBUILDING THE T CELL COMPARTMENT

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The T cells which reside in the blood and lymphoid organs (i.e., T cell compartment) mature in the thymus. Maturation of T cells in the thymus includes positive selection of cells bearing antigen receptors capable of recognizing self-MHC and negative selection of T cells bearing antigen receptors that bind too strongly to self-MHC. The most important aspect of selection, however, involves the nurturing of T cells bearing receptors that are sufficiently cross-reactive to be able to recognize novel peptides associated with self-MHC. Depletion of the T cell compartment, as sometimes seen following irradiation or treatment with anti-T cell agents or with aging, can lead to lasting immunodeficiency or to spontaneous restoration. In the latter case, the T cell compartment is initially restored by expansion of existing mature T cells and later by T cells newly emerging from the thymus. To the extent that the T cell compartment is restored by expansion of existing mature T cells, rather than production of new T cells, the diversity of the TCR repertoire is contracted. This contracted repertoire of T cells may lack specificity for some antigens and may be subject to clonal exhaustion or clonal senescence further narrowing diversity of the repertoire and immune competence over time. One potential means to improve immune function following depletion of the T cell compartment is to enhance the production of new T cells in the thymus. I will describe here several strategies for achieving this end.

EVOLUTION OF TECHNOLOGY FOR HUMAN IDENTIFICATION ANALYSIS

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I will discuss the development of new techniques and methodologies for use within an integrated forensic environment whilst also speculating on the adaptation of technologies for other applications.

DETECTION OF BOTULINUM TOXINS USING MICROMECHANICAL AND FLUORESCENCE–BASED SENSORS

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Botulinum neurototoxins (BoNTs) are the most lethal of all known human toxins, exerting their actions by cleaving the soluble N-ethyl maleimide-sensitive fusion protein attachment protein receptors (SNAREs) required for neurotransmitter release.[1,2] Indeed, the potency and ease of distribution of BoNTs make them a potential threat as biological weapons. The Center for Disease Control and Prevention (CDC) in Atlanta, Georgia, lists botulism as one of the six most dangerous bioterrorist threats.[3] In parallel, however, BoNTs have emerged as important therapeutic tools in medicine due to their specific actions. Early detection of these toxins is important for appropriate medical treatment. Traditional assays to detect BoNT activity monitor the effects of the toxins on a mammalian organism (observing signs of botulism in mice), or identify cleaved substrate molecules (electrophoresis and immunoblot). Similarly, enzyme-linked assays have been used for screening potential toxin inhibitors in vitro in an attempt to select antitoxins that could be used for therapy. Furthermore, there have been recent reports of the development of two distinct techniques to monitor BoNTs activity in vitro and in living cells.[4,5] In vitro detection was carried out using a micromechanosensor that relies on the attachment of a bead to the micromachined cantilever through interactions between SNARE proteins, with synaptobrevin 2 deposited onto beads and syntaxin 1A deposited onto cantilevers. The presence of toxin is indicated by detachment of the bead, resulting from cleavage of synaptobrevin 2. An alternate in vitro detection system is possible using fluorescent sensors constructed by inserting linkers, containing fragments of SNARE proteins, acting as toxin substrates, between cyan and yellow fluorescent proteins (CFP and YFP). Toxins cause the cleavage of these linkers and thereby abolish fluorescence resonance energy transfer between CFP and YFP. This approach, combined with an additional sensor based on subcellular redistribution of YFP fluorescence in cells, has been used for cell-based screening of toxin activity.

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ERRONEOUS REFUTATION OF ROMANOV DNA TESTING RESULTS

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A paper by Knight et al. relating to ancient DNA[1] has created a stir by attempting to cast doubt on the DNA analysis of nine skeletons officially identified as those of Tsar Nicholas II, the empress Alexandra, three of their daughters, their doctor, and three servants.[2] While claiming to be an attempt to replicate the findings of the original DNA study,[3] Knight et al. actually only tested a new putative reference sample for Alexandra: the purported 80 year old finger of her sister. Their results were unremarkable: a mixture of mtDNA sequences were recovered by cloning some amplicons, with minority components matching a rare sequence motif from the skeletal remains identified as Alexandra. More remarkable is the notion that this reference sample should be preferred to the one analyzed previously: a modern day blood sample collected from Prince Phillip, Duke of Edinburgh. Nevertheless, Knight et al. conclude definitively that the results of Gill et al. were due to contamination with "fresh" DNA, solely because of the amplicon sizes involved. Our presentation will primarily examine this assertion in the light of forensic DNA and ancient DNA experience, the range of criteria that are involved in determining authenticity of results, and the molecular characteristics encountered when testing of Tsar Nicholas II was replicated in an independent laboratory.[4] We will discuss the specific PCR technique used by Gill et al., and how that relates to the issue of successful recovery and amplicon sizes. Finally, we will discuss the hurdles that prevent unspecified allegations of intentional contamination from being reasonable alternative explanations for the DNA data. The presentation will highlight parallels and divergences between the ancient DNA field and the forensic DNA field, with regard to criteria for authenticity, standards for laboratory work, and the need to deal with spurious counter claims.

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TOLL-LIKE RECEPTORS AND THE BALANCE OF RESISTANCE AND INJURY

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Toll-like receptors are generally thought to provide a sensory apparatus for detecting bacterial products such as lipopolysaccharide. Stimulation of this apparatus by exogenous substances may account for some, perhaps many, cases of the sepsis syndrome and may promote immune responses to microorganisms. However, stimulation of toll-like receptors by this mechanism fails to account for some cases of sepsis syndrome and the utilization of this receptor system for the triggering of immune responses to tumors, transplants and viruses. In sum, the classical concepts fails to explain how the toll-like receptor system can participate in the broad range of biological activities recently connected with it. Two recent discoveries help explain the role of toll-like receptors in normal biological responses, host defense and the pathogenesis of disease. The first discovery concerns the ability of endogenous substances to stimulate toll-like receptors. The second concerns endogenous controls of toll-like receptor signaling that help to avoid pathophysiologic and pathogenic responses. A new model for the biology of toll-like receptors will be presented.

IMMUNE TOLERANCE: PROS AND CONS

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Central to the functioning of the immune system is the control of immune responses so that they can be directed at the microbial organisms, tumors, toxins and other foreign threats but not at normal cells. Recent insights into the mechanisms by which lymphocytes are activated appear to provide a mechanistic basis for understanding how the immune system selectively targets things that are foreign (that is, the basis for immune tolerance) but not things that are self. However, the immune system can also recognize autologous cells and substances, at least to some extent. Hence, the apparent failure of the immune system to direct effector functions at autologous cells and to engender tissue injury as a result must reflect a more complex process underlying activation of immunity and/or tolerance. Observations from a variety of biological systems now point to the possibility that the activation and progression of immune responses and the control of those responses reflects iterative interactions that determine whether protective immunity or tolerance ensues. These interactions can now be demonstrated in a variety of transplant settings and the failure of these interactions may be linked to disease.

BIOTERRORISM: THE THREAT AND REVIEW OF NEW COUNTERMEASURES

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In this talk, we will review the open source publicly available information on state–sponsored bioweapons programs, particularly the Russian and Iraqi bioweapons programs over the last twenty years. The importance of this information is to demonstrate the capability, intent, reality of the threat, and the needed countermeasures to biologic weapons. In addition, we will review current research in our laboratory aimed at assessing and developing existing and novel vaccines against anthrax and smallpox.

Suggested reading

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• Poland G, Grabenstein J, Neff J. (2005) The US smallpox vaccination program: a review of a large modern era smallpox vaccination implementation program. Vaccine 23, 2078-2081.

DNA ANALYSIS IN IDENTIFICATION OF WAR VICTIMS

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For years now, different methods of forensic DNA testing (known as DNA fingerprinting) have been widely established and accepted as the standard procedure in various police and court investigations. These methods offer great help in identification of war and mass disaster victims as well. DNA typing for forensic purposes is based on the same techniques that are routinely employed in a wide variety of medical and genetic situations, such as diagnosis and gene mapping. The identification of human remains found in mass graves employs various methods: identification of remains by direct facial recognition, fingerprint analysis, dentition analysis, identification of special features (scars or tattoos) recognition of clothing and belongings, autopsy findings, the analysis of skeletal remains by forensic anthropologists (estimating gender, age, race, stature of the remains and length of time since death), reconstruction of facial features from skulls, hair comparisons and, finally, DNA analysis. The latest war conflict in Croatia resulted in more than 11.000 missing persons. Therefore, significant efforts have been underway to identify missing individuals discovered in 135 of mass graves situated throughout Croatia. Due to lack of ante mortem data and body decomposition, common methods for human identification were not sufficient in some 47 percent of all cases and DNA identification was requested. Genetic typing by analysis of PCR-amplifiable STR loci is the most promising approach for forensic DNA profiling and has become a method of choice for identification of human remains. DNA typing from skeletal remains is among the most challenging parts of the entire identification process. Its success depends mostly on several factors: DNA extraction methods, DNA degradation level, the type of skeletal remains, the percentage of enzyme inhibitors per reaction, size of STR products, DNA concentration, etc. For the first time our country was confronted by the difficult task of applying DNA technology to such cases. Unfortunately, identification is not over, but it has become more successful as DNA technology develops, particularly the automated equipment and emergent multiplex technology. Laboratories in Croatia are striving to keep up these developments.

DNA EVIDENCE IN COURT – A JUDICIAL PERSPECTIVE

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The ever increasing use of DNA evidence in court has provided the justice system with a valuable tool in the search for justice and the truth. It has assisted in convicting the guilty and exonerating or excluding the innocent. But for effective use of DNA evidence, it must be presented so that ordinary citizens who sit on juries can understand its importance and accuracy. Attorneys and scientists must be able to communicate with each other and work to understand their respective disciplines to present DNA evidence to juries effectively. This presentation will provide a judge's perspective on effective and ineffective presentations of evidence, emphasizing clarity and simplicity as an approach that will not confuse the jury. Admissibility of DNA evidence will also be discussed, but only to the extent that it has almost become a non-issue. The so-called "CSI Effect" on jurors will also be discussed. Most lawyers and judges do not understand the science of DNA and its technology. Very few have a background in the sciences. With DNA evidence being used in more cases in court, it is important that lawyers and judges at least have a basic understanding of the science and the legal issues they may encounter. The speaker will describe and present a National Institute of Justice project to develop a CD–ROM for training judges and lawyers in the use and admissibility of various types of DNA evidence. Forensic science is changing the way trials are conducted in the justice system. However, judges, lawyers, scientists, and law enforcement personnel are overwhelmed by the amount of science and technology information required to meet various legal issues. Another National Institute of Justice project is seeking to not only bridge the gap between law and science, but also to assemble the available scientific, technological, and relevant legal resources into a comprehensive "one-step" searchable database with free access for all. The presentation will provide information on the National Clearinghouse for Science, Technology, and the Law, and how it can assist scientists as well as legal professionals in dealing with forensic science and technology issues.

HEMATOPOIETIC STEM CELL TRANSPLANTATION ACROSS MAJOR GENETIC BARRIERS

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Studies in mice and humans demonstrate that transplantation of hematopoietic progenitors in numbers larger than commonly used ("megadose" transplants) overcomes major genetic barriers. In vitro studies suggest that veto cells, within the population of hematopoietic progenitors, facilitate this favorable outcome. This tolerizing activity, mediated by apoptosis, can be blocked by TNF–neutralizing antibodies. Considering the limited availability of human CD34-positive cells, the availability of other types of veto cells or immuneregulatory cells is crucial for further application of allogeneic stem cell transplantation under reduced intensity conditioning. Perhaps the most potent veto cell is the CD8-positive cytotoxic T lymphocyte (CTL). However, this cell is also associated with marked graft-versus-host disease (GVHD). GVHD can be separated from the veto activity by generating anti-third party CTLs under IL-2 deprivation. Under such selective pressure only the stimulated clones, which make IL-2, can survive, while anti-host clones die. In vivo studies show that such anti-third party veto CTLs can be used safely for tolerance induction without GVHD. Very recently we demonstrated the potential synergism between the effect of rapamycin, which interferes with IL-2–receptor signaling, and the veto activity of anti-third party CTLs that operate via Fas–FasL mechanism. Moreover, we found that $CD4^+CD25^+$ T cells (regulatory cells), could also contribute to the enhancement of bone marrow allografting by veto CTL and rapamycin. This synergism might prove effective if tested in recipients of HLA matched or even mismatched megadose CD34 stem cell transplants for whom lethal conditioning is unacceptable.

EMBRYONIC TISSUE SPECIFIC STEM CELLS AS A NEW SOURCE FOR ORGAN TRANSPLANTATION

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The number of human kidney transplants has increased rapidly in recent years, but the demand greatly exceeds organ availability. Induction of appropriate kidney differentiation and growth out of stem cell or progenitor cell populations represents an attractive option to combat chronic kidney donor shortage. In an analogy to hematopoietic stem cells which are much more efficient in giving rise to blood than to other cell types, if any at all, renal stem cells could afford an unlimited source for regenerating nephrons. While a single nephrogenic stem cell has not been characterized, indirect evidence suggests that a renal stem cell population is contained within the metanephric mesenchyme, which along with a branch of the Wolffian duct represents the direct precursor of the mature kidney. Human tissue fragments derived from these developing precursors can regenerate renal structures when grafted into mice. Moreover, recent data pinpoints a window of time in human and pig kidney development that may be optimal for transplantation into mature recipients. “Window” transplants are defined by their remarkable ability to grow, differentiate and undergo vascularization, achieving successful organogenesis of urine–producing miniature kidneys with no evidence of transdifferentiation into non-renal cell types, lack of tumorigenicity and reduced immunogenicity compared to adult counterparts. In contrast, “non-window” transplants (earlier or later in gestation) can respectively form teratomas or are more prone to immune rejection, and are both less suitable for organogenesis. More recent results suggest that by using the same approach, “window” transplants can be defined successfully for embryonic pig liver and pancreas. Hopefully, the use of stage-specific early human and porcine kidney precursors to cultivate mature organs and tissue in vivo, possibly in conjunction with other modalities of stem cell technology and tissue engineering, will prove valuable to sustain life in patients with failing organs.

READING THE SIGNATURES OF POPULATION HISTORIES: FROM LANGUAGE TO DNA

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Historical processes are laboratories where human populations are created. Today we face a plethora of intriguing information on different problems of (macro)evolution and hypotheses and theories about current human diversity. These intellectual constructs are usually based on grossly insufficient information. I will discuss the rich data collected during three decades of field research in the isolated populations of Adriatic island; these data can provide meaningful interpretation and provoke discussion about the justification of (micro)evolutionary hypotheses. The underlying methodology has been based on the notion that research in population anthropology is much more than collecting and elaborating the collected data, that models must help in the reduction of information and its interpretation and that we have immense new possibilities and methods of research (from analyses of metric, morphological traits in the fifties to very fine physiological and biochemical analyses and until today almost unimaginable possibilities for molecular genetic research). The results of our study of numerous population traits show that different traits respond to (micro)evolutionary impacts differently. Some traits are more likely connected to specific different selective pressures, others more likely reveal selective inertia, while for the third group of traits a reasonable genetic interpretation cannot always be provided because of their extreme phenotypic plasticity. One should accept that the humans are social and cultural beings in addition to the physical being and that they contribute to their own biological state that is limited both by natural laws and sociocultural restraints.

FUTURE OF FORENSIC TESTING OF NON-HUMAN DNA

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In the limited time afforded to the presentations in the non-human DNA section of the meeting, we have looked at the use of non-human DNA in three areas: to identify a plant species (marijuana), two domestic animals (dogs and cats), and select agents causing diseases such as anthrax, plague and brucellosis. However, this just skims the surface of the forensic applications of non-human DNA testing. The potential of linking a perpetrator to a crime scene using non-human DNA sources, started with the DNA profiling of seed pods from a Palos Verde tree found in the back of a pickup truck of a suspected murderer and compared to trees near the crime scene. Are there other potential sources? The determining of time of death in decomposing bodies relies on the use of maggots from various flesh–eating flies. Until recently it has not been possible to separate these different species. DNA analysis of different mtDNA regions or random amplified polymorphic DNA (RAPD) can now separate different species. The ability to characterize both bacteria and viruses allows the forensic scientist or public health worker to track intentional transfer of diseases such as HIV infection to a specific individual, or to trace the dumping of environmentally hazardous bacteria. The advent of the endangered species act has lead to the development of forensic DNA laboratories that specialize in the testing of non-human DNA for the identification of endangered species. However, this work has been expanded to include everything from the DNA profiling of caviar to make sure of its origin, to the misappropriation of cattle. The wide spread use of DNA identification of agricultural species has allowed for the investigation of fraud in the sale of horses and cattle and the identification of the source of a “downer” cow that was identified with mad cow disease. It appears that we have just begun to use non-human DNA in forensic applications.

MICROARRAY ANALYSIS OF GENE EXPRESSION

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One of the many benefits of the Human Genome Project is a complete catalog of the 27,000 human genes. Among the technologies instrumental in the completion of this project was the development of sufficiently powerful and fast computers to handle and manipulate the three billion base pairs that comprise the human genome. To create faster computer chips to manage all this data, technologies were developed to reduce the size of features on a computer chip. Using the same photolithographic techniques used to etch a computer chip, companies like Affymetrix can now synthesize small glass chips that contain millions of oligonucleotide probes, which can then be used to interrogate the expression of all 27,000 human genes simultaneously. Alternative strategies utilized by Agilent use high–density laser jets to synthesize longer oligonucleotides that can also interrogate the expression of all human genes. By comparing the expression of all human genes in normal tissues and corresponding disease tissue one can begin to determine genes that are aberrantly expressed in the disease state. I am going to use the example of microarray analysis of gene expression in cancer cells to describe how this technology will have a dramatic impact on the study of cancer. For example, one can begin to identify genes that could prove to be very useful and effective markers for the early detection of cancer. In addition, by comparing tumors from patients who were highly responsive to chemotherapeutic regimens we may be able to develop signatures of gene expression that tell us something about a specific cancer prior to chemotherapy treatment. As computer chips are being developed with even greater numbers of features it is now possible to not only interrogate the portion of the genome that encodes for expressed genes but the entire genome. This type of analysis is just beginning to reveal that a much greater proportion of the genome encodes useful transcripts that are somehow important for normal cellular regulation. An analysis of the dysregulation of these transcripts should yield important insights into the total genomic alterations that underlie the development of cancer and many other diseases.

POSITIONAL CLONING TO IDENTIFY IMPORTANT DISEASE-RELATED GENES

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Positional cloning is a strategy to identify genes when one has no information about the protein encoded by those genes but some information about the chromosomal localization of that gene. This information could be obtained from genetic linkage analysis when alterations in the gene can be identified in families predisposed to develop a specific disease. Alternatively, this could be derived from cytogenetic analysis when recurrent chromosomal alterations (such as translocations) are observed in affected individuals. Finally, consistent chromosomal deletions or amplifications, frequently observed in cancer cells, can be used to begin to pinpoint the chromosomal location of the affected gene. The international effort to map and then sequence the human genome has greatly facilitated positional cloning. In addition, utilizing the reagents that are currently available (including the complete human genome sequence) it is now considerably easier to identify disease-related genes using this strategy. I will discuss the history of the Human Genome Project and the identification of important disease-related genes that has been facilitated by the tools generated from this project. I will use the example of identification of important tumor suppressors and oncogenes that play an important role in cancer development. I will compare the identification of genes such as retinoblastoma and neuroblastoma (completed before the human genome project began) to the strategies that are currently employed to identify important cancer related genes. An important part of this is the resources and techniques now available to facilitate this analysis. This includes publicly available databases containing information about all known genes present in defined chromosomal regions as well as new methodologies to rapidly localize small, defined chromosomal regions that contain these genes. Finally, I will discuss the future of positional cloning based upon new methodologies currently being developed.

DNA: CHAINS TO FREEDOM

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DNA is today's miracle. There are so many ways that it has been used and new ways that are coming to light. In medicine DNA can be used to discover the cause of certain diseases, it can be used to help in the search for new medicines and can help in understanding the individual patient. For those with lost loved ones such as in natural or man made disasters, runaway or kidnapped family members or with discovered remains, DNA offers identification that solves the mystery. For crime fighting agencies, DNA brings the newest and best tool to their investigative arsenal. For this stay–at–home mother of two, it brought freedom from the suicidal tendencies brought on by rape and the freedom of validation. DNA did more than restoring me to who I was–it brought new strength and determination. This presentation will show a value of DNA that most people, especially those working the closest with it, never know.

HUMANS – THE NEXT GENETICALLY MODIFIED ORGANISMS?

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Technologies that enable us to deliberately alter the genome of complex organisms are rapidly developing and becoming more efficient. It is, thus, only a matter of time before these technologies become applicable to humans. Today we can envision two possible genetic interventions into humans: additive and subtractive. Additive interventions such as gene therapies and stem cell-based therapies including therapeutic cloning are on the verge of becoming applicable in clinical practice. The elimination of genetically challenged embryos and fetuses following preimplantation diagnosis and prenatal screening could be considered to be subtractive, though one could also envision the future use of cell and gene therapy to clinically intervene in such situations. These technologies and their possible applications have provoked sociological, ethical and religious confrontations like no other biomedical discoveries before and these conflicts are often based on a poor understanding of the underlying biology. To fully appreciate the possibilities of such technologies and the inherent dangers to individuals and society, it is essential that we clearly understand their limitations and visualize the possible short–term and long–term advances and their consequences.

INTERACTIVE PROTEOMICS INVOLVING MEMBRANE PROTEINS: THE FIRST SIX YEARS AND BEYOND

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Proteins associated with membranes total approximately one third of all proteins in a typical eukaryotic cell. Due to their pivotal role in many cellular processes, their direct link to human disease and often their extracellular accessibility towards drugs, an understanding of membrane protein function is desirable. The hydrophobic nature of membrane proteins often renders the proteins insoluble resulting in difficult isolation that hinders the determination of their composition and function. Previously we have developed a yeast-based proteomic technology for the in vivo detection of membrane protein interactions, the so-called membrane-based yeast two-hybrid (MbYTH) system. Our current effort is directed to the generation of a comprehensive protein interaction network of the selected yeast membrane proteins whose human homologs are involved in the onset of human diseases as well as the majority of the human G-protein coupled receptors. I will report on the latest achievements on this project and will highlight the latest interaction and functional data.

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HUMAN ORIGINS THROUGH MOLECULAR ANTHROPOLOGY

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Who are our nearest living relatives? How did our species originate? These two fundamental questions about the nature of humans, although subject to intense investigation and discussion for centuries, were only recently answered. And, the answers came not from traditional lines of anthropological inquiry, but rather from analyses of molecular genetic variation. I will discuss how molecular anthropology has answered these questions, and also indicate why such seemingly simple questions proved so difficult to answer.

REMOVING BOTTLENECKS IN GENE EXPRESSION ANALYSIS FROM WHOLE GENOME TO SINGLE GENE

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Applied Biosystems has developed a series of integrated genome analysis tools in which curated genomic information from multiple sources is integrated into state–of–the–art technology. Millions of ready–to–use assays are now available for gene expression and SNP genotyping analysis, to improve and accelerate research related to human genome. For gene expression analysis, we present a fully integrated system enabling analysis from whole genome to single gene level. The system includes chemiluminescence microarrays and Taqman® real–time PCR technologies where every microarray probe and every Taqman® assay are designed against the same curated gene. An integrated workflow allows easy analysis of expression of some 30,000 curated and annotated genes in human, mouse and rat, each one coming with existing Panther™ and GO ontology functional information, orthologue cross mapping, cross mapping with any other array platform or database. Integration of information with technology makes it possible today to remove bottlenecks in research such as understanding what gene is targeted by a probe, designing an assay against the same gene, understanding the biology of our experiments. This gene expression system has recently been enlarged with the introduction of a panel of Taqman® assays for 180 human miRNAs to complete the capability of genome investigation.

EMBRYONIC STEM CELLS IN CARDIOGENESIS AND TISSUE REPAIR

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Current therapeutic modalities for myocardial infarction provide palliative care but do not adequately contribute to repair. Discovery of cell populations that exhibit a proclivity to differentiate into specialized tissue types has provided the foundation for cell-based regenerative medicine. A case in point is the embryonic stem cell, recognized for its distinctive regenerative potential imparted by unequaled pluripotency and mitotic capacity. Embryonic stem cells differentiate readily into cardiomyocytes, and on implantation can provide a source for de novo cardiogenesis. However, the promise of embryonic stem cells has remained unfulfilled, limited by the threatening propensity for unguided transformation inherent to pluripotency upon separation from the nurturing guidance of the embryonic niche. Cardiac transformation of the mesoderm requires endodermal signaling, which is difficult to recapitulate without the formation of germinal layers. Therefore, the risk of misdirected neoplastic growth outweighs potential therapeutic benefit upon transplantation into host heart. To this end, we here present a strategy to hone stem cell plasticity, secure cardiogenesis, and elude malignancy-prone unguided differentiation. Our studies have detected an endoderm-dependent process during differentiation whereby pluripotency is directed towards cardiac pre-determination, termed “cardiopoiesis”, eliminating the risk for uncontrolled growth and tumorigenic predisposition. Thus, lineage-specific recruitment of embryonic stem cell-derived pro-cardiogenic intermediates can serve as a safe and efficacious means to overcome the shortcomings of embryonic stem cell therapy.

METHODS AND PROBLEMS IN ADVANCED CELLULAR GRAFT ENGINEERING

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Advances in cell biology, immunology, hematology and developmental biology have raised the hope that cell transplantation can treat malignant diseases, inborn errors of metabolism, autoimmunity, neuronal injuries, and others. As a result, the past decade has witnessed the emergence of regenerative medicine that is partially based on the application of stem cells and progenitors of terminally differentiated cells. These advances together with the emergence of cellular immunotherapy and novel modes of blood and marrow transplantation require validation by clinical trials. In response to this need, medical centers and cell therapy industry have been developing cell graft engineering methods and facilities for preparation of clinical–grade cellular grafts. Organization and implementation of clinical-grade graft preparation (manufacturing) proved, however, to be a formidable task due in part to the many difficulties inherent the very nature of graft engineering. This discipline spans the distance from the proof of scientific principle (obtained by ordinary laboratory methods) to the final product (obtained by standardized manufacturing methods that yield cellular products with predictable function, viability and phenotype in full compliance with the current regulations, economy of manufacturing, etc). Cell graft manufacturing brings a new culture that is neither basic science nor medical practice nor traditional pharmaceutical industry nor biotechnology, but draws from them all. I will explore the role of translational research, biotechnology and cell therapy industry and development of on site manufacturing in implementation of graft engineering methods for clinical trials that comply with all ethical, scientific, medical and legal requirements. As examples of graft engineer's problems in this scientifically young field, I will review the attempts at applying the cells from bone marrow and blood in therapy of heart diseases and preparation of allogeneic T cell grafts devoid of the ability to induce graft–versus–host disease.

CHARGESWITCH® TECHNOLOGY– A NOVEL HIGHLY SENSITIVE DNA PURIFICATION TECHNOLOGY, OPTIMISED FOR FORENSIC APPLICATIONS

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Following large–scale automated and manual validation, ChargeSwitch®, a novel technology for the purification of DNA, is now in use in high throughput European forensic labs. This presentation describes the technology and the experience gained from large–scale validation on forensic casework, scene of crime and criminal justice studies. ChargeSwitch® Technology was developed by DNA Research Innovations, Ltd. (now a wholly owned subsidiary of Invitrogen) to address sensitivity and performance limitations inherent in other conventional methods of purification such as Chelex and silica. Operating as a pH dependent ionic switch, ChargeSwitch® is a surface coating that enables purification by means of charge density attraction. The protocol avoids the use of chaotropic salts and alcohols, which can interfere with downstream reactions if co-purified. In trials, ChargeSwitch® demonstrated significantly reduced failure rates and partial profiles in scene of crime samples including challenging touch materials and complex samples including laser microdissected sperm cells.

PROSECUTORIAL CHALLENGES IN DNA APPLICATION

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Law enforcement has used forensic DNA–related evidence to solve crimes in the United States for over fifteen years. Despite its widespread acceptance, prosecutors still face rigorous challenges in the application of DNA in the courtroom. Generally speaking, there are two types of DNA admissibility challenges: defense attacks on the science or defense attacks on the statistics. Further, enhanced technologies enable DNA to be identified in "cold cases" thus creating additional challenges in reviving these cases for trial. Statutes, rules of evidence or case law regulate the admissibility of scientific evidence and expert testimony in American criminal cases. The goal of this presentation is to address issues arising from current challenges to DNA admissibility in American courts and how working closely with one's lab analyst can greatly assist overcoming these challenges. Finally, this presentation will discuss proactively addressing defense challenges to DNA admissibility.

Suggested reading:
- Forensic DNA Fundamentals for the Prosecutor – Be Not Afraid
- DNA Evidence Policy Considerations for the Prosecutor
  Both published by APRI and available on-line at: www.NDAA-APRI.org under DNA Forensics Program.

ABSTRACTS OF POSTERS
THE FOURTH EUROPEAN-AMERICAN SCHOOL IN FORENSIC GENETICS

Presentation number: F1

DEVELOPMENT OF A COMPLETELY ROBOTIC HIGH–THROUGHPUT SYSTEM FOR STR TYPING OF MODERATELY CHALLENGED ORAL SWABS

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The Armed Forces DNA Identification Laboratory was challenged with the development of a system to expedite the analysis of several thousand oral swabs collected on various substrates, without standard procedures, and warehoused under unknown storage conditions for as long as two and half years. Due to the varied history of these samples it was necessary to anticipate a wide range of DNA yields and quality. Robotic platforms were validated for extraction, amplification and analysis. These included the Qiagen 9604, the Corbett Robotics CAS-1200 and the AB 3100. In advance of a lab-wide transition from AmpFLSTR® Profiler Plus® and AmpFLSTR® COfiler® to a single multiplex, we also validated PowerPlex® 16. Additionally, because a high–throughput formatted quantification system did not exist in-house, the system was intentionally designed without quantification or subsequent normalization steps. To assist with data management and data review we employed the use of LISA v. 7.02.1, an information management system designed and developed by Future Technologies, Inc. and the expert system TrueAllele® v. 2.6.30 designed and developed by Cybergenetics. Profile success, data quality considerations, validation parameters, data management strategies and discordant results will be discussed.

Presentation number: F2

CREATION OF DNA PROFILES DATA BASE

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World practice of genotype database creation is various, but principles of its development are similar. There are two main directions of DNA-profiles data base modulation: 1. Formation of DNA data base on the basis of profiles received as a result of expert research that is the additional practical appliance of them. 2. Creation of DNA data base on the basis of entering face profiles that are not included into the sphere of legal procedures. Realization of the first direction consists of the following stages: a) choosing the examples of biological tracks from things-bearers found on the crime scene, distinguishing their DNA-profiles and entering them into data base; b) entering into the data base the profiles of criminals, which condemned for heave crimes such as murder, sexual assaults, violent attack and so on. Each of DNA-profiles entered into the data base is given a personal code number with reserve broadening for entering required notices. The work on the project started in 2005, mainly directed on developing approaches of genetic passportization for identifying ethnos of the individuals and unknown corpses on the basis of analyzing biomaterials and automatization of DNA data base by means of applied software creation. Practical realization of this project in the field of nuclear DNA data base formation is already available in the beginning stage of its performance by means of including existing DNA-profiles into the data base, which were established earlier during the DNA expertise in the connection with verification of criminal matters regarding to the terrorist acts, murders, sexual assaults. Information about significant quantity of DNA is already collected and the most of them are personificated. Except the well-known individuals that have committed crimes, in perspective it is planned to include DNA-profiles of all unidentified individuals, including killed during terrorist acts, mass collapses, Significance of the second direction of the DNA data base elaboration is in the opportunity of simple identification of individuals whose profession is connected with risk of life (military personnel, policemen, operative service officers and so on). This trend requires the existence of normative base, for example, in the form of the Law “About DNA data base” and special financial support.

Presentation number: F3

Y-CHROMOSOME HAPLOGROUPS IN EASTERN EUROPEAN POPULATIONS

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According to archeological and genetic data the Balkan territory was continuously settled since the Paleolithic, and during the last Glacial Maximum (LGM) has been an important refugee area. The Y-chromosome haplogroup (Hg)I distribution suggested a central role of this refugee in the process of the post LGM recolonization of Europe. In addition, as a crossroad among three continents, this region was affected by different Asian groups (some Slavic clans during the 6th and 7th century and the Turks in the 15th century) that could have had a major impact on the gene pool of local populations. To evaluate the contribution of the Balkan refugee(s) to the genetic landscape of Europe, the variation at 30 Y-specific biallelic markers was examined in about 650 males of different populations from the Balkans (Slovenia, Croatia, Bosnia and Herzegovina, Albania, FYRM, Greece) and in 237 from East Europe (Poland, Czech Rep., Hungary) and 100 from Caucasus. The three commonest haplogroups, differently distributed, were the haplogroups I, R-M17 and R-M269. Haplogroup I, almost exclusively accounted for by its sub-haplogroup I-P37*, was observed at high frequency in the Northern Balkan populations (30-48% with a pick of about 70% in the Bosnian Croats). The R-M17, which is the prevalent R sub-haplogroup in Eastern European populations, shows in the Balkans a north-south decreasing gradient (from ~35% in Slovenia to 2.4% in FYRM). Interestingly, the prevalent western European haplogroup R-M269 has a frequency around 20% in the northern and southern Balkans, but its incidence drastically decreased in the area of Croatia and Bosnia-Herzegovina characterized by the highest frequencies of HgI. Haplogroups E and J, which are known to mark migrations from the Middle East occurred in Neolithic times, were observed almost exclusively in southern Balkans and mainly accounted for by the sub-clades E-M78 and J-M172.

Presentation number: F4

FORENSIC DNA KITS BY DIFFERENT MANUFACTURERS RESULT IN "DIFFERENT" PROFILES

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Standard kinship analysis was performed with the aim of paternity establishing. DNA extraction from buccal swabs and blood samples of mother, child and expected father was carried out with Chelex-100. PCR was performed with Identifiler Kit (Applied Biosystems) for genotyping of 15 STR loci and Amelogenin. Capillary electrophoresis was performed on 3100 Genetic Analyzer; data was analyzed with Genescan and Genotyper software. The results in 14 loci proved paternity. The only exception was vWA locus with 14/17 mother, 14 allele child and 17 allele father. Another multiplex kit FFv triplex (Promega) for 3 loci (vWA, FESFPS, F13A01) analysis was used. Amplification products of FFv triplex were studied in denaturizing 4% PAGE with silver staining visualization. Additional study revealed following profiles in vWA locus: mother – 14/17, child – 14/18, father – 17/18. Observed difference in vWA profiles obtained with commercial kits from two different producers may be explained by different primer sites used by them for vWA amplification and probable mutation in Applied Biosystems primer site on father's chromosome being inherited by the child. Peak heights of child's 14 allele and father's 17 allele in vWA locus from Identifiler panel under careful examination did not looked like homozygous profile, but like one alleles of heterozygous profiles. This situation leads to conclusion concerning main part of our work – forensic cases. Opportunity of possible mutation should be considered in forensic cases of unknown bodies identification when situation is accomplished with absence of one of the parents, for example in cases of newborn murders when few loci screening of possible mothers is being made. Only at least two loci exclusion may be enough for negative report. Peak heights of homozygous profiles (especially in loci with kinship exclusion) should be considered as well because mutations in primer sites are possible.

Presentation number: F5

DOMESTIC CAT HAIRS IDENTIFICATION IN MURDER CASE WITH DISMEMBERING OF THE BODY

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Four bags with parts of dismembered male body were found near metro stations of Minsk city. Only head and hands were absent to make person identification difficult. Shed white domestic cat hairs were found on victim's clothes. In three months another bags with parts of another dismembered male body without head and hands were found. Police has succeeded in searching of second offender. This man was suspected in first crime committing, but no biological evidences corresponding to first body were found in his apartment except of his white male cat. The MeowPlex panel of 11 tetranucleotide STR loci of cat genome and gender marker was used for investigation of cat hairs collected on victim's clothes. Marilyn Menotti-Raymond, Laboratory of Genomic Diversity, National Cancer Institute at Frederick, MD, kindly provided us method have been developed in the laboratory. Chelex-100, Proteinaze K, DTT extraction was used for DNA isolation from cat hair roots as well as from reference blood samples and freshly plucked hairs. From two to five hairs (collected in close area) were combined in one tube extraction as amount of total genome DNA being extracted from shed hair roots have been expected to be very low. Twelve primer pairs marked with 6-FAM, VIC, PET, NED fluorescent dyes in appropriate concentrations have been added to PCR reaction mix. Standard 28 cycles PCR was performed. Capillary electrophoresis was carried out on 310 and 3100 DNA Analyzers (Applied Biosystems). Blood and hair reference samples revealed the whole 12-loci profile as only one sample from "crime scene" showed very small peak of male SRY marker. Changes in PCR conditions (60 min post-PCR incubation) and gradual PCR cycles multiplying up to number of 38 at least resulted in achieving of readable profiles in three "crime scene" samples. It was realized that suspect's cat could not be the source of hairs of evidence. Meowplex kit proved to be useful tool in forensic investigations. Risk of contamination during domestic cat evidences examination in "human" forensic laboratory is not very high, this allows expert to use method modifications (such as multiplying of cycles number) for sensitivity increasing.

Presentation number: F6

SOME PROBLEMS IN USING DNA PROFILE AS EVIDENCE OF CRIME

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As it is written by some American writers, some years ago, every country has its own way to go out with truth. In some counties true comes out before, in some after the trial. In Croatia there were two interesting cases involving one man and two crimes. Using DNA he was found guilty for one of them. He was found not guilty for another. Young man was suspected to be rapist of 11 and 17- year old girl. During police investigation asked for plea guilty but he said that he didn't do it. That he wasn't at the crime scene. There was no identification because victims said that they didn't see him. During the polygraph test he was talking things that only attacker could know. At the end of police investigation he said: 'Ok. I did it'- but his counsel for the defense was not present. Then, he came to the court of enquiry. He was asked if he did that crimes, but there he said: 'No. I didn't do it.' Investigative judge wanted to see if his story is really truth. Young man had to give his blood to be tested. After two DNA testing, DNA analyze showed no match of his DNA profile and profile of the victims, so he was free to go. But, some months after, one old lady was murdered. Police took cigarette from the crime scene. Using DNA test, they found that this young man was in ladies room that day she died. Court found him guilty and put him in prison only based on this DNA profile found on cigarette. What does that proof? Only that he was in ladies room, not that he killed her. Especially, because court had no other evidence. Only some leads. There is big problem of taking DNA profile as relevant evidence in criminal procedure on court. It is not enough to take only one DNA profile and say: "He's the one". But, also in some cases there is enough to take only DNA profile to say: "Ok, he's the one".

Presentation number: F7

PATERNITY TESTING ON EXHUMED BODIES: OLD PROBLEMS AND NEW STRATEGIES

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There is an increasing demand for paternity testing from exhumed bodies in our routine. DNA extraction from human bones is known to be a difficult and time consuming procedure, and all the cases investigated in this study showed these typical difficulties. We focused our attention on nails as an alternative source of DNA in those cases when no result was obtained from bones; commercial DNA extraction columns were also tested on bones in comparison to standard DNA extraction procedures. DNA extraction was performed from compact diaphyseal femoral bone tissues using either the organic technique or commercial columns. DNA from nails was extracted with organic method. Standard PCR was performed using commercial forensic kits and the amplified fragments were resolved on automatic instruments. Nails proved to be a good alternative source of DNA, both in terms of DNA yield and of reduced amount of time requested for analysis. Commercial columns gave better results when compared to standard organic extraction on bone extracts. Other advantages and disadvantages of this approach are discussed.

Presentation number: F8

TESTING DNA OF THAILAND TSUNAMI VICTIMS BY THE INTERNATIONAL COMMISSION ON MISSING PERSONS

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The tsunami disaster left approximately 5,000 persons reported missing in Thailand. In the first six months of the identification effort, thousands of victims were identified by fingerprints and dental records, but few by DNA testing. In June 2005 the International Commission on Missing Persons (ICMP) was asked to assist the Thailand Tsunami Victim Identification (TTVI) effort. ICMP contributed both in DNA-STR testing of bone samples as well as in matching of STR profiles between victims and family reference samples. DNA was isolated from bone samples by a silica based extraction method, quantified by the Applied Biosystems Quantifiler™ system, and then amplified by the AmpFLSTR® Identifiler® PCR Amplification Kit. Most bone samples from Thailand contained significant levels of genomic DNA, but a small number contained very little. DNA testing results as well and DNA matches will be presented.

Presentation number: F9

DNA-BASED IDENTIFICATION OF THE FINNISH TSUNAMI VICTIMS

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After the Asian Tsunami disaster on December 26th 2004, 178 Finnish tourists were reported missing. Seventy-five extended families were affected, resulting in a bimodal age distribution among the victims. The identification of the Finnish victims is led by the Disaster Victim Identification (DVI) team of the National Bureau of Investigation. The local section of this DVI team has been mustering large amounts of diverse ante mortem (AM) data, which is then compared with the post mortem (PM) data collected in Thailand by international teams. Five months after the catastrophe, 135 victims have been identified and repatriated with the aid of dental records and fingerprints. However, a majority of the missing children lack conclusive dental AM data due to intact teeth. This has led to a bias in the age distribution among the identified victims - c. 80% of the persons missing after five months are children. For these, the identification can be achieved only through DNA-based methods. The challenges of the DNA-based identification of Tsunami victims are due to a lack of direct AM-DNA-samples as no national DNA or tissue databank exists. Although for some victims the direct DNA profiles can be obtained from AM samples stored in hospitals or from personal belongings, for most cases reference samples from close relatives are required. For the children, the most informative reference sample donors (mother and father) were among the Tsunami victims. This poses another challenge: to reliably extract and analyze DNA from the PM tissues of the identified victims. Many international laboratories have already reported difficulties in obtaining results from different types of PM tissue (rib bone, teeth, muscle). At the Department of Forensic Medicine, University of Helsinki, PM femur bone samples have been taken in the autopsies performed for all the repatriated bodies, immediately followed by DNA extraction and profiling for fifteen STR loci and for one gender-specific locus. We have achieved a very high success rate (>99%) in DNA profiling through appropriate choice of the sample tissue, careful phenol-chloroform based extraction method and overall rapid processing after sampling. The data analysis and actual identification of the remaining victims are underway.

Presentation number: F10

FORENSIC RESPONSE VEHICLE: RAPID ANALYSIS OF EVIDENCE AT THE SCENE OF A CRIME

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The first hours of a criminal investigation can be the most important. A suspect who is arrested soon after a crime has less time to remove evidence from their person, possibly allowing stronger forensic ties between the individual and the crime scene. We have developed a mobile laboratory (the Forensic Response Vehicle) that offers a complete package of services and expertise to bring forensic science to the crime scene. This vehicle has designated work areas for the intelligent searching of small items, DNA, electronic, fingerprint and footwear analysis. The DNA analysis processes utilizes a combination of novel and off the shelf equipment, consumables and instrumentation to rapidly extract, PCR, separate and detect the fluorescently labeled STR amplicons. Data collected is directly imported from the electrophoresis instrument to I3(TM) expert system software to interpret the STR profiles to provide an automated solution to profile designation. Subsequently, an SGM+ profile can be produced and compared to the National DNA Database(R) in approximately 5 hours from start to finish, potentially providing the police with valuable intelligence early in the investigation of a crime. The vehicle also carries the capability for the interrogation of personal electronic items such as mobile phones, and the satellite communication systems allow direct connection with the FSS computer network allowing images of fingermarks and footwear marks to be searched against the appropriate databases.

Presentation number: F11

ENTIRE mtDNA CONTROL REGION DATABASES FROM CENTRAL ASIA: AFGHANISTAN, KAZAKHSTAN, KYRGYZSTAN, TAJIKISTAN, TURKMENISTAN, AND UZBEKISTAN

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We report here new databases of entire control region sequences representing individuals originating from six Central Asian countries, comprising approximately 1500 individual sequences. All samples were obtained from individuals living in Uzbekistan, but including individuals with direct ancestry in neighboring Central Asian countries. Additionally, individuals of Uzbek ancestry were sampled from five distinct regions: Fergana, Karakalpakistan, Xorezm, Qashkadarya, and Tashkent. The aim of this study is to represent in some depth the range of genetic variation present in the mtDNA lineages of Central Asia, enabling proper interpretation of forensic mtDNA testing in Uzbekistan and surrounding regions. We have sequenced the entire control region, using a high throughput system that relies on laboratory robotics and a robust bioinformatic infrastructure. The system is designed to avoid the most common sources of "phantom mutations" (errors) that are common in some forensic and academic mtDNA databases. We will present population genetic and mtDNA haplogroup analyses of the ~1500 sequences in this study. We will discuss differences in the distribution of mtDNA types amongst the various population samples, and their implications for interpretation and application of mtDNA testing in the Central Asian region.

Presentation number: F12

INITIAL EXPERIENCE IN COLLECTING TRACES FOR FORENSIC DNA ANALYSES FROM FIRE ARMS IN MONTENEGRO

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In regard to crimes committed by fire weapons, in addition to some other things, there are also specific traces identified as thrown fire arms, cartridges (cases), projectiles, gunpowder particles, etc. Modern investigation procedure asks for multi-disciplinary examination of all traces from the crime scene, as well as of fire arms traces. Therefore, during the last few years, DNA analyses of the biological originating traces have been carried out, which can be found on the fire arms, projectile, paraffin gloves and on other objects. Due to the lack of DNA laboratory in the Republic of Montenegro, from the objects used for committing crimes with fire weapons, sampling - smear taking is carried out, as well as smear taking from the suspects, and analyses are carried out in the DNA laboratories in the Republic of Slovenia, Federal Republic of Germany and Republic of Serbia. For the purpose of illustration, five examples were taken relating to the murder cases, and attempt of murder cases with unknown perpetrator. During committing of individual crimes, more than one piece of fire arms was used. In these cases, in addition to some other forensic procedures, DNA traces were examined and sampling was done. On the fire arms and other traces with the reasonable suspicion of their originating from the perpetrator or person linked with perpetrator of those crimes, sampling was done aimed at providing evidence of the presence of the biological traces. The above mentioned sampling involved taking smears from the fire arms in question, as well as other traces, or their proper identification during the crime scene investigation, and in the form they were found, they were sent to the foreign labs. In the three out of the five cases, DNA profiles were identified on the two pieces of fire arms, involving two of the above mentioned cases, and result was negative in two cases. In the three above mentioned cases, two perpetrators were identified, involving murder case with the use of fire arms and in one case in spite of identifying DNA profile, the case remained unsolved and it is under investigation. Pioneer attempts are carried out in Montenegro, aimed at implementing this method, primarily in regard to proper detection, documentation, taking and submitting traces to be subjected to these analyses. For the short past period, good results were achieved in terms of forensic examination carried out by the Labs in Wiesbaden and Ljubljana. Also, it is very important, both in the regard to the experience exchange and presenting, that those types of analyses were carried out on different places, which can point out new knowledge, the labs quality, and international cooperation in the field of fighting crime. The Police in Montenegro, together with the forensic lab, will continue with the specialized education in regard to DNA analyses, as well as to further international cooperation with the relevant foreign labs aimed at more efficient sampling of traces and crimes committed by fire arms.

Presentation number: F13

LEGAL ASPECTS OF FORENSIC DNA ANALYSIS IN A RAPE CASES (CASE REPORT)

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During the August 2004 year in Skopje the capital of Macedonia serial rapes were done. On the clothes on one of the victim the traces of semen were found. From the police Photo portrait for suspected person was made, according to the descriptions of the victims. One man was arrested who looking like a person on the Photo portrait. The police found that traces of the semen on the clothes of the victim were B group. In the driving license of the suspected person B blood group was written. He was arrested in a jail for 30 days during the investigation procedure. His advocate was asking a DNA analysis to be made to prove his innocence. In the DNA laboratory of the Institute for forensic medicine in Skopje from the suspected person blood and buccal swab was taken. It was confirmed that the suspected person has blood group A. Then DNA analysis from the traces of the semen and from the blood of the suspected person with AmpIdentifilerTM kit was done. It was found that gene profile of the semen traces on the clothes of the victim and blood from the suspected person has no match. Consequently suspected person was realized.

Presentation number: F14

DNA EVIDENCE AND PERSONAL SAFETY: THE CRIME SCENE INVESTIGATORS AND TECHNICIANS' LEVEL OF KNOWLEDGE AND SAFETY MEASURES IN CROATIA

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Crime scene investigators and technicians face the risk of contracting HIV or HBV infection during search, collection and preservation of biological evidence. This presentation shows the results of the survey the purpose of which was to determine: (1) general knowledge of HIV/AIDS and hepatitis; (2) the extent of training received; (3) the extent of personal protective equipment (PPE) use; (4) self-assessment of the risk for blood-borne pathogen infection; (5) attitudes toward working together with HIV positive colleagues. The survey was conducted as the first part of a project aimed to establish training program and guidelines for procedures and uses of PPE for crime scene investigators and technicians in Croatia. An anonymous, nonobligatory questionnaire was distributed to all crime scene investigators (63) and crime scene technicians (369) employed in 20 Police Departments. The overall response rate was 72,7%. Subjects showed good general knowledge of blood-borne pathogens and their modes of transmission, with the exception of transmission by casual contact. Less than 50% of subjects have learned about precautionary measures during professional training. Latex gloves and protective overalls are in frequent use, but other items are used inconsistently or not at all. A majority of subjects stated that the main reason for not using PPE is not being equipped with it (63,5%). Although a majority of subject stated that they have frequent contact with biological evidence, 11% forget to use PPE, and 11% think it is unnecessary. A large majority of subjects assessed the risk of infection as high (83,8%). An even larger majority (84%) expressed a need for additional training. Crime scene investigators and technicians should be provided with PPE designed to protect them against blood-borne pathogen hazards. The development of blood-borne disease training programme and written guidelines for precautionary measures and use of PPE for crime scene investigators and technicians are needed to increase their preventive practices.

Presentation number: F15

DNA USED TO IDENTIFY A PRESIDENT'S BODY

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In February 2004 there was a plane crash in the area of Mostar. Plane belonged to the Macedonian government and Macedonian president Boris Trajkovski was one of the nine people on the board. The plane was heading from the Macedonian capital, Skopje, for the town of Mostar, where Mr. Trajkovski and the group of advisers were to attend an investment conference. The crash happened in the area of the mine fields, so the search took more time than expected. All nine died and the remains were found two days after. They were so badly damaged which made classical identification almost impossible and DNA analysis remains the only tool for their identification. Only one body was not burned and was identified by dental record, height and age. Samples from eight remains (tissue and bone) were taken to the lab for DNA analysis. To determine identity of each body, blood samples from relatives of missing persons were taken. DNA from bones was isolated by organic extraction method and from tissue using QIAamp kit. DNA from blood stains was isolated with Chelex-100 reagent and QIAamp kit. PowerPlex 16 System, PowerPlex Y System and AmpFlSTR Identifiler kits were used for PCR amplification of STR loci. President's body was identified from blood samples provided by his father and twin brother. Seven other people were identified by DNA test as well.

Presentation number: F16

OVERVIEW OF POPULATION GENETIC STUDIES BASED ON PHENOTYPE AND MOLECULAR MARKERS IN BOSNIA AND HERZEGOVINA DURING THE LAST THREE CENTURIES

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Many historical episodes marked Bosnia and Herzegovina as major ethnic crossroads, which makes it very interesting object for various population studies. The roots of anthropo-genetic and population-genetic studies in Bosnia and Herzegovina may be recognized in preliminary works accomplished 120 years ago. In fact, the first known bio-anthropological studies, initiated by Austro-Hungarian army physicians at the end of the 19th century, present initial phase of exploration of human genetic diversity in Bosnia and Herzegovina. The first stages of this long term and complex process were based on observation of numerous phenotype markers: blood types, color blindness, PTC sensitivity, lobulus auricilae shape, thumb extensibility, etc. This period lasted over one hundred years and was illustrated by various studies. These studies varied mainly in type and number of the observed markers, type of examined populations and the level of analyzed data. The following phase that was relatively short was dominated by different cytogenetic markers. Finally, at the beginning of this century, molecular-genetic diversity of BH population became the focus of modern research. Autosomal and Y-STR markers were initially used in examination of isolated as well as complete human population of modern Bosnia and Herzegovina. Mitochondrial and haplogroup diversity of isolated mountain populations was also described and compared with similar regional populations. The most recent study describes distribution of Y-chromosome haplogroups in the three main ethnic groups of Bosnia and Herzegovina and generates preliminary scenario for the process of peopling this area. The analysis of Y-chromosome variation in the three major ethnic groups in the modern Bosnia and Herzegovina reveals that Bosnian Croats, Bosnian Serbs and Bosniacs harbor haplogroups that they share with many other European nations. This experience may and ought to be used as solid foundation for further efforts in examination of notable genetic diversity in human populations in such a small country.

Presentation number: F17

VALIDATION OF A NOVEL mtDNA SEQUENCING STRATEGY AND THE AB-3100 GENETIC ANALYZER FOR PROCESSING ANCIENT SKELETAL REMAINS

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The objective was to validate a novel mtDNA sequencing strategy and the ABI PRISM® 3100 Genetic Analyzer for processing ancient skeletal remains in a high-throughput manner. This sequencing strategy calls for a DNA mass ladder to be run along with the amplified products. The intensity of the amplified products is compared to 20ng and 60ng bands, to determine the final centricon and sequencing template volumes. Preliminary experiments assessed data quality when using Edge BioSystems Performa® DTR 96 well standard filtration plates (standard plate) versus the Edge BioSystems Performa® DTR 96 well short plates (EDGE BioSystems, Gaithersburg MD) for purification of full strength, half strength and quarter strength Applied Biosystems BigDye® version 1.1 sequencing products. Standardization of sequencing volumes coupled with the ability to sequence in a 96-well format using ½ reaction BigDye® v1.1 and the 96-well standard plates for purification was more cost effective and reduced the number of man hours needed to process samples. The new procedure should help alleviate the sequencing bottleneck to process mtDNA case samples by lowering the amount of resequencing due to miscalculations in input template DNA. In addition, a linear reduction in relative fluorescent units was observed when samples were re-injected at 7 and 5 seconds instead of 15 seconds, again saving time and money by preventing the need to re-sequence. Finally, this presentation will also discuss anomalies observed during the mixture portion of this validation. The views expressed in this abstract are those of the author and do not reflect the official policy of the Department of the Army, the Department of Defense or the U.S. Government.

Presentation number: F18

LOW DENSITY MICROARRAYS FOR FORENSIC DNA ANALYSIS

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The aim of this work was to develop an oligonucleotide microarray for forensic studies. The developed microarray allows analyzing 9 alleles of gene HLA-DQA1, 5 alleles of gene ABO and 2 alleles of gene AMEL and thus can present some useful searching information about blood group and sex of crime suspect. The procedure includes two-stage PCR with fluorescently labeled primers and hybridization with oligonucleotide microarray. The fluorescent signal was analyzed using special chip-reader equipped with CCD-camera. The developed microarray can determine 0101, 0102, 0103, 0106, 0201, 0301, 0401,0501, 0601 alleles of gene DQA1; A, B, O1, O1V, O2 alleles of gene ABO; AMELX and AMELY. The method allows sorting all people out 1350 groups. In future we plan to extend the limits of the assay. The present microarray was tested with DNA samples isolated from fresh blood, saliva and spot of blood from locus delicti. Also some samples were sequenced and/or analyzed by allele specific PCR-kit (“DNA-Technology”, Moscow, Russia). The sensitivity of the method is about 10-100 pg per analysis. CONCLUSIONS: The microarray was developed for person identification by analyzing HLA-DQA1, ABO, AMEL loci. The principle possibility to use gel-based microarray technology in forensic studies has been shown. Now, we plan to carry out the population study of the allele frequencies for DQA1 and ABO loci in Moscow region. This work was supported by ISTC (3086).

Presentation number: F19

IDENTIFICATION OF SPECIES OF BLOODSTAINS BY THE USE OF GEL ELECTROPHORESIS

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One of the most important evidence at the crime scene is the bloodstain. It can provide valuable data that help in the identification of the suspect. So, it is important that bloodstain to be properly collected and preserved whatever the amount with avoiding bacterial contamination. Sometimes, the murderer can dispose of the victim's body and try to clean any trace evidence including blood. Though, tiny particles will cling to most surfaces for many years and can detected by special tests such as luminol test, orthotoulidine test, and leucholmalachite test. The detection of the species of bloodstain is essential to forensic scientists. Such knowledge is employed in solving cases ranging from minor hunting offences to assault or murder investigations. This study was conducted to study the possibility of identification of the species of bloodstain by using sodium dodecyl sulphate polyacrylamide gel electrophoresis. Four parameters were taken into consideration; including, the number of bands, rate of flow (Rf), molecular weight (MW), and the areas of no bands. This study was performed on 22 samples of bloodstain, which were divided into human and animal groups. In this study, some samples showed identical rate of flow and molecular weight at the same time. The areas of no bands could be as an additional feature of this study for proper identification of the species of the bloodstain. The use of SDS-PAGE is more advantageous than other methods used for identification of the species of the bloodstain as less expensive chemicals were used, no need for large number of antibodies in addition to rapidity of the test.

Presentation number: F20

SEQUENCE ANALYSIS OF MITOCHONDRIAL DNA HYPERVARIABLE REGIONS IN THREE IRANIAN MATERNAL GENERATIONS: AN APPROACH TO PERSONAL IDENTIFICATION

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Mitochondrial DNA (mtDNA) sequence analysis of the hypervariable control region has been shown to be an effective tool for personal identification. The high copy and maternal mode of inheritance make mtDNA analysis particularly useful when old age or degradation of biological sample prohibits the detection of nuclear DNA analysis. This study reports mtDNA polymorphisms in both HV1 and HV2 of the non coding D-loop region from 30 Iranian persons (10 unrelated families in 3 sequential maternal generations). 81 polymorphic nucleotide positions were found, 32 in HV1 and 49 in HV2. The sequence of HV1 and HV2 and occurred polymorphism were completely similar in each family, except for heteroplasmy in 5 positions in HV2. The average numbers of nucleotide differences between families were 5.2 nucleotide in HV1 and 2.8 nucleotide in HV2, so we expect 8 nucleotide differences in sequence of both HV1 and HV2 in two unknown, unrelated Iranian samples.

Presentation number: F21

RECOVERY OF TRACE DNA USING HYDROSOLUBLE TAPE AND THE NVITROGEN CHARGESWITCH® FORENSIC DNA PURIFICATION KIT

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DNA analysis of forensic biology samples requires a separation of biological material from sample collection devices such as swabs, adhesive tape or fabric during the extraction process. This separation occurs by a transfer or filtration step; however some biological material can remain on the collection medium or filters. The successful analysis of trace DNA, the DNA that is transferred to surfaces by handling or skin contact, largely depends on the amount and quality of transferred DNA available for analysis. This study examines the use of hydrosoluble adhesive tape for the recovery of trace DNA and saliva transferred to skin and drinking bottles, with DNA isolation performed using the Invitrogen ChargeSwitch® forensic DNA purification kit. DNA is released directly into lysis solutions by dissolution of the tape; therefore all collected DNA is available for analysis. DNA is isolated and purified using the ChargeSwitch® magnetic bead system. Trace DNA profiles of the handlers were recovered after ten seconds of hand-to-arm contact and bottle handling. Deposited saliva was readily recovered and profiled from the arms of volunteers and bottle mouths. The tape and ChargeSwitch® combination allows a direct flow from sample collection, cell lysis and DNA purification. No column purification, vacuum filtration or additional tube transfer steps are required. This method has been shown to be a sensitive tool for forensic DNA analysis and has potential for automation.

Presentation number: F22

NEW EXPERIENCES WITH DNA EXTRACTION FROM SKELETAL REMAINS OF VARIOUS AGE

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DNA extraction and subsequent identification of skeletal remains continue to be very problematic not only in cases of old historical bones but in cases of recent skeletal remains concerning the forensic cases, as well. Obtaining the DNA in a sufficient amount and quality is the crucial step during DNA analysis of bone remains. The tested material is often contaminated with the DNA coming from some other biological species (bacteria, fungi, etc.) which can disable the whole DNA analysis. That is why, the success of the DNA analysis of bone remains does not often depend only on the age of these remains. In this project we focused on the DNA extraction from skeletal remains of various age with using of different extraction methods. The oldest tested bones came from the bronze age and the youngest ones from the half of the 20th century.

Presentation number: F23

SIMULTANEOUS TYPING OF mtDNA A, B, C, D AND X HAPLOTYPES USING A MULTIPLEX MINISEQUENCING BASED METHOD

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Mitochondrial DNA (mtDNA) is a small circular molecule, present in high copy number per cell and maternally inherited as a haplotype; these characteristics make it an ideal tool in the attempt to understand populations' migration through the analysis of both living individuals and ancient human remains. Restriction Fragment Length Polymorphism (RFLP), for mtDNA haplotyping, has recently been replaced by single nucleotide primer extension, or minisequencing, through the use of the SNaPShot kit. This allows the simultaneous typing of regions that may lay far apart on the genome thus making the analytical process faster and less labor intensive. Furthermore sensitivity and automation are enhanced by the aid of fluorescent capillary electrophoresis. The application of the SNaPshot kit to mtDNA haplotyping has been previously reported for Caucasian population but not for Native Americans. We developed a new rapid and robust essay based on multiplex amplification of five different fragments, that include the polymorphic sites, followed by minisequencing, in order to screen mtDNA for haplotypes A,B,C,D and X for the analysis of Native American populations. This method was developed for cost-effective, high throughput analysis of DNA samples from living individuals and for the future application in the analysis of ancient mtDNA extracted from bones found at different archeological sites in Central America. Since ancient samples generally yield highly degraded DNA, 5 different primer sets were designed to generate amplicons all shorter than 190bp; 5 minisequencing primers, annealing one base short from each polymorphic site, were then simultaneously used for the final haplotype determination. Results obtained with the minisequencing reaction were confirmed with cycle sequencing analysis and sensitivity tests were conducted showing that 0.001ng of DNA could be successfully typed. Furthermore, this multiplex essay can be implemented with the analysis of other polymorphic regions of the mtDNA thus increasing the significance of the information that can be obtained from each sample with a single procedure. In conclusion this method has proven to be robust, cost-effective and highly sensitive and we believe that it can be successfully applied for mtDNA haplotype screening of samples from living individuals as well as from ancient human remains.

Presentation number: F24

COMPARATIVE ANALYSIS OF THREE DIFFERENT APPROACHES IN DNA EXTRACTION FROM VARIOUS BLOOD STAINS

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With only a slight modification, the standard molecular techniques can be very useful for obtaining and interpreting the final results in the field of forensic genetics. Successful DNA typing of various biological evidences found on crime scene is based on optimization of numerous factors. One of the most important critical phases of whole procedure is determining the most convenient DNA extraction procedure. There are plenty DNA extraction protocols that can provide enough DNA for following analysis. Nevertheless, deciding on a proper DNA isolation procedure still presents a certain challenge in forensic casework. Referent blood samples (3.5 ml) were collected from voluntary donor by use of BD Vacutainer System with EDTA anticoagulants (Beliver Industrial Estate, Plymouth UK) and immediately transported to the laboratory at the Institute for Genetic Engineering and Biotechnology. The samples were stored at -80^{o}C until DNA was extracted. These samples were used in preparing six bloodstains on various materials (woody table surface, glass, cotton shirt, filter paper, FTA card and jeans). For extraction of DNA from all of these samples, three different procedures were used: the Qiagen DNaeasy^{TM} Tissue Kit (Qiagen Co. 2001), water elution procedure and standard organic extraction. DNA was detected and quantified by QuantiBlot; assay. The PowerPlex 16 kit (Promega Corp., Madison, WI) was used for simultaneous PCR amplification of 15 STR loci. Amplification was carried out as described previously (Promega Corp. 2001). The total volume of each reaction was 25μl. The PCR amplification was carried out in PE Gene Amp PCR System Thermal Cycler (ABI, Foster City, CA). Electrophoresis of the amplification products was preformed on an ABI PRISM 377 genetic analyzer (ABI, Foster City, CA), using 5% bis-acrilamide gel. The results of quantification, the quality of obtained STR profiles, the time required for processing all samples and calculated costs indicate advantages and disadvantages of examined methods.

Presentation number: F25

GRIFFON VULTURES POISONING

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On the 17th of December 2004, ten dead griffon voltures were found on the uninhabited part of the island Rab, Punti Sorinju. All ten griffon voltures were delivered to the Faculty of Veterinary Medicine so pathology search and toxicology analyses can be done. With x-ray search it was established that the voltures had not been killed with a canister shot. It was assumed that the voltures were poisoned with food. Vomit, livers, contents of the crops and stomach were delivered to the private forensic laboratory 'DAN-JAR' for toxicology analyses, total 24 samples. Extraction and isolation were accomplished on all delivered samples. Given extracts were analysed by gas chromatography with the mass detector (GC 6890N, MSD 5973 Agilent Technologies). The temperature of column (HP-5MS, 30m) ranged from 90 ºC to 250 ºC and increased by 15ºC per minute. Helium flew at 1.5 mL/min. Other conditions included: the column, HP-5MS, 30 m; injector, splitless 250 ºC and the detector, MSD, 300 ºC transfer line. Chemical compounds were found with the following mass: m/z 164, 149, 122, 221 that are the mass of carbofuran; m/z 137, 180, 162 that are the mass of the metabolite hydroxycarbofuran; m/z 164, 131, 147 that are the mass of phenol carbofuran. Carbofuran was identified in crops contents 10x, hydroxycarbofuran was identified in livers 2x and phenol carbofuran was identified in almost all samples, except in the intestines and crops No.6.

Presentation number: F26

SHORT TANDEM REPEAT POLYMORPHISMS FREQUENCIES IN CZECH GENERAL POPULATION

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Preparing of the short tandem repeat polymorphisms frequencies database for Czech general population was the main aim of these molecular genetic analyses. The collection of DNA samples was completed from 967 individuals of Czech general population from the regions of North, South and East Bohemia and North and South Moravia. The genetic loci used in this presentation are as following: TPOX, D13S1358, FGA, D5S818, CSF1PO, D7S820, D8S1179, TH01, VWA, D13S317, D16S539, D18S51, D21S11. Molecular genetic techniques used in this population genetic testing are basic equipment of molecular genetic laboratory. We extracted deoxyribonucleic acid by classical isolation procedure from human peripheral blood leukocytes. Amplification reactions of the tested chromosomal regions were realized by symmetric type of polymerase chain reaction (PCR). The visualization of polymorphic DNA fragments was carried out by using the capillary electrophoresis. Statistic assessment of genetic polymorphism frequencies in Czech general population and several parts of Bohemia and Moravia were calculated by following statistic programs: The result of this presentation is the comparison of allele and genotype frequencies between Czech general population and central, south, west and north European populations, but also the comparison of differences between parts of Bohemia and Moravia. These allelic frequencies will transfer to forensic genetics practice like the population genetic database for paternity testing and crime scene identification in Czech Republic. These population data results complete the CR situation in large population collection. These results were supported partially by grant no.: LN00B107, Ministry of Education, Youth and Sport, Czech Republic

Presentation number: F27

ELECTRONIC CHAIN OF CUSTODY FOR HIGH–THROUGHPUT FORENSIC LABORATORIES

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INTRODUCTION In the early 1990's armed conflicts erupted in the former Yugoslavia, resulting in a tremendous loss of human life. By the time the conflicts ended in 1999, up to an estimated 40,000 persons remained missing. The International Commission on Missing Persons (ICMP) was founded at the G-7 conference in Lyon France in 1996 to help resolve the fate of the missing in the former Yugoslavia. By June of 2000, ICMP began collecting blood samples from family members who were missing loved ones. In concert with this action, four DNA laboratories, each designed to address a specific DNA testing need, were constructed. The creation of a population based DNA-led identification program was unique and many obstacles were faced. INITIAL PROBLEMS The necessity to preserve a forensically sound Chain of Custody (COC) is a critical part of laboratory practice. As the ICMP continued to improve its extraction protocols, the number of profiles extracted from bone samples quickly grew. The amount of labor required to track these samples grew in parallel. The need became apparent that a more efficient mechanism was needed to track the COC and also to provide Quality Assurance. The previous method used in ICMP's Laboratories to track the COC was entirely paper based. This required that each sample container had to be manually labeled in conjunction with the accompanying COC paper work. This part of the process was seriously impacting the performance and capacity of the Laboratories. SOLUTION The ideal solution was to develop an electronic COC and Quality Assurance for all samples as well as the functionality to view and report on the progress and status of any sample in ICMP laboratories at any time. To accomplish this efficiently the software needed to electronically identify samples, technicians, workstations, equipment and chemicals used throughout the entire process. Data collection, monitoring adherence to protocols, Quality Assurance and reporting were the core values used in the prototyping of the Laboratory Information Management system (LIMs).

Presentation number: F28

TAQ POLYMERASE REVERSES INHIBITION OF QRT-PCR BY HUMIC ACID

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Aim: To investigate the influence of humic acid (HA) and extra addition of Taq polymerase on Quantitation Real Time PCR (QRT-PCR) and test method on DNA extracted from ancient bones. Methods: Weak amplification of human target and no amplification of Internal PCR Control (IPC) may indicate a partial PCR inhibition in the sample. When the template is extracted directly from bone or other samples, humic acid can inhibit Taq polymerase. Inhibition is an especially significant problem when DNA has to be extracted from old and ancient material. In this study, we described the dose-response effect of humic acid on QRT-PCR inhibition and the effect of extra addition of Taq polymerase in overcoming the humic acid inhibition. By performing that method we also evaluated ten ancient DNA extracts on the quantitation by QRT-PCR. Results: The addition 10 - 75 ng of synthetic humic acid (Fluka) can inhibit QRT- PCR while the addition of 100 ng of synthetic humic acid completely inhibits QRT- PCR. The addition of 1,25 Unit (U) of Taq polymerase per assay (25ml) appeared to be the optimum amount in overcoming the humic acid inhibition. The best results were obtained when crude DNA extracts containing humic substances were quantified by QRT-PCR, with extra addition of 1,25 Unit (U) of Taq polymerase per assay. Conclusion: This modified procedure (with extra addition of Taq polymerase) should allow more effective QRT-PCR analysis in humic acid-containing samples.

Presentation number: F29

GENOTYPING OF THE POLYMORPHIC STR LOCI HUMVWA, HUMFES, HUMTPO, AND HUMTH01 IN IRANIAN POPULATION: A STUDY FROM THE HISTORICAL PROVINCE OF ISFAHAN

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Genotyping for four short tandem repeat (STR) loci HUMvWA, HUMFES, HUMTPO, and HUMTH01 was examined in 110 unrelated individuals from the population of Isfahan province of IR Iran. The loci were genotyped using the polymerase chain reaction (PCR) followed by polyacrylamide gel electrophoresis (PAGE) and silver staining. The data demonstrated that the tetranucleotide repeat markers were all highly informative in the population examined. The STR markers were also found to have a relatively high degree of heterozygosity. Forensic and paternity indices including power of discrimination and exclusion as well as polymorphism information content and typical paternity index were determined for the STR alleles examined. Therefore, the STR loci studied have proven useful for paternity testing and individual identification in Iranian population, and the data could be used in construction of a genetic database.

ABSTRACTS OF POSTERS
MAYO CLINIC COURSE IN ADVANCED MOLECULAR AND CELLULAR MEDICINE

Presentation number: M1

EXPRESSION OF FERROPORTIN (IREG1) AND IL-6 GENES IN MACROPHAGES OF HEALTHY C57BL/6 AND BALB/C MICE STIMULATED BY KILLED TRYPANOSOMA CONGOLENSE PARASITES

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BALB/c mice show high susceptibility to Trypanosoma congolense infection compared to relatively resistant C57BL/6 mice. Evidence shows that susceptibility to this infection depend upon high level of iron in the body, and resistant mice show anaemia. The aim of present study was to examine if killed parasite has ability to influence iron release in macrophages of susceptible and resistant mice. Macrophages were isolated from spleen of healthy mice and cultured in the present of IFN-γ and LPS and exposed to human red blood cells (RBC) opsonized with anti-human RBC. Positive culture wells were stimulated with 1×10^6 killed T. congolense parasites. Total RNA extracted from spleen macrophages of C57BL/6 and BALB/c mice by TRIZOL. cDNA was obtained using reverse transcriptase and amplified by quantitative (real-time)-PCR using IREG1 and IL-6 primers. Expression of IREG1 (a putative transporter of iron from enterocyte to circulation) and IL-6 (a type II cytokine which was included because of its predicted effects on IREG1) genes were compared by relative quantification. The results showed that expression of IREG1 and IL-6 genes are significantly up-regulated in spleen cells of resistant C57BL/6 mice compared to susceptible BALB/c mice, either stimulated or non-stimulated with killed T. congolense. Moreover, significantly down-regulated expression of IL-6 gene was shown in cells of BALB/c mice induced by T. congolense compared to cells without stimulation. These results indicate that healthy resistant mice have ability to increase iron level via up-regulation of IREG1 gene, compared to susceptible mice. In addition, killed parasites have no influence on expression of IREG1 gene in resistant mice. However, simultaneously up-regulated expression of IL-6 gene in macrophages of resistant mice may be indication for a balance needed for haemostasis of iron in cells, because up-regulation of IL-6 gene expression may results in increased production of hepcidin, which leads in blocking iron release from macrophages. Moreover, a down-regulated expression of IL-6 gene in BALB/c mice spleen cells stimulated with killed parasite in comparison to non stimulated cells showed that there may be susceptibility in this strain of mice to increase iron in the presence of killed T. congolense parasites via down-regulation of IL-6 gene which results in decrease of hepcidin production in macrophages.

Presentation number: M2

MODULATION OF IMMUNE-RESPONSE RELEVANT GENES EXPRESSION IN TREFOIL FACTOR 2 (Tff2) KNOCK-OUT ANIMALS

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The human trefoil factor family is differentially expressed along the gastrointestinal (GI) tract and is considered to play an important role in maintaining the integrity of the mucosa. While Tff1 and Tff3 knock-out mice show serious functional disturbance in stomach and gut, Tff2-/- constructs do not reveal specific gross or microscopic abnormalities. To determine the function of the Tff2 protein we determined molecular changes following Tff2 deficiency. We analyzed differences in expression of mRNA of 12.000 genes (Affymetrix) in Tff2 expressing GI tissue (pyloric anthrum/ Brunner's glands), of wild type and Tff2-/- animals (n=3). The genes with the significantly changed expression where identified using the Significance Analysis of Microarrays (SAM) program. We found 70 genes to be up regulated and 50 genes down regulated by factor >1.6. The expression of several interesting genes involved in immuno-response was analysed by quantitative real time PCR (qPCR) in animals (n=12) using Sybergreen detection in several tissues: (1) pyloric anthrum with Brunner's glands, (2) duodenum and (3) jejunum/ileum. qPCR measurements were normalized vs. 2 most stable out of 3 house keeping genes (Gapdh, Gusb, Mhkg) using GeNorm program. We found that gene expression of several components of MHC class I presentation pathway (proteasomal subunits: LMP2, LMP7 and PSMB5; TAP1 transporter responsible for importing degraded antigens in ER, and chaperone modulating gene product BAG2) were modulated. These specific changes, together with significant up-regulation of several intestinal microbicidal proteins (cryptdins), demonstrate modulation of genes involved in important mechanisms of the immune system and demonstrate an indirect interplay of TFF2 with immune response.

Presentation number: M3

QUALITY OF RNA OBTAINED FROM MICRODISSECTIONS USING UV CUTTING

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Microdissection has been widely used in scientific research as a tool for procuring pure cell populations for high fidelity molecular analysis. Different types of lasers have been utilized for microdissection. However, the effects of UV laser exposure on the quality of nucleic acids—especially RNA due to its fragile nature—has not been thoroughly investigated. In this report, we evaluate the effects of microdissection using UV laser cutting on the quality of total cellular RNA and explore how such effects may alter down-stream analysis. Variables studied include the type of laser used for microdissection and size of microdissected sample ranging from single cells to large areas.

Presentation number: M4

THE ROLE OF EBV IN SPONTANEOUS ABORTION

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The incidence of spontaneous abortions is 12-15%, and 25-60% are with chromosome abnormalities. In most cases that abnormalities include triploidy, tetraploidy and polyploidy. The theory of “two hits” for one unstable cell cycle resulting with aneuploidy is still in the basis of these events. From the genetic counseling archive we retrospectively analyzed the documentation from 500 couples. Chromosome analysis of both parents from peripheral blood shows karyotypes. Serological analysis shows significant findings for EBV in mother’s blood samples, as a result of a reactivation during pregnancy. Reactivation was confirmed by positive IgM VCA and/or IgG VCA and/or EA IgM or IgG antibody, with positive EBNA IgG antibody. Aneuploidy in aborted material was analysed by flow cytometry. A retrospective analysis was done in couples with one or more spontaneous abortions, and 296 paraffin embedded samples of aborted material were available. 41 placenta was analyzed by flow cytometry, of which 27 (66%) were diploid, and 14 (34%) were aneuploid. Thirty percent had IgM or/and high IgG for EBV. All paraffin embedded samples of aborted material were analyzed for EBV by PCR-real time. Ten samples were positive. We could not confirm EBV by in situ hybridization. We conclude that the placental EBV infection was not the direct cause of the abortion. We discussed about the causes of nondisjunction in spontaneous abortions. The theory of “two hits” was in the basis of our explanation. The first hit could be one of the viruses (EBV). The second hit could be other genital infections and/or environmental agents. In the causes of recurrent spontaneous abortions with de novo chromosomal changes there must be a big place for viruses and other environmental mutagens.

Presentation number: M5

ANALYSIS OF AZF GENE COMPLEX IN IDIOPATHIC MALE INFERTILITY

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Ten to twenty five percent couples encounter difficulty to procreate. Microdeletion of the long arm of the Y chromosome, are associated with spermatogenic failure and have been used to define three regions on Yq (AZFa, AZFb and AZFc) which are critical for spermatogenesis. In the last few years considerable progress has been made towards understanding sperm physiology and the biology of gamete interaction to understand the pathophysiology of male infertility. Also with advent of Assisted Reproductive Technology (ART) and Intracytoplasmic sperm injection (ICSI) knowledge about the various factors leading to spermatogenic impairment is one of the most important aspects of scientific research. About 50% of couple infertilities are of male origin, some of them caused by genetic abnormalities of the Y chromosome and have been used to define three regions on Yq (AZFa, AZFb and AZFc) which are critical for spermatogenesis. Deletions in AZF region can cause severe spermatogenic defects ranging from non-obstructive azoospermia to oligospermia These 3 loci act at different stages of germ cell development and deletion of each loci result in a characteristic phenotype. Deletion of AZFa, AZFb, AZFc results in Sertoli Cell Only syndrome (SCO), maturation arrest and hypospermatogenesis respectively. So far four groups from India have published their data on male infertility using molecular methods. Idiopathic oligozoospermic and azoospermic cases were included in this study. Cytogenetic and semen analysis was done in each case. Testicular Fine Needle aspiration Cytology was collected whenever possible. On cytogenetic analysis Klinefelter Syndrome (KFS) was the major cytogenetic anomaly followed by mosaic KF, variant Klinefelter, and cases with other numerical and structural cytogenetic abnormalities. In cytogenetically normal cases microdeletion analysis was done using STS-PCR approach using primers sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc). Microdeletions spanning the AZF loci were found in 8% cases and genotype, phenotype correlations exists. Cases with AZFa and AZFb microdeletions were associated with Sertoli cell Only Type I syndrome (SCO type I) and cases with AZFc deletions had a variable phenotype ranging from Sertoli cell Only Type II syndrome to hypospermatogenesis to maturation arrest. Variation in testicular phenotype in cases with AZFc deletion is due to multiple copies of the gene and presence of autosomal homologues. Cases with AZFc microdeletion show a progressive decline in semen quality thus these cases are counseled to go in for semen cryoconservation at the earliest age should they opt for ICSI at a later date. Thus various factors genetic, epigenetic and environmental modulate the effect of these genes. The FSH and LH levels were elevated in these cases. In a large number of cases with AZFc microdeletion there is a population of germ cells with XO cell line and these cases are counseled prior to going in for ART about the risk of male offspring being born with sexual ambiguity, turner phenotype in addition to being infertile. Deletion on Y chromosome makes the Y chromosome more prone to secondary larger deletions resulting in worsening of testicular phenotype. Thus in a large number of idiopathic cases of male infertility there is a genetic basis and in the other normal cases need to further evaluated. Therefore presence of cytogentic anomalies and detection of Yq microdeletions encompassing the AZF loci determines the prognosis and management of these infertile cases.

Presentation number: M6

MUTAGENIC EFFECTS OF CARTAP HYDROCHLORIDE ON PERIPHERAL BLOOD CULTURES

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Pesticides of worldwide application are used in agriculture in vast amounts each year. Cartap is a very commonly used pesticide and there are very few studies on the cytotoxic and genotoxic effect of these pesticides. In this study we analyzed the effect of increasing dose of Cartap on peripheral blood cultures by studying the mitotic index and chromosomal aberrations like breaks, gaps, acrocentric associations, polyploidy, acentric fragments and endoreduplication. There was a significant increase in chromosomal aberrations and significant decrease in mitotic index with increase in concentration of cartap hydrochloride. In men occupationally engaged in farming and using this pesticide measures should be taken to minimize or prevent exposure to Cartap as long term use may lead to bioaccumulation in the body and several adverse effects like cancers. In light of several recent studies highlighting the side effects of these pesticides the debate around pesticide exposure and the onset of cancer that has lead to controversial bylaws regulating pesticide use. It seems timely to review the carcinogenic and genotoxic potential of commonly used pesticides like Cartap. The purpose of this study is to present some debated epidemiological research that deals with the relationship between pesticide exposure and cancer, and to review and update the literature on in vitro mammalian carcinogenic and genotoxic potential of these pesticides.

Presentation number: M7

CHROMOSOMAL ABERRATIONS AND REPRODUCTIVE FAILURE

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Chromosomal abnormalities and aneuploidy are found to be associated with and have a higher prevalence in infertile males than in the general population. These aberrations not only result in partial or complete spermatogenic arrest but may also result in implantation failure and consequently failure of In Vitro fertilization (IVF). Assisted Reproductive Technology (ART) has revolutionized the management of infertility and allows infertile couples to procreate. Cytogenetic and molecular analysis was done in 165 infertile males and 14 couples going in for IVF. Twenty five well spread G banded metaphases were karyotyped using image analyser (Cytovision, Applied Imaging). In mosaic cases 50 metaphases were analyzed. Chromosomal abnormalities were found in 46 infertile males. We found 16 cases with Klinefelter Syndrome (KFS), 20 cases were KF mosaics and 6 were mosaic variants, three cases with 46, XY 1qh+ and one case with 46, XY16h+. In one of the 14 infertile couples opting for assisted reproduction cytogenetic analysis in the female partner revealed 46, XXq- chromosomal complement. Though polymorphism of heterochromatin region of chromosome 1, 9, 16 and Y are well known but we found that in our study a significant correlation between additional heterochromatin (1qh+) and spermatogenic arrest. Deletion of long arm of X chromosome (Xq-) in the female partner might have resulted in repeated failure of blastocyt development. This couple had gone in for 4 IVF cycles which had failed due to failure of blastocyst development. The male partner was cytogenetically normal and also had no Yq microdeletion spanning the AZF loci. In cases with sex chromosomal and autosomal aberrations there is probability of poor embryo development and consequently poor implantation, which may be a result of high segregation abnormalities and may negatively affect the outcome of assisted reproductive techniques. Thus these infertile couples should be counseled prior to going in for ART about the importance of genetic analysis, and how the presence of genetic anomalies results in poor IVF outcome and vertical iatrogenic transmission of these anomalies to the offspring born through ART.

Presentation number: M8

KLINEFELTER'S SYNDROME AND ITS ASSOCIATION WITH HAEMATOLOGICAL MALIGNANCY

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Klinefelter's syndrome is the most common genetic cause of hypogonadism and male infertility. However many cases remain undiagnosed because of substantial variation in clinical presentation and insufficient awareness of the syndrome itself. It is the commonest sex chromosomal anomaly. These cases have one or more extra X chromosomes and has an incidence of 1:800 males.. It is an abnormality of sexual development characterized by infertility, seminiferous tubular dysgenesis, decreased virilization, tall stature and gynaeconmastia. In this syndrome the patients IQ tends to be below average and learning disabilities include dyslexia are common in these cases. These cases have low testosterone and elevated gonadotrophin levels. Lichiardopol C et al (2004) reported that the commonest cause of mortality in KFS is diabetes, cardiovascular, respiratory and digestive diseases. There is an increased incidence of breast cancer in men with KFS which is believed to be a marker of KFS but till date few cases with KFS and haematological malignancy have been reported. In our study of 125 infertile men we identified 29 cases with 47, XXY chromosomal complement. Of these 29 cases two cases had haematological malignancy. We report two KFS cases with Acute lymphocytic leukemia (ALL) and B type lymphoblastic lymphoma respectively.

Presentation number: M9

GENE EXPRESSION PROFILING OF MICRODISSECTED HUMAN BREAST CANCER CELLS FROM FORMALIN FIXED PARAFFIN EMBEDDED SECTIONS USING MICROARRAYS

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Tumor biopsies are commonly preserved as formalin fixed paraffin embedded (FFPE) tissues. Enabling high-throughput gene expression analysis of FFPE samples using microarrays will revolutionize the way tumors are studied. However, since the macromolecules in FFPE tissues are cross-linked, efficient extraction and isolation of RNA with quality adequate for microarray analysis from such samples poses a challenge. We have recently developed and optimized a novel process that integrates efficient isolation of total cellular RNA from FFPE sections, linear amplification of isolated RNA, and synthesis of labeled aRNA for microarray analysis. We have also designed custom microarrays that optimize performance of such samples. We have compared matching frozen and formalin-fixed samples to study the fidelity of gene expression ratios derived from both samples. Here we demonstrate that microdissected cells from FFPE sections can be used to study native expression levels of genes, and that replicate microdissections of pure tumor cell populations can be utilized to generate highly reproducible expression profiles. Further, we have successfully utilized this platform to identify molecular signatures from archived breast cancer biopsies, suggesting future application to clinical analysis.

Presentation number: M10

FRIEDREICH'S ATAXIA: ANALYSIS OF MITOTIC INSTABILITY

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Friedreich's ataxia is an autosomal recessive disease and it is the most common among inherited ataxias, with a frequency of 1 in 50 000 in the Caucasian population. Friedreich's ataxia is primarily caused by a homozygous GAA repeat-expansion mutation within intron 1 of the FRDA gene (96% of patients). Approximately 4% of patients are compound heterozygotes for this expansion and a point mutation within the same gene. The number of GAA repeats in normal alleles range from 5 to 33. Alleles with 34-65 GAA repeats are not associated with an abnormal phenotype but they have significant propensity to expand during parental transmission. Disease-causing alleles are characterized by the huge number of GAA repeats (66-1700) and somatic instability. Expanded alleles show a length-dependent increase in somatic variability. Routine genetic testing is performed by PCR based GAA repeat length analysis. In our laboratory we established more sensitive and precise method: small pool, long range PCR based Southern blot method. It is used for study of intra- and intertissue mosaicism and intergenerational instability of disease-causing alleles. Studying mosaicism in peripheral leukocytes in vivo, we have noticed that disease-causing expanded alleles are extremely unstable and have a strong natural tendency to contract. Understanding molecular mechanism(s) that cause contractions would be a chance to intervene in those mechanism(s) and develop potential therapeutic strategy.

Presentation number: M11

LIPOSOMES WITH SUPEROXIDE DISMUTASE PROTECT A2182 CELLS AGAINST OXIDATIVE STRESS

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Superoxide dismutase (SOD), an antioxidant enzyme, was encapsulated into liposomes in order to increase its releasing time and to facilitate its cellular penetration. Positively, neutrally, and negatively charged liposomes were prepared by the proliposome method. Differently charged liposomes were examined for the efficiency of delivery of Cu/Zn superoxide dismutase (CuZnSOD) to human lung epithelial cells, A2182, and their prospects of cell protection from oxidative agents. Untreated cells and cells pre-treated with liposome-encapsulated CuZnSOD were exposed to oxidative stress caused by xanthine/xanthine oxidase. Cellular antioxidant response was monitored for 4 or 24 h after the beginning of oxidative stress induced by the activity of superoxide dismutase (SOD) and total glutathione concentration. CuZnSOD-loaded liposomes increased the SOD activity of A2182 cells 24 h after treatment. The highest increase of cellular SOD, by 108%, was achieved using anionic liposomes. Exposure of untreated cells to oxidative stress increased the cellular glutathione level after 24 h. Cells pre-treated with liposome encapsulated CuZnSOD were protected from oxidative stress, as shown by the unchanged concentration of cellular glutathione.

Presentation number: M12

ALPHA$_{1}$-ANTITRYPSIN Z AND S ALLELES IN NORMAL SUBJECTS AND DISEASES RELATED TO ALPHA1-ANTITRYPSIN DEFFICIENCIES IN IRAN

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The distribution of high–risk phenotypes of alpha1-antitrypsin (AAT) in some regions of the world such as USA, Europe, and West Asia has been well studied; no reliable reports are available about these phenotypes and the related diseases in the Iranian population. Thus, we studied 307 whole blood samples of the patients suspected for diseases related to AAT deficiency (AATD) and 156 healthy persons. AAT phenotypes and genotypes (classified according to the Protease Inhibitor System) were determined by isoelectric focusing (IEF) and PCR-RFLP, respectively. Primers p7553 (5'-CGT TTA GGC ATG AAT AAC TTC CAG C-3') and P7720 (5'-GAT GAT ATC GTG GGT GAG AAC ATT T-3') were used to amplify a 149-bp fragment from the S mutation, while primers P9966 (5'-ATA AGG CTG TGC TGA CCA TCG T-3') and P10063 (5'-GAA CTT GAC CTC GAG GGG GAT AGA C-3') were used to amplify a 97-bp fragment from the Z mutation. Alleles and phenotype frequencies were compared between patients and normal subjects. For further reliability, a family study of patients and normal groups was also carried out. We found that among patients the frequency of the Z allele was 8.63 percent and of the S allele 7.49 percent. The corresponding values among normal subjects were 4.17 percent and 5.13 percent, respectively. Our results show that the frequency of S allele in Iran is in agreement with the frequency reported by the WHO. However, the frequency of Z allele in Iran seems to be twofold higher than the reported values.

Presentation number: M13

FRAMESHIFT MUTATION IN ZHFX1B GENE IN A CROATIAN GIRL WITH HIRSCHPRUNG DISEASE, MENTAL RETARDATION AND EPILEPSY (MOWAT WILSON SYNDROME)

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Mowat Wilson syndrome (MWS) includes distinctive facial appearance, intellectual impairment, seizures and other malformations such as Hirschprung disease, congenital heart defects and agenesis of the corpus callosum. It is caused by heterozygous deletions or truncating mutations of the ZFHX1B gene on chromosome 2q22. We would like to present a 13-years-old Croatian girl born to healthy and unrelated parents. Case report: The girl was born from uncomplicated pregnancy and delivered in term. Because of the short segment Hirschprung disease emergency colostomy was performed at 2nd day of life. Dysmorphia, hypotonia and failure to thrive have also been observed. Since the 1st year of life the antiepileptic therapy was introduced because of the seizures and pathologic changes on EEG. Brain MRI showed hypoplastic corpus callosum. At the age of 13 years she was normocephalic, but with severe mental delay, happy disposition and distinctive dysmorphic features: sparse hair and eyebrows, sloping forehead, telecanthus, broad nasal bridge, large ears, pointed chin and long fingers and toes. We suspected clinically to the possible mutation in ZHFXB1 gene and de novo frameshift mutation 1352delC (exon 8) in the heterozygous state was found. It corresponds to the delet ion of the nucleotide C 1352 of the gene coding sequence and results in a frameshift creating a STOP codon at position 453 of the protein. Conclusion is that all children with syndromic Hirschprung disease should be tested for mutation or deletion in ZFHX gene. The early diagnosis of MWS enables adequate genetic counseling and neuropediatric follow-up because in majority of children with MWS the epilepsy could appear during the first year of life, before the recognizable facial dysmorphic features characteristic for this syndrome.

Presentation number: M14

LINKAGE DISEQUILIBRIUM BETWEEN HLA-B, TNF-A AND MICA IN THE CROATIAN POPULATION

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The three nucleotide repeat locus MICA and dinucleotide repeat locus TNF-a situated in the HLA class I region were investigated in the Croatian population. One hundred and sixty-five healthy individuals previously typed for HLA class I and class II alleles were tested for 5 MICA alleles and 13 TNFά alleles on 6% polyacrylamide gel in an automated sequencer (ALFexpress). The strongest two loci associations were those of MICA-A4 with B*27 and B*18, MICA-A5 with B*15 and B*35, MICA-5.1 with B*0702, B*08 and B*44, MICA-A6 with B*44 and B*51, while MICA-A9 was associated with B*38 and B*35. Analysis of TNFa/HLA-B associations showed the strongest LD values for following combinations: TNFa-a2 with B*08, *15 and *51, TNFa-a6 with B*27, TNFa-a7 with B*44 and *51, TNFa-a10 with B*18, B*35 and B*38 and TNFa-a11 with B*0702. These data are important for the identification of disease associations and for a better definition of donor-recipient compatibility in bone-marrow transplantation program.

Presentation number: M15

MITOTIC EFFECTS OF ANTI-SNAKEBITE SERUM IN HUMAN LYMPHOCYTES AND ALLIUM CEPA ROOT-TIP CELLS

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Snakebite envenommation is a medical emergency calling for immediate treatment with antivenom. In our research we aimed on evaluation of mitotic activity in human lymphocytes and Allium cepa root-tip cells after cultivation and treatment with different concentrations of anti-snakebite serum. Allium test was performed to evaluate potential genotoxic effects of anti-snakebite serum, such as irregular phases of mitosis, multipolarity, laggards, C-mitosis. Mitotic index was calculated after observing at least 1000 cells per treatment and counting all stages of mitosis. In cultures of human peripheral blood lymphocytes, micronucleus cytokinesis blocked assay was performed. At least 1000 binuclear cells per treatment were counted and frequency of micronuclei determined. Nuclear division index was calculated on 500 cells. Results will be presented.

Presentation number: M16

IMPACT OF ENVIRONMENT XENOBIOTICS IN WAR AND POSTWAR PERIOD ON GENETIC CONSTITUTION OF EXPOSED BOSNIAN POPULATION

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As a result of war activities, citizens of Bosnia and Herzegovina were exposed to various potential genotoxic agents such as waste products of used ammunition, food conservancies, pharmaceuticals and food of suspicious origin and quality. All of those, together with everyday psychological burden, effected genetically constitution of exposed individuals. In our research 70 samples of peripheral blood from individuals directly exposed to various xenobiotics of environment during the war and postwar period were examined. Forty samples were taken from individuals additionally exposed to depleted uranium. Those persons inhabited area where depleted uranium contamination was confirmed by UNEP investigation (2003). In this research for assessment of genotoxicity, micronucleus cytokinesis blocked assay was performed. Results have shown increased number of binuclear cells with micronuclei as well as total number of micronuclei in local population exposed to depleted uranium.

Presentation number: M17

THYMIDYLATE SYNTHASE GENE POLYMORPHISMS IN CROATIAN POPULATION

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Thymidylate synthase (TS) is crucial enzyme in the nucleotide biosynthetic pathway because it catalyzes the reductive methylation of dUMP by 5,10-methylentetrahydrofolate to form dTMP which is very important reaction for cell proliferation. Thus, TS gene has been an important target for a variety of chemotherapeutic drugs auch as 5-FU. Inhibition of TS by such an agent causes cytotoxicity leading to thymineless death or sometimes chronic uracil misincorporation into DNA. Resistance to fluoropyrimidines which is not rare arises from many different mechanisms including TS protein expression. The human TS promoter region includes a cis-acting enhancer which is polymorphic containing two or three 28-bp tandem repeats and has been implicated in affecting on TS mRNA expression as well as TS mRNA translational efficiency. The majority of individual human TS alleles harbor either a double repeat (2R) or a triple repeat (3R) for this polymorphism, creating genotypes of 2R/2R, 2R/3R i 3R/3R. Individuals who are homozygous for the 3R were found to have elevated intratumoral TS mRNA and protein level compared with 2R homozygous. A novel G→C SNP in the second repeat of the 3R alleles identified recently has shown that the 3R sequence with G has three to four times greater efficiency of translation than the 3R with C and the 2R sequence. Genotypes 2R/3G, 3C/3G, 3G/3G are associated with high expression of TS and genotypes 2R/2R, 2R/3C and 3C/3C with low expression. Due to associations of the TS polymorphisms with the prognosis of several tumor types, we performed a study to determine the distribution of TS polymorphisms in Croatian population. A total of 125 healthy unrelated individuals were genotyped for the TS 5' UTR polymorphisms using PCR-RFLP method with HaeIII restriction enzyme. Genotype frequencies for 5' UTR TS polymorphisms were 26,4 %, 16%, 2,4%, 42,4%, 8,8% and 4% for 2R/3G, 3G/3C, 3G/3G, 2R/2R, 2R/3C, 3C/3C genotype respectively. Our results showed that in Croatian population low TS expression genotypes were more frequent (55.2%) than high TS expression genotypes (44.8%) but not significant.

Presentation number: M18

ASSOCIATION STUDY OF C5178A MITOCHONDRIAL DNA POLYMORPHISM IN SCHIZOPHRENIA PATIENTS FROM BOSNIA AND HERZEGOVINA

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Relationship between mitochondrial polymorphisms and certain neuropsychiatric disorders have been already assessed in various genomic and expression studies. C5178A polymorphism is located within region of NADH subunit-2 gene and was investigated for its relationship with psychotic disorders and personality subtypes. Suggestive connection of variants of 5178 mitochondrial polymorphism with major brain functions sets this site as an interesting to explore within the light of schizophrenia population sample. The frequencies of genotypes were observed in both, case and control samples (N=100) and observed against age at onset, familial history and other data relevant for disease.

Presentation number: M19

ULTRA RAPID DETECTION OF SALMONELLA TYPHI BY MULTIPLEX PCR

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Typhoid is a major health problem throughout the world. Globally, there are more than 16 million cases of typhoid fever each year, with more than 600,000 deaths. It is especially prevalent in developing countries. Rapid detection of the disease is very important for its control, but unfortunately, definitive diagnostic procedures are not available. Different techniques are used for the diagnosis of typhoid bacteria, including culture, biochemical identification, serological tests and ELISA, Widal and immunofluorescence. Molecular techniques target the pathogen itself (not antibodies produced against it), so they can be useful for the early detection of the bacteria. PCR technology has provided unparalleled sensitivity and specificity for the diagnosis of typhoid. In this study, we used the sets of 6 PCR primers for O, H, and Vi antigen genes (invA, tyv, prt) for rapid and specific identification of Salmonella enterica serovars typhi with multiplex PCR. The results showed that this assay correctly identified Salmonella serovars typhi and was able to distinguish these bacteria from other Enterobacteriaceae. To examine possible cross reactions of the selected primers among major enteric pathogens, including the several genera of the family Enterobacteriaceae, some strains were tested by the multiplex PCR assay; none showed positive results. This test was able to differentiate typhoid fever agents from other family of salmonella like: Salmonella typhimurium, S. parathyphi A, S. parathyphi B, S. parathyphi C, S. infantis, S. havana and S. enteritidis by the combinations of the different size bands produced. Standards and clinical isolates of Salmonella were examined and were accurately identified by this assay. Specificity of the assay was evaluated by different gram negative and gram positive bacteria. The sensitivity of assay was measured by colony count and DNA concentration and revealed that this test was able to detect 1-10 copy number or CFU of bacteria. By optimization of PCR program with standard thermocycler used in research laboratories (gradient Eppendorff thermocyler) we were able to shorten the PCR time from 2.5 hrs to 35 minutes in 20 cycles comprising of 7 seconds of denaturation, annealing and extension times. Taken together, by the methods described here it is possible to rapidly detect and identify S. typhi bacteria within 90 minutes of the arrival of specimens in the diagnostic microbiology laboratory. Other interesting finding of this study was possibility of distinguishing between S. infantis and S. havana. Sequence resulted from this study are registered in the GenBank under accession numbers: AY771363, AY771362 and AY771364.

Presentation number: M20

LINKAGE ANALYSIS BY MICROSATELLITE REPEATS ON A DUCHENNE MUSCULAR DYSTROPHY FAMILY: A CASE REPORT

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Duchenne Muscular Dystrophy (DMD) is an X-linked genetic disorder resulting from mutations in the dystrophin gene. The prevalence of DMD is 1 in 3500 live born males, making it the most frequent muscle disease in children. Even though clinical examination, muscle biopsy and biochemical analyses are, in most cases, sufficient to diagnose someone with DMD, genetic testing for deletions (present in 65% of DMD cases) in the dystrophin gene is inevitable tool either for definite diagnosis of DMD or for prenatal analysis in families with affected males. In families with DMD but without an identified deletion, a linkage analysis involving DNA markers in and around the dystrophin gene is necessary for genetic counseling. Linkage analysis is useful even in families where DMD patients are not available. In this study we analyzed six short tandem repeat (STR) loci: 5'DYSII, STR44, STR45, STR49, STR50 and 3'DYSCA in the dystrophin gene in order to determine a possible carrier status of a pregnant woman coming from a DMD family, but without DMD patients available for genetic testing. Her two uncles from mother's side both died diagnosed with DMD. She was the only child of her mother, and in the time of the analysis she was in 16th week of gestation, carrying a male child. Six STR markers were amplified using PCR, and then analyzed on silver-stained 6% denaturing PAGE together with sequenced allelic ladders. All STR loci were informative in the analyzed family. The results of linkage analysis have shown that a proband inherited an X chromosome haplotype from her mother, the same that her mother inherited from her father, implying that she was not a carrier of a DMD mutation enrolled in her family. A carrier status of a proband's mother remained unsolved. Linkage analysis using STR loci is very useful in genetic counseling of families bearing DMD, either in families carrying deletions in the dystrophin gene or in families without known mutations. Moreover, linkage analysis is of great importance in DMD families when patients are not available.

Presentation number: M21

QUANTIFICATION OF CELL-FREE FETAL DNA IN MATERNAL CIRCULATION AND ITS DIAGNOSTIC POTENCIAL

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Multiple studies have demonstrated that cell-free DNA of fetal origin circulates in the blood of pregnant women. Elevated concentrations of this DNA in maternal plasma have been found in various complications of pregnancy including preeclampsia. In our study, the concentrations of fetal DNA in maternal plasma have been measured during all trimesters of pregnancy and compared with clinical data. Fetal SRY-specific DNA quantification was carried out by real-time PCR method on the ABI PRISM 5700 and 7000 machines using the MGB probe and a couple of primers. Cell-free DNAs isolated from plasma of 200 pregnant women were tested. We followed the changes in the concentrations of free fetal DNA in maternal plasma in pregnancies from the 5th to 42nd gestation week. To determine the proportion of fetal DNA in maternal circulation, we used GAPDH/SRY ratio. We have found that both SRY concentrations and GAPDH/SRY vary in different ways during pregnancy. The SRY values in females with clinically diagnosed preeclampsia were not elevated significantly when compared with the pregnancies of the same gestation age (Wilcoxon test, p = 1.00). No significant elevations of cell-free fetal DNA concentrations in maternal circulation have been found in twin pregnancies, in pregnancies with IUGR and hypertension. In fetal sex determination, we achieved 96% sensitivity and 91.1% specificity. It has been reported that concentrations of cell-free fetal DNA are elevated before clinical onset of and during preeclampsia. We examined the preeclamptic patients at the time of diagnosis, twin pregnancies, pregnancies with IUGR and hypertension. No significant elevations of these values in our patients were detected when compared with the control pregnancies of the same gestational age. It is difficult to interpret the variations in the concentration of cell-free fetal DNA during the same pregnancy and the differences among different physiological pregnancies, because the physiological and pathological factors affecting release of fetal DNA into and clearance from maternal circulation are poor understood. Probably, a complex multifactorial trait results in the final concentration of fetal DNA in maternal plasma.

Presentation number: M22

MOLECULAR DIAGNOSTICS IN HEMATO-ONCOLOGY TODAY

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Several novel aspects of molecular diagnostics and prognostics in hematological oncology have emerged in the recent period. First relates to established molecular biomarkers arising from genomic alterations through nonrandom chromosome aberrations. These aberrations are found in approximately 55% of adults with acute leukemia and have long been recognized as independent predictors for clinical outcome. However, the molecular methods and tests were not standardized until recently when Europe Against Cancer Network and BIOMED Concerted Action Program announced proposals for standardized, controlled assays for detecting most frequent molecular markers of leukemia and lymphoma. For leukemia genetic markers it is by now well established that quantification by real-time PCR, although somewhat less sensitive than nested-qualitative assays, provides dynamic molecular profile that is more informative in the clinical setting, especially in terms of monitoring minimal residual disease. For the large proportion of leukemia patients with normal cytogenetic discovery of mutations in FLT3, and CEBPA genes, partial tandem duplication in MLL gene and expression of WT-1 gene has generally proven related to adverse prognosis, but due to their relatively low frequency of 10-30% their clinical value is still evaluated. Another exciting new discovery made early this year was the detection of single nucleotide substitution in the JAK2 gene (V617F mutation) that leads to constitutive activation of this tyrosine kinase associated with cytokine receptors. It soon became evident that the significant proportion of non-Philadelphia-positive chronic myeloproliferative disorders had this mutation in their stem cells which makes this the first specific genetic mutation detected in this large group of diseases. We and others have found V617F present in 80-90% of Polycythemia vera, but also in aprox. 50% of Idiopathic myelofibrosis and 60% of Essential thrombocythemia patients. No other known genetic aberration matches JAK2 mutation frequency and therefore the questions on the clinical significance (disease characteristics, prognosis, use of JAK2 inhibitors in the treatment) and biology (are there and which are other additional specific genetic aberrations in IMF and ET?) are awaited to be answered in the near future.

Presentation number: M23

IMMUNOHISTOCHEMICAL EXPRESSION AND LOH OF NM23 IN ENDOMETRIAL CARCINOMA

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Aim is to analyze the association between immunoreactivity and LOH in nm23 gene in endometrial carcinoma and to examine their correlation with traditional clinicopathological factors such as clinical stage, grade, myometrial invasion and histological type. Expression of the nm23 gene product was examined by immunohistochemistry in formalin fixed, paraffin-embedded specimens from 69 endometrial carcinomas. Moderate and strong staining was considered positive. Loss of heterozygosity assay was analyzed by PCR using specific oligonucleotide primers. The statistical comparison was performed with the chi Square test. There was no significant association between immunohistochemical expression and LOH of nm23 (p=0.348). Nm23 was positive in 41 carcinomas (59.4%). The expression of nm23 protein was significantly associated with histological type (p=0.033) whereas its association with grade was of borderline significance (p=0.063). No significant correlation was found of nm23 expression with FIGO clinical stage (p=0.57) and myometrial invasion (p=0.899). Among 69 samples 44 of them (63.7%) were heterozygotes (informative). LOH of nm23 was observed in 19 of 44 (43.2%) informative endometrial carcinomas. LOH was more frequent in endometrioid type of cancer and in FIGO clinical stage I (73.7%) than in other histological types and clinical stages. No association was found between immunohistochemical expression and LOH of nm23. Expression of nm23 protein was associated only with histological type. LOH of nm23 didn't show significant association with clinicopathological factors.

Presentation number: M24

GENETIC SCREENING FOR TYPE 1 DIABETES RISK IN ALBANIAN POPULATION

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T1DM is caused by the interaction of genetic factors and environmental factors. A substancial part of the transnational variation in the incidence of childhood-onset Type I diabetes in Europe is explained by variations between populations in the distribution of particular DQ genotypes which confer a high risk of Type I diabetes in the general population. The objective to this study was to determine the a ssociation of HLA-DQA1 alleles with Type I diabetes mellitus and the frequencies of these alleles in the Albanian population. The study group consisted of 65 T1DM patients (29 females and 36 males) with age between 11 months and 32 years (20 cases 0-14 years old and 45 cases above 15 years old). We also studied frequencies of T1DM associated HLA-DQA1 in 193 Albanian control subjects and 1784 greek control subjects representing background populations. The samples were typed for 6 HLA-DQA1 alleles (0201, 0302, 0301, 0602,0603 e 0604) using a method that utilizes time-resolved fluorometry to detect the hybridization of lantadine-labeled allele specific oligonucleotide probes with amplified gene product. The 0201,0302 risk genotype had a frequency of 7,69% at the T1DM patients and 2,07% at the control subjects ($p<0,05$). The 0201,X risk genotype had the frequencies 53,8% and 11,39% respectively ($p<0,001$). The 0302,X genotype had a frequency of 6,15% and 5,18% ($p>0,05$). HLA DQA1 allele 0201, conferring increased risk to the development of T1DM had a frequency of 23,8% among Albanians and 27,13% among Greeks ($p<0,05$). The risk allele 0302 had a frequency of 10,8% among Albanians and 12,05% among Greeks ($p>0,05$). The protective allele 0301 had a frequency of 45% among Albanians and 52,91% among Greeks ($p>0,05$), whereas the protective alleles 0602-0603 were more frequent among Albanians (21,2%) than Greeks(10,99%) ($p<0,05$). Statisitical analysis showed that 0201,X and 0201, 0302 are high risk genotypes for the Albanian population. There is a genetic basis for the difference in the T1DM incidence among Mediterranean countries that have a low annual one in comparison with North European countries.

Presentation number: M25

CYTOGENETIC AND FISH STUDIES OF LYMPHOMA BY FINE NEEDLE ASPIRATION

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The detection of chromosomal abnormalities characteristic of lymphomas, is important in the diagnostic workup of aggressive lymphomas given its impact on treatment strategies and prognosis. Recently this has been accomplished using FISH. In confirmation with other methods for collecting samples the fine needle aspiration (FNA) was attractive for diagnosis. We report the cytogenetic investigation in series of 59 patients with lymphoma (28 woman and 31 men, median age 40, ranged 3-90 years). In our series of the specimens, 54 (91.5%) yielded sufficient numbers of analyzable metaphases. Among the 54 successful karyotyped specimens 42 (77%) showed clonal karyotypic abnormalities. Numerical changes in 4, numerical with structural changes in 17 and structural changes in 21 cases. Trisomies 3, 7, 8, 12, X and monosomy 1 were most frequent. The NHL cases were typically characterized by structural rather than numerical aberrations with chromosome arms 1p/q, 3p/q, 6q, 11q and 14q most frequently involved. The expected t(8;14)(q24;34) and t(14;18)(q34;q21) were present in 11 (34%) NHL. The detected abnormal clones in HD were typically very complex and comprised only a small percentage of metaphases. FISH permitted to detect loss or gain of genetic material and revealed rearrangements suspected by conventional abnormalities in 14 (26%) cases. In conclusion, a cell culture sampled by FNA of lymph nodes is an adequate method for chromosomal analysis.

Presentation number: M26

MODULATION OF THE RIBAVIRIN INDUCED CYTOTOXICITY IN HARVESTED HUMAN LYMPHOCYTES IN VITRO

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The present investigation was undertaken to evaluate effect of vitamin B12 on ribavirin induced cytotoxicity in phytohemaglutinin-stimulated human lymphocytes. Blood samples, obtained from healthy volunteers (males, aged 31 years, non-smokers) are used for examination. Blood cells were treated with increasing doses of ribavirin: 0.05, 0.17, 0.32, 0.47 and 0.65μmol/ml at three different incubation times: 2, 4 and 17 hours. Duplicate ribavirin treated cultures were supplemented with 50 μl of B12 during the drug treatment. Supplementation with B12 lowers the MN frequency and slightly recovers the proliferation potential of treated cells in each treatment period of time except for the highest concentration and the longest treatment. Presence of ribavirin during entire S phase of the cell cycle strongly blocks cell cycle progression. This study have shown that B12 posses unique beneficial effects in reducing the toxic effects of ribavirin. Mechanism of beneficial effects of ribavirin should be examined further.

Presentation number: M27

NIJMEGEN BREKAGE SYNDROME WITH MYELODYSPLASIA

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Nijmegen Breakage Syndrome (NBS) (MIM:251260) is an autosomal recessive chromosomal instability syndrome characterized by short stature, progressive microcephaly, characteristic facial features, development delay with loss of cognitive skills, immunodeficiency and cancer predisposition particularly lymphomas (International Nijmegen Breakage Syndrome Study Group, 2000.). Mutations of the NBS1 gene are detected in nearly all patients. This genetic defect affects protein nibrin on chromosome 8q21 involved in the processing repair of DNA double strand breaks and cell cycle regulation. Case report: An 8-years old boy presented with low-set ears, prominent nasal bridge, micrognathia and left skin syndactyly (III-IV toes). He is the first child of healthy non-consanguineous young parents, born as the second twin (first was stillbirth), by Caesarean at 39 weeks of gestation with low birth weight, length and head circumpherence. At 18 months of age low levels of IgG (2.2 g/l) first were registered; than at 20 months growth and developmental delay and facial dysmorphisms. Clinical examination at 7-years of age shows failure to thrive, extreme microcephaly, frequent respiratory infection, low gamma globulin levels, and pancytopenia with myelodysplasia. Low levels of total IgG (3.3 g/l), IgA (0.2 g/l) and IgM (0.36 g/l) were detected. Cytogenetically with standard GTG-banded chromosome analysis, of peripheral blood lymphocyte, 46, XY karyotype with unusually high level of spontaneous chromosome breakage, with typically inversions and translocations was found. Molecular analysis shows that the child is homozygous for a 5-bp deletion (657del 5) in exon 6 of the NBS1 gene. He developed progressive bone marrow failure and was treated transient with substitution of immunoglobulins, different antibiotics, G-CSF and erythropoietin.

Presentation number: M28

CEREBRAL SINUS THROMBOSIS ASSOCIATED WITH PROTHROMBIN G20210A MUTATION

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Cerebral venous and sinus thrombosis is a rare but alarming disease that usually begins with severe headache. Prothrombotic states, both hereditary and acquired (protein C or S deficiency, factor V Leiden mutation, pregnancy and puerperium) are regarded as risk factors, but in many cases the cause remains unknown. Case report: A 37-year-old man was admitted to neurological emergency room because of severe headache. In the familial disease history it was found that patient's mother had thrombosis of the right arm and his brother had thrombophlebitis of low extremities. Two years ago, the patient suffered from thrombophlebitis of the right leg and he started anticoagulation warfarin therapy. Two months prior to the headache, due to the patient's good general condition, anticoagulation therapy was replaced with antiaggregation ticlopidine therapy. However, patient ceased taking ticlopidine after several days, without informing his physician. Computerized tomography (CT) that was performed immediately after hospitalization suggested the possibility of cerebral sinuses thrombosis. Subsequent cerebral panangiography revealed that both tissue perfusion and drainage of major sinuses were significantly retarded. Drainage occurred first through the smaller cerebral veins and much later through the communications with the cavernous sinus. In addition, a number of filling defects appeared during angiography within the frontal and superior parietal part of sagittal sinuses, which indicated potential thrombosis. We were unable to visualize inferior sagittal sinus, as well as rectal, transversal and confluent sinus, due to severe formation of thrombi within them. The same condition was found in carotid and vertebrobasilar sinuses. The patient was placed into intensive care unit and received heparin (24000 units in the 240 ml of saline, the rate of 1000 units/h i.v.). Tramadol (400 mg, per os) and metamizol (5 g in 120 ml of saline, the rate of 5 ml/h) were used for the treatment of pain. Genetic analysis was performed to test for potential point mutations. The patient was heterozygous for factor II (G20210 mutation) and was the wild type for factor V Leiden (there was no evidence for Arg506Gln substitution).

Presentation number: M29

THE INFLUENCE OF PESTICIDES ON DEVELOPING OF DYSGENESIS OF GONADS

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The dysgenesis of gonads belongs to genetically stipulated forms of the retardation of human genital development. In spite of the rare existence the population the problems of dysgenesia of gonads is of very importance in the whole world. The aim is to define the effect of pesticides on developing the dysgenesia of gonads. We examined 55 female patients in the age of 13 – 20 with dysgenesia of gonads. We made the complex clinical and laboratory investigation. 15 (27,2%) patients were from the city of Samarkand and 40 (72,7%) – from rural districts of Samarkand region. We revealed that in 45 (81,8%) families the patients had aggravated anamnesis: dead birth -5 (11,1%), sanguin relative marriage – 10 (22,2%), professional injuriousness of parents – 30 (66,6%). On karyotype we determined: Swyer's syndrome – 2 (3,63%) – 46,XY; mixed form (45,XO/46,XY) – 4 (7,27%), typical form or Shereshevsky-Turner's syndrome (45,XO) – 10 (18,1%), mosaic form (45,XO/46,XX) – 14 (25,4%), pure form (46,XX) – 25 (45,4%). Among them patients' parents – 10 (33,3%) were from Urgut district. Till the moment of conception they worked with tobacco. The rest patients' mothers from various rural disticts contacted with pesticides. Thus obtained data of aggravated anamnesis in patients with dysgenesia of gonads in 77,7% cases allow to determine the correlation of this pathology and pesticides used in 80-th in the Republic of Uzbekistan.

Presentation number: M30

DETECTION OF TWO MOST COMMON FORMS OF INHERITED NEUROPATHIES CHARCOT-MARIE-TOOTH (CMT) USING QUANTITATIVE MULTIPLEX REAL-TIME PCR

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Inherited peripheral neuropathies - diseases Charcot-Marie-Tooth (CMT) - represent one of the most common degenerative neurological disorders. Two most frequent forms of CMT, both associated with PNS demyelination, are CMT1A and hereditary neuropathy with liability to pressure palsies (HNPP). CMT1A is caused by duplication of a region on chromosome 17 encompassing the peripheral myelin protein 22 gene (PMP22). HNPP is caused by its deletion. CMT1A patients carry 3 PMP22 copies, HNPP patients only 1 copy. Routine analysis of CMT1A/HNPP is done by the analysis of microsatellite markers flanking PMP22. Our aim was to define non-overlapping ranges of DDCt values and thus reliably distinguish samples with 1, 2 and 3 PMP22 copies using quantitative multiplex real-time PCR. We tested 110 genomic DNA samples which were previously analyzed by the microsatellite markers analysis: HNPP patients (n=33) (1 PMP22 copy), healthy controls (n=32) (2 PMP22 copies), CMT1A patients (n=45) (3 PMP22 copies). Samples were run on the ABI 7000 instrument in multiplex reaction using two primer pairs and fluorescent probes for PMP22 exon 3 of and albumin exon 12 (endogenous control gene). Calculation of PMP22 copy number was carried out using the relative comparative Ct method ($\Delta\Delta$Ct). Further on we tested 59 DNA samples of CMT patients with unknown molecular genetic cause who were previously tested negative for HNPP/CMT1A and one family (3 patients) with ambiguous results of microsatellite analysis. The ranges of $\Delta\Delta$Ct values are: <1.15 for HNPP patients, 1.8-3 for healthy controls, >3.4 for CMT1A patients. In 56 out of 59 patients with unknown molecular diagnosis, the previous finding of 2 PMP22 copies was confirmed. In 1 patient, the $\Delta\Delta$C value predicts PMP22 deletion, in 2 patients PMP22 duplication. These 3 samples are currently subject to study by MLPA. In the family with inconclusive result of the microsatellite analysis, real-time PCR detected PMP22 duplication and MLPA proved untypical duplication of the region. Quantitative multiplex real-time PCR can be used for fast and reliable CMT1A/HNPP testing, for reevaluation of microsatellite analysis findings and for detection of untypical CMT1A duplications/HNPP deletions.

Presentation number: M31

MECHANICAL SINGLE MOLECULE PROBING OF THE SNARE PROTEIN INTERACTIONS AND THE DEVELOPMENT OF BOTULINUM TOXIN TYPE B MICROMECHANOSENSOR

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Exocytotic release of neurotransmitters is mediated by the ternary SNARE complex, comprised of syntaxin (Sx), synaptosome-associated protein of 25 kDa (SNAP25) and synaptobrevin 2 (Sb2). Since exocytosis is an event with the process of association and dissociation of the bonds between molecules of the SNARE complex being inherently a non-equilibrium process, dynamic measurements at the single molecule level are necessary for a detailed understanding of intermolecular interactions of proteins within the SNARE complex. To address this issue, we used the Atomic Force Microscope (AFM) in force spectroscopy mode to show from single molecule investigations of the SNARE complex, that Sx1A and Sb2 are zippered throughout their entire SNARE motifs without involvement of SNAP25. Note that single intermolecular syntaxin-synaptobrevin interaction, is sufficient to hang a 41 μm diameter bead off the cantilever tip, given the buoyancy of the bead in the fluid and the measured Sx1A-Sb2 interaction strength of 250 pN. This was used to develop a Botulinum neurotoxin (BoNT) type B sensor. Presently available tests for detection of BoNTs, while sensitive, require hours to days. We report a BoNT-B sensor whose properties allow detection of BoNT-B within minutes. The technique relies on the detection of an agarose bead detachment from the tip of a micromachined cantilever resulting from BoNT-B action on its substratum, Sb2 attached to the beads. The mechanical resonance frequency of the cantilever is monitored for the detection. To suspend the bead off the cantilever we utilize synaptobrevin's molecular interaction with Sx1A that was deposited onto the cantilever tip. Additionally, this bead detachment technique is general and can be used in any displacement reaction, such as in receptor-ligand pairs, where the introduction of one chemical leads to the displacement of another. The technique is of broad interest and will find uses outside toxicology.

Presentation number: M32

PARTIAL MONOSOMY 18p: CYTOGENETIC AND CLINICAL PRESENTATION IN FOUR PATIENTS

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Partial monosomy 18p is considered the second most frequently occurring autosomal deletion. This deletion occur de novo in about 85% of patients, and in 15% of cases is a result of a segregation of familial rearrangements. Most reported cases are due to terminal deletion of short arm. Occasionally deletion of the long and the short arm with fusion of the ends results in ring chromosome. The phenotype is highly variable and depends on the extent of deletion. We present the results of cytogenetic and clinical evaluation in a four patients with partial monosomy of 18p. First patient is 8 years old girl with facial dismorphism and mild mental retardation. Cytogenetic analysis revealed aberrant karyotype with 45 chromosomes, missing chromosomes 15 and 18, and the presence of the rearranged chromosome. Banding pattern and fluorescence in situ hybridization (FISH) analysis showed that aberrant chromosome was composed of the long arms of chromosomes 15 and 18, with the presence of the centromeric region of both chromosomes involved. Second patient is 3 months old girl with mild dismorphic traits and congenital heart defect. Cytogenetic studies showed a karyotype with 46 chromosomes missing whole short arm of chromosome 18. Third patient is 2 years old girl with dimorphic face, cleft lip and palate (CLP) and developmental delay. Classical cytognetics identified ring chromosome 18(p11.2;q23). Fourth patient is 9 years old boy with dysmorphic traits, cleft palate (CP) and mental and growth retardation. Routine cytogenetic studies demonstrated a mosaic kariotype 46,XY,r(18)(p11.3q22)/46,XY. Clinical presentation in our patients is compared with the literature data on patients with identical genotype.

Presentation number: M33

CHROMOSOME ABERRATION IN SPONTANEOUS MISCARRIAGES IN SUBOTICA 2000-2004

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The study presents the frequency of chromosome aberration in spontaneous miscarriages in the first three months of pregnancy. We tested 134 chorion and fetal tissues aged eight to 12 weeks of pregnancy collected at the Health Center in Subotica between 2000 and 2004. Cytogenetic analyses have been done on the chorion and, when possible, on fetal tissues (lungs for their high mytotic index) by direct analysis with no cultivation. Cytogenetic analysis from direct material is possible as the tissue is viable and cells are spontaneously dividing in cytotrophoblastic layer, so the risk of bacterial contamination is less and the results are obtained more quickly. Out of 143 analyzed fetal tissues and choriones, chromosomopathy has been found in 38 (28%). Trisomy has been the most frequent, 16 and triploidy. Other chromosomic aberrations have been thrisomy 13, 18, 8, 9, 45, XO. Considering the presence of chromosomopathy according to age, a considerable increase has been noticed for pathologic karyotype of miscarriaged fetus. During 2001, 7% of chromosomopathy has been found; in 2002, 14%; in 2003, 18%; and in 2004, 21% of chromosomopathy. The importance of cytogenetic analysis of miscarried fetus is indication of parents' karyotype where chromosomopathy of fetus has been confirmed. It leads to revelation of balanced structural aberrations of chromosome that absolutely indicates the choriocenthesis or amniocenthesis in the following pregnancy, regardless the age of pregnant woman. The control of presence of chromosome aberration in miscarried fetus is an indication of general population risk, which shows the directions of preventive measures, at the same time.

Presentation number: M34

FV, FII, MTHFR AND PAI-1 POLYMORPHISMS IN PATIENTS WITH DEEP VENOUS THROMBOSIS

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Coagulation factors V (FV), II (FII), plasminogen activator inhibitor-1 (PAI-1) together with the methyltetrahydrofolate reductase (MTHFR) are considered to be risk factors for coagulation and fibrinolysis disorders. Aim is to investigate frequency of these polymorphisms in patients with deep venous trombosis (DVT) and to determine whether there is a difference in polymorphisms distribution between healthy controls and DVT patients. Methods: Polymorphisms for FV, FII and MTHFR were determined using PCR-RFLP method, while PAI-1 genotype is determined with PCR-SSCP. Genotype distributions for FV polymorphism (N=215) were 94%, 5.1% and 0.9% in healthy group vs. 87.3%, 12.7% and 0% in the DVT group (N=181) for wt/wt, wt/mut and mut/mut. Allele frequencies were 96.5% and 3.5% in healthy group vs. 93.6% and 6.4% in DVT group for wt and mut. Distributions for FII genotypes (N=87) were 97.7%, 2.3% and 0% in healthy group vs. 93.9%, 6.1% and 0% in the DVT group (N=180) for wt/wt, wt/mut and mut/mut. Allele frequencies were 98.9% and 1.1% in healthy group vs. 96.9% and 3.1% in DVT group. Genotype distributions for MTHFR polymorphism (N=103) were 35%, 57.3% and 7.8% in healthy group vs. 46.4%, 41.8% and 11.8% in the DVT group (N=153) for wt/wt, wt/mut and mut/mut. Allele frequencies were 63.6% and 63.4% in healthy group vs. 67.3% and 32.7% in DVT group. Distributions for PAI-1 polymorphism (N=104) were 30.8%, 52.9% and 16.3% in healthy group vs. 31.9%, 41.2% and 20.8% in the DVT group (N=72) for wt/wt, wt/mut and mut/mut. Allele frequencies were 57.2% and 42.8% in healthy group vs. 55.6% and 44.4% in DVT group. Distribution comparison between DVT patients and healthy controls showed significant difference only for the FV genotype (p=0.034 Chi square test; OR=2.26 with the 95% CI=1.11-4.60) while other investigated genotypes showed no difference in distribution between these groups. Our investigation showed only FV mutation to be a risk factor for DVT.

Presentation number: M35

MOLECULAR CYTOGENETIC FINDINGS IN CHILDREN WITH DEVELOPMENTAL DELAY

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Croatia Chromosomal abnormalities are the most common cause of mental retardation and present in 4-28% of patients. Standard cytogenetic investigation using high resolution banding techniques cannot detect genomic abnormalities smaller than 5-10Mb. Rearrangements involving smaller interstitial and subtelomeric chromosomal regions may be detected by molecular cytogenetic methods. In this report we present the results of chromosome analysis in 170 children referred to cytogenetic laboratory for developmental delay, dysmorphism and malformations. Chromosome analysis was performed on slides obtained from synchronized peripheral blood lymphocyte culture. All samples were studied by GTG-, RBG- and CBG-banding methods, and fluorescence in situ hybridization (FISH) method with appropriate DNA probe. Structural chromosome aberrations were detected in 12 (7.0%) out of 170 children by classical cytogenetic banding methods. Interstitial microdeletions were identified by two-colour FISH in 14 (11.0%) out of 127 children with phenotype suggestive of microdeletion syndromes. Subtle rearrangements of chromosome subtelomeric regions were detected by multi-probe FISH screening method in 2 (6.45%) out of 31 patient, or in three (9.1%) out of 33 persons including parents. The results of this study indicate the following: 1. Submicroscopic chromosome abnormalities are a significant cause of developmental delay. 2. FISH with multiple subtelomeric probes is useful test for detecting cryptic rearrangements and establishing diagnosis in patients with unexplained developmental disorder. 3. The evaluation of patients with mental retardation and dysmorphism requires stepwise testing including high resolution chromosome identification and FISH analysis to exclude a particular microdeletion syndrome before screening for subtelomeric aberrations is performed. 4. The positive result with subtelomeric screening requires the confirmation with single FISH probe and cytogenetic investigations of both parents. 5. FISH analysis is useful in precise identification, understanding the mechanism of origin of structural chromosome rearrangements and assessment of chromosomal breakpoint positions.

Presentation number: M36

DETECTION OF GENETICAL MUTATIONS AT THE INHABITANTS OF THE CHEMICAL CENTER WITH THE PURPOSES OF GENETICAL MONITORING

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Ecological intensity in industrial regions conducts to contact of the population with potentially harmful polluting factors of environment, which can enhance the mutation influence, can damage a genome in gametes and somatic cells of the man. Sterlitamak is large industrial city of the Bashkir Republic, where are submitted the potent chemical industry and oil processing. The problem of the evaluation of influence of the factors of environment on the genome of the townspeople is especially important. The perennial epidemiological and genetic-biochemical researches of the population of Sterlitamak with the purposes of genetical monitoring testify to augmentation of genetic infringements at the population. In the submitted work with the purposes of detection of genetic mutations are studied fingerprints with DNA of bacteriophage M 13 of inhabitants. The bank DNA of the townspeople is being created, which now contains 397 samples. The experimental group (EG) include of the members 53 families (both of parents and them children), selected with allowing for professional contact with toxic materials even of one of the parents during not less half-year up to a conception of the child. The control group (CG) - 30 families, where the parents by virtue of the professional work had no similar contact. The fingerprints with DNA of bacteriophage M 13 of the members 14 experimental and 6 control families is carried out. The fields of 11 radioautographs with lengths from 2027 up to 21226 were used for visual comparison. We believed that the bands are identical, if in an electrophoretic profile the bands differed less, than on 1 mm. The average of restriction fragments at the adults in EG were 24.9 and 23.9 fragments at the children. 29 fragments were on the average at the parents and 26.4 at children in the control group. In CG both at children and at the adult the number of restriction fragments is a little bit more, than in EG. However, statistically difference was not revealed. We considered as mutation events the absence of fragment in comparison of the strip at the level of parent (M "-"), and presence at him of the restriction fragment which is not conterminous on molecular weight to strips of the parents (M "+"). Number of mutations M "+" and M "-" was summarized. This size counted as general number of mutations. Amount of bands in a profile of children, which were not referred to parents, was unexpectedly above the concerning similar researches: 4.25 new bands for the child in experimental group and 6.50 - in monitoring. This fact can be conditioned as by methodological features of the given research, so by actual effect on gametes of high concentrations of mutagens of environment.

Presentation number: M37

WHOLE TRANSCRIPT PROFILING OF TUMOR CELLS FROM FORMALIN FIXED PARAFFIN EMBEDDED SECTIONS USING QUANTITATIVE REAL TIME PCR

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Tumor biopsies are commonly preserved in clinic as formalin fixed paraffin embedded (FFPE) tissues. Enabling whole transcript (non-3'-biased) profiling of FFPE samples using quantitative real-time PCR (QRT-PCR) will revolutionize the way tumors are diagnosed and treated. However, since the macromolecules in FFPE tissues are cross-linked and often fragmented, efficient extraction and isolation of RNA with quality adequate for whole transcript profiling poses a technical challenge. We have recently developed and optimized a novel process that integrates efficient isolation of total cellular RNA from FFPE sections, followed by reverse transcription of whole transcripts for QRT-PCR analysis. We have demonstrated sensitivity of the process down to one nanogram of total RNA. Further, we present data demonstrating whole transcript representation and quantification of low, medium and high abundance transcripts. The process we have developed can be applied in clinical research to understand the biological mechanisms underlying cancer, and the role abnormal splice variants play in tumorigenesis and metastasis.

Presentation number: M38

BONE PHENOTYPE OF IL-7 TRANSGENIC MICE

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The aim of this study was to investigate the bone phenotype of mice with the insertion of pDOI 5-IL7. IL-7 transgenic mice express a lymphoproliferative disorder (the expansion of cells at an early stage of B-cell development) which could influence the bone cell microenvironment in long bones. Tibiae from 6 or 12 week-old mice were processed for histology. Serial sections were stained with Goldner's trichrome stain and the percentage of volume of trabecular bone was measured using Osteomeasure software. Osteoclasts were identified by tartrate-resistant acid phosphatase (TRAP) staining, counted on serial sections and presented as number of osteoclasts per millimeter of bone length. Murine bone marrow cells were cultured to stimulate osteoclast-like cells (OCL) formation. Bone marrow cell populations were identified by flow-cytometric analysis using anti CD45R and CD19 (B-cell development markers), CD11b (monocyte-macrophage population marker), and CD3 (T-cell surface marker). Our results showed that trabecular bone volume was lower in IL-7 transgenic mice compared with wild type mice (wild type vs. IL-7 transgenics: 9.03 ± 4.39 vs. 1.81 ± 1.01). The mean number of TRAP positive osteoclasts was higher on bone surfaces and in IL-7 transgenics wild type vs. IL-7 transgenics: 58 ± 8 vs. 137 ± 54), as well as in in vitro culture of OCLs from bone marrow (wild type vs. IL-7 transgenics: 270 ± 30 vs. 468 ± 41). The proportion of CD45R positive cells was increased three fold in bone marrow of IL-7 transgenic mice but decreased in peripheral blood. The number of CD11b-positive monocyte-macrophage cells was lower in bone marrow and peripheral blood of IL-7 transgenic mice. Our results suggest that IL-7 transgenic mice have a specific bone phenotype of decreased trabecular bone volume, which is, as we presume, the result of an increased osteoclast number and activity. The relationship between the changes in the hematopoietic cell populations and the bone marrow microenvironment needs further clarification.

Presentation number: M39

THE EFFECTS OF IMMUNOSUPPRESSANTS CYCLOSPORIN A AND DEXAMETHASONE ON T-LYMPHOCYTE ACTIVATION, CELL-CYCLE AND APOPTOSIS

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The aim of this study was to investigate the effects immunosuppressants cyclosporin A (CsA) and dexamethasone (DEX) on T-lymphocyte activation, cell-cycle progression and apoptosis in the in vitro model of mitogen-stimulated T-lymphocyte leukemic Jurkat cell-line and separated peripheral blood mononuclear cells (PBMC). Jurkat cells or PBMC were pretreated with different doses of CsA and DEX for 1 hour at 37 °C, activated with previously optimized doses of mitogen phytohemagglutinin (2.5 and 0.625 µg/ml for Jurkat and 5 and 20 µg/ml for PBMC), and cultured for 24, 48 or 72 hrs. Activation, cell-cycle and apoptosis were measured using fluorescent labeling, followed by flow-cytometric analysis. The expression of CD69 (24 or 48 hrs), an early cell-surface activation marker, was analyzed by flow cytometry, and propidium-iodide staining was used for cell-cycle analysis (48 or 72 hrs) and nick-translation assay for the detection of apoptosis (72 hrs). Expression of CD69 activation-marker decreased in a dose-dependant manner after immunosuppressant pretreatment. Cell-cycle analysis revealed an inverse correlation between the number of cells in S/G2-phase and the dose of CsA and DEX. Decrease in activation, as well as in number of cells in S/G2-phase, was higher when cells were treated with CsA than with DEX, and more pronounced additive effect was detected in the cultures of PBMC than Jurkat cells. Cell apoptosis significantly increased with CsA- and DEX-pretreatment, and CsA showed stronger effect than DEX. The additive effect on cell apoptosis was observed when the combination of CsA and DEX was used. Our results suggest that both CsA and DEX inhibit T-lymphocyte activation and block cells in G0/G1-phase of the cell-cycle, but CsA has a more potent effect. Additive effect of CsA and DEX may have an important role in immunosuppressive combination therapy which aims at minimizing side effects of any single drug.

Presentation number: M40

BLEEDING AS A COMPLICATION OF WARFARIN THERAPY

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Cytochrome P450 CYP2C9 is the main enzyme for metabolism of S-warfarin, which is the drug of choice among oral anticoagulants. The aim of the study was to determine the association of bleeding as the most important complication of warfarin therapy with CYP2C9 polymorphism, warfarin dose, INR at induction, and underlying illnesses. The study included 181 patients treated with warfarin for at least a month. According to the bleeding events the patients were divided into the three subgroups: without, with minor and with major bleeding. According to the daily warfarin dose they were divided into the two groups: dose of 1.5 mg or less (n=24) and dose of more than 1.5 mg (n=157). According to the INR at induction they were divided into the three subgroups: 3.5 or less, between 3.6 and 5.0 and more than 5.0. Genotyping of cytochrome P450 CYP2C9 (alleles *1, *2 and *3) was performed by PCR-RFLP procedure. One hundred and sixtythree of the 181 patients were free of bleeding (90.1 %), minor bleeding occurred in 14 (7.7%) patients and 4 patients (2.2%) experienced major bleedings. Bleeding occurred more frequently in those taking 1.5 mg or less of warfarin. Subgroups according to the genotype did not differ significantly in bleeding events frequency. Presence of *1 allele was not a risk for bleeding event, neither was presence of *2 allele nor *3 allele. Bleeding occurred the most frequently in the subgroup of patients with INR more than 5.0. The subgroups of patients divided according to the bleeding events differed significantly in the optimal warfarin dose. The subgroups of patients according to the underlying illnesses did not differ in the bleeding frequency.

Presentation number: M41

DETECTION OF THE 35delG MUTATION IN THE CONNEXIN 26 (GJB2) GENE IN CROATIAN PATIENTS WITH NONSYNDROMIC HEARING IMPAIRMENT

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Hearing loss is one of the most common congenital disorders affecting 1 to 3 of 1000 newborns. NSHI (nonsyndromic hearing impairment) is observed in about 70% of cases with a genetic background and is mostly caused by autosomal recessive mutations. Mutations in GJB2 gene, encoding gap junction beta 2 protein (connexin 26) are found in 50% Caucasians with profound NSHI. 35delG is the most frequent GJB2 mutation leading to NSHI in European populations, especially of Mediterranean descent, with carrier frequency up to 1/30. The aim of this study was to determine the allelic frequency of 35delG mutation in patients with NSHI and in normal hearing individuals in the Croatian population. The method we used is based on the principle of PCR-mediated site-directed mutagenesis, followed by a BsiYI digestion. PCR products were subsequently size-separated by electrophoresis on a 4% agarose gel and analyzed. Results: We analyzed 29 unrelated individuals with nonsyndromic sensory deafness and 342 normal hearing individuals. Among the patients with NSHI the 35delG mutation was found on 48.27% alleles. The carrier frequency among the healthy control individuals was 5 in 342 (1.46%). Conclusion: The relatively high prevalence of 35delG mutation in GJB2 gene among Croatian patients with NSHI shows that this mutation is one of the leading causes of NSHI in Croatia. Hence, early screening for 35delG mutation would greatly improve the genetic counseling, prevention and treatment strategies in our patients with NSHI.

Presentation number: M42

AN ANTISENSE APPROACH FOR EMBRYO GENE THERAPY

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Preimplantation human embryos are characterized by various degrees of cytoplasmic fragmentation. The degree of fragmentation during early cleavage is a perfect indicator of an embryo quality during human in vitro fertilization treatment. Extensive fragmentation has been associated with reduced capacity of blastocyst formation and implantation, and a high incidence of developmental arrest before the blastocyst stage. Recent observations suggest that cellular fragmentation in a subset of human preimplantation embryos could be regulated by certain components of a genetic program of cell death. The potential for modulating gene expression by the use of antisense oligonucleotides (AsODNs) has become increasingly interesting in resent years. AsODNs are complementary nucleic acid fragments that hybridize to target sequences within RNA to form a DNA-RNA duplex, resulting in the block of translation of messenger RNA into the protein. The aim of the present study was an examination of AsODNs specific to mRNA of the one of apoptosis-related genes as therapeutic agents for embryo gene therapy. Human oocytes and then preimplantation embryos from 28/28 patient cycles were incubated with/without specific natural AsODNs for 3 days. Embryos were classified on days 2-3 according to the percentage of fragmentation and degree of developmental abnormalities. Good morphology 3-5 days embryos were transferred in uterus. Treatment with AsODNs significantly increased total amount of embryos without fragmentation on days 2 and 3 ($P<0,05$). Also after treatment developmental abnormalities on day 2 were significantly lower, ($P<0,05$). The pregnancy rate in the study group was higher (13/28, 46,4%) compared with control group (8/28, 28,6%). These findings demonstrate that this antisense approach is a useful tool to improve embryo condition during human in vitro fertilization treatment. The higher pregnancy rate in the study group demonstrates that embryos after therapeutic influence have an improved developmental potential.

Presentation number: M43

QUANTIFICATION OF TSPY GENE AND PROBABLE IDENTIFICATION OF GONOSOMAL ABERRATIONS IN INFERTILE MEN USING CAPILLARY ELECTROPHORESIS

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TSPY gene family has 20-40 gene copies that vary from individual to individual and occur in at least six locations on the human Y chromosome; with each cluster containing a unique combination of variants. TSPY gene is 2.8 kb long with six exons and five introns. Its prototypic coding sequence is 924 bp and a sequence divergence of 10% has been reported in Human TSPY sequences. TSPY gene is known for its role in testicular carcinomas and seminomas; prostate carcinoma and probably also has role in spermiogenesis. Furthermore, the topology of testicular expression in the adult testis and the homology of TSPY with members of the TTSN-family that are involved in cell cycle control, also suggest the role of TSPY in spermatogonial proliferation. Immunohistochemical studies suggest that TSPY protein expression may be the point that differentiates mitotic proliferation and meiotic differentiation. We studied the number of TSPY copies in fertile and idiopathic infertile males. Blood samples from normospermic, idiopathic oligospermic and idiopathic azoospermic men were obtained. All the patients were screened for the presence of any systemic disease, chromosomal aberrations, microdeletions of Y chromosome, trauma or any kind of carcinoma. RQF PCR (Refined quantitative fluorescent PCR) was performed on all samples to study the number of TSPY copies in each. AMEL genes on X and Y chromosome were used as internal controls. In preliminary studies we found a difference between relative number of copies of TSPY gene when different populations were compared. In addition to the number of copies we also identified chromosomal aberrations and mosaics of sex chromosomes. Our study further strengthens the role of TSPY gene in spermatogenesis. Also the number of copies of TSPY might have a dosage effect in the process of spermatogenesis, thus number of copies of TSPY in association with other factors may influence male fertility.

Presentation number: M44

CYP2C9 GENETIC POLYMORPHISM AND WARFARIN THERAPEUTIC DOSE PREDICTING BY MULTIPLE LINEAR REGRESSION MODEL

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Warfarin is an anticoagulant drug, whose dosage is carefully titrated to avoid the risk of serious side effect like life threatening bleeding. This risk exists at least in part due to genetic polymorphism in CYP2C9 - major enzyme of warfarin metabolism. Besides wild type allele CYP2C9*1, most common mutant alleles CYP2C9*2 and CYP2C9*3, code for enzymes with only 16-20% and 5% of total wild type activity, respectively. Three types of metabolic phenotype can be derived from genotype information (Poor metabolizer - PM, with both alleles mutant, Intermediate metabolizer - IM, with one mutated allele, and Extensive metabolizer, with both wild type alleles). The aim of the study was to find multiple regression model that could be used for dose prediction and to assess the importance of CYP2C9 genotyping in patients receiving warfarin anticoagulant therapy. We genotyped 181 patients (56.4% males, mean age 62) with PCR-RFLP method, who were receiving warfarin in doses needed for maintaining prothrombin time value within INR range 1.5-2.5. Other factors like gender, age, influence of other used drugs and the influence of different diagnoses were also included in the model assessment. Those factors showed to be of no importance for the model. Best multiple linear regression model showed to be a model which estimate optimal warfarin dose from standard dose at the beginning of therapy, patient's EM and PM phenotype, and the ratio of target INR value/INR derived 72 hours after therapy initiation. Within +/- 1mg deviation, this model is able to predict drug dose for about 76% of patients. In this model, IM phenotype contributes for 6%, and PM phenotype for 9% decrement of standard dose. This study confirmed that compared to 1/1 genotype, CYP2C9 impairment significantly decreases optimal warfarin therapy maintenance dose: 2/2 genotype decreases dose to 66%, and genotype 3/3 to 33% (P=0.025). IM phenotype decreases the dose to 88% (P=0.008), and PM to only 55%. Probability that CYP2C9*3 allele carriers will take doses smaller than 3mg is four times higher than non carriers (OR=4.14; 95%CI: 1.7-10.3). CYP2C9 allele, genotype and phenotype distribution in healthy population is concordant to other authors: 11.8% CYP2C9*2 alleles and 4.0% CYP2C9*3 alleles, genotype 1/1 (68.8%), 1/2 (22.6%), 1/3 (8.1%), 2/2 (0.5%), and genotype 2/3 and 3/3 (0.0%). Study conclusion is that information derived from CYP2C9 genotyping can indicate to increased risk of warfarin therapy side effects, and that the use of proposed regression

Presentation number: M45

RELATION OF C-KIT EXPRESSION AND DUAL ESTERASE ACTIVITY TO FISH PHILADELPHIA CHROMOSOME POSITIVITY IN PATIENTS WITH CML

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Chronic myelogenous leukemia (CML) is a myeloproliferative disease with t(9;22) and/or BCR/ABL fusion gene. Treatment of CML patients with imatinib mesylate (Gleevec) is based on the inhibition of BCR/ABL and c-kit (CD117) tyrosine kinase activity. The aim of the study was to analyze immunocytochemical expression of CD117 expression and cytochemical dual esterase activity in bone marrow hematopoietic cells (HCs) of CML patients on Gleevec therapy and compare the results with FISH Ph+ positivity. Immunocytochemical APAAP CD117 expression and cytochemical dual esterase activity in HCs were evaluated in 22 patients with CML on Gleveec therapy and in 10 patients without hematological disease (control group). Medians and ranges of CD117+ and dual esterase+ HCs were similar in patients with CML and the control group and without statistical difference. Although medians of CD117+ and FISH Ph+ HCs were highest in CML patients during the first 6 months of Gleveec treatment, correlation of these two parameters was low. FISH Ph+ HCs were significantly lower in CML patients with longer Gleevec therapy. CD117+ HCs were significantly higher in patients with abnormal peripheral blood leukocyte findings. In conclusion, results indicated that Ph+ HCs declined in majority of CML patients on Gleevec therapy. Also, slightly increased CD117+ HCs in CML patients could be connected to inferior response to Gleevec.

Presentation number: M46

HLA MICROSATELLITE ASSOCIATIONS WITH TYPE 1 DIABETES IN THE CROATIAN POPULATION

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Present study extends previous investigations about association of HLA region with Type 1 Diabetes (T1D). Distribution of four HLA microsatellites (D6S265, D6S273, MIB, MICA) was studied among 100 patients with T1D and 184 healthy matched controls. DNA was isolated from peripheral blood using DNA extraction kit. After PCR amplification, samples were run on 6% polyacrylamide gel in ALFexpress sequencer. Comparison of allele distribution among patients and controls revealed differences for D6S273 and MIB loci, while there was no difference observed for D6S265 and MICA loci. Alleles D6S273-126bp (p=0,026), D6S273-138bp (p=0,019), D6S273-140bp (p<0,001), MIB-348bp (p=0,013), MIB-350bp (p=0,007) and MIB-352bp (p=0,023) were present with increased frequency in patients, while D6S273-130bp (p=0,029) and MIB-334bp (p=0,001) appeared with decreased frequency. We examined possibility that these microsatellite alleles showed association with disease due to their linkage disequilibrium with HLA class II genes, by excluding individuals positive for DRB1*03 and/or DRB1*04. Difference was observed only for MIB-350bp (p=0,012). In the same time, the presence of DRB1*01 and MICA-A9, was found in 40% of DRB1*03 and DRB1*04 negative patients which was significantly higher than in matched controls (p=0,003). In conclusion, our results suggest that MIB is additional genetic marker for developing T1D and that DRB1*01 in presence of MICA-A9 might be considered as predisposing genetic factor for T1D in DRB1*03 and DRB1*04 negative patients.

Presentation number: M47

THE ROLE OF PHARMACOGENOTYPING IN THERAPY OPTIMIZATION

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Variability in the efficacy and toxicity of drug therapy in individual is often associated with polymorphisms in genes encoding drug-metabolizing enzymes, transporters, or drug targets. Pharmacogenetics studies the variations in candidate gene(s) or the network of genes that determine an individual’s response to drug therapy and aims to identify individuals predisposed to high risk of toxicity from conventional doses of therapeutic agents. Genetic polymorphism based on drug metabolism ability is associated with three phenotype classes. The extensive drug metabolizer phenotype is characteristic of normal population. The poor metabolizer phenotype is associated with the accumulation of specific drug substrates in the body due to mutation and/or deletion of both alleles responsible for phenotypic expression. The ultrarapid metabolizer phenotype is characterized by enhanced drug metabolism due to gene amplification. Genetic variability for drug absorption forms the basis for slow and rapid drug absorption phenotypes, as well as in drug interactions with receptors result in poor or efficient receptor interaction phenotype. There are two approaches of pharmacogenetics testing in clinical use. The first approach implies making a specific hypothesis on the genes that cause therapeutic response modification and their testing in all individuals irrespective of their therapeutic response (gene candidates). The second approach implies the search for so-called SNP profile (SNP prints) associated with efficient or adverse events in a respective population (pharmacogenetic approach). Examples of SNP profiles of specific genes modifying drug response, which can currently be used in clinical practice are the genes encoding for drug metabolizing enzymes from the families CYP450, CYP2D6, 2C19 and 2C9, then phase II enzymes NAT2 and TPMT, B2-AR receptors, and some enzymes involved in the metabolism of antitumor drugs.

Presentation number: M48

DNA DEMETHYLATING AGENT 5-AZACYTIDINE REDUCES TUMOUR GROWTH IN EXPERIMENTAL TERATOMA AND TERATOCARCINOMA ORIGINATING FROM GRAFTED POSTIMPLANTATION EMBRYOS

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Various types of undifferentiated cells have been considered for cell transplantation in regenerative medicine. One of the problems in using undifferentiated cells, that might altogether compromise the therapeutic effect, is their potential to produce malignant tumors. In this work experimentally induced tumor in grafts of early postimplantation rat and mouse embryos were treated with a DNA demethylating agent to try to influence tumor development epigenetically. 9,5-days-old Fisher rat embryos and 7,5-days-old C3H mouse embryos were microsurgically isolated under the dissecting microscope. Adult males of respective strains were anesthetized and embryos were grafted under the kidney capsule for 5 weeks. 5 mg/kg 5-azacytidine was administered intraperitoneally once or twice a week to experimental animals. Controls were treated with PBS in the same way. After five weeks, animals were sacrificed, tumors and male gonads were weighted and fixed in St. Marie solution. As expected, grafted embryos gave rise to teratomas in the rat, while in the mouse teratocarcinomas developed. In the rat, as well as in the mouse, 5azaC significantly reduced tumor growth when applied twice a week. Moreover, in the mouse, when 5azaC was applied only once a week, significantly smaller tumors were found. It seems that in the mouse some grafted embryos did not develop tumors at all, while in controls almost all grafts developed tumors. Beside reduced tumor growth, significantly reduced gonad weights were determined in both rat and mouse. It can be concluded that the DNA demethylating agent 5azaC reduces growth of teratoma and teratocarcinoma in the rat and in the mouse, respectively, but also has a deleterious effect upon male gonads. This investigation points out that the regulation of gene expression through DNA methylation is crucial for development of teratoma and teratocarcinoma. It was established that vast majority of so far investigated cancers have methylation abnormalities being often deficient in global methylation levels, but hypermethylated in tumor suppressor genes. Therefore it is possible that in teratomas and teratocarcinomas, investigated in this work, 5azaC induced demethylation of tumor-supressor genes, too.

Presentation number: M49

IMPACT OF CAPILLARY ELECTROPHORESIS PARAMETERS ON PRECISE QUANTITATIVE ANALYSIS OF FREE FETAL DNA IN MATERNAL PLASMA

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The reliability and reproducibility of DNA quantitative analyses on the capillary electrophoresis are crucially influenced by many factors (capillary wear, base line level, matrix quality, analysis software, dose of fluorescent signal, voltage and time of injection etc.). Control algorithm creation and assessment of the influence of tested parameters are necessary for more sensitive and precise quantitative diagnostics such as: free fetal DNA in maternal plasma, rare mosaic detections, chimerism analyses, assess of copies in the repetitive genes. This work summarizes our experience with the quantification of STR and gonosomal loci and describes examples of failure or distortion of the quantitative analyses.

Presentation number: M50

APPLICATION OF QUANTITATIVE ANALYSIS OF CHROMOSOME Y SEQUENCES IN GONOSOMAL ABERRANT PATIENTS

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Chromosome Y sequences in TS patients may induce tumorogenesis. Abnormal expression of TSPY gene was reported in gonadoblastomas. Our project analysed percentage of TSPY and AMELY loci in different tissues of Y positive Turner syndrome patients. DNA from 135 peripheral blood samples, from seven ovarian and tumorous samples embedded in paraffin (4 resp. 3), two RNA specimens from dysgenetic,Y positive patients were collected. QF PCR in TSPY loci and RQF PCR in AMELY were used for quantitation. Six out of 130 TS samples were positive for Y sequences ranging from 0,46 % to 63,8 %. Eleven out of 135 patients were positive for TSPY gene with extreme range of mosaicism or number of copies. Four ovarian and tumorous samples out of 7 were TSPY positive in range from 0,01 % to 6 %. Tests of 2 cDNA samples were mild positive compare to negative control. Role of TSPY gene is not fully understood yet, the gene is supposed to take part in spermatogonies directing into the meiosis. It is significant candidate for GBY with possible role in multistage gonadoblastoma and dysgerminoma development in TS patient's dysgenetic gonads. TSPY gene exists in multiple copies in several clusters, with assumed splicing variability. Diagnostic analysis of Y sequences in TS patients should involve TSPY locus. For better understanding of gonadoblastoma tumour genesis more detailed express study of TSPY gene will be required.

Presentation number: M51

ISOELECTRIC FOCUSING AND PCR-RFLP FOR DETECTION OF ALPHA1-ANTITRYPSIN DEFICIENCY

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It is well established that alpha1-antitrypsin deficiency (AATD) is related to many diseases including those of the liver and lung and rheumatoid arthritis. We studied the ZZ, MZ, ZS, SS, and MS phenotypes in 53 persons suspected of harboring AATD by use of serum protein electrophoresis (SPE), measurement of trypsin inhibiting capacity (TIC), isoelectric focusing (IEF), polymerase chain reaction (PCR), and the combined IEF/PCR-RFLP technique. We used primer–induced restriction analysis (PIRA) for gene fragment amplification. By SPE and TIC, we found AATD in 35.9 percent and 50.1 percent of patients, respectively. However, when we employed the combined IEF/PCR-RFLP, we detected AATD in 100 percent of patients. We suggest that the sensitivity of the latter method results from the combined powers of IEF and PCR-RFLP that can detect AATD both at the level of gene structure and protein expression. Hence for detection of AATD the IEF/PCR-RFLP technique is recommended.

Presentation number: M52

COAGULATION FACTOR GENE POLYMORPHISMS – ROLE IN THROMBOSIS

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Hypercoagulability, a state of hightened activation of the coagulation system plays a major role in pathogenesis of venous thromboembolism (VTE). Hypercoagulability also likely plays some less clearly defined role in the pathogenesis of various forms of arterial thrombosis. The inherited hypercoagulable states are associated with venous rather than arterial thrombosis. It is well known today that mutations in genes that code for regulatory proteins in the process of coagulation and fibrinolysis have an impact on structure and function of these proteins. Until recently, genetic defects could be identified in about 10% of patients with hereditary thrombophilia with antithrombin, protein C and protein S genes considered relevant for venous thrombosis. The discovery of important genetic predisposition factors for VTE ten years ago - FV R506Q and the prothrombin G20210A variant - explain the cause of about 60 percent of familial thrombophilia. The role of other genetic polymorphisms among coagulation factors genes as possible risk factors for VTE has not yet been proven. Various studies have stressed the importance of genetic factors for the development of arterial thrombosis with myocardial infarction and ischemic stroke as complication. Coagulation factors contribute to the generation of arteriosclerosis by formation of blood clots that occlude arteries but the data on mutations and polymorphisms in genes that code for these factors gave inconclusive results. Protective effect on the development of myocardial infarction was shown in several studies for FXIIIVal34Leu polymorphism only. FV R506Q, FVII GR353Q and VWF Thr789Ala polymorphisms are candidates that can contribute a certain percentage to the total risk of this complex process.

Presentation number: M53

URGENCY OF THE MOLECULAR METHODS FOR DIAGNOSIS SPECIFICATION IN THE SUDDEN DEATH VICTIMS

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Approximately 5% of patients with unexpected cardiac death have no evident structural heart disease and are classified to have idiopathic ventricular fibrillation (IVF)/ polymorphic ventricular tachycardia (PVT). Mutations in the human cardiac ionic channels are responsible for primary electrical diseases with high risk of the sudden cardiac death (SCD). We have been analyzed pedigrees of the 54 unrelated families with inherited arrhythmias (Long QT syndrome, Brugada syndrome, IVF, Cardiac conduction defect) caused by discovered mutations in the KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 genes. Mutations screening of the indicated genes have been performed using PCR-SSCP analysis with direct sequencing of the abnormal conformers. Detailed pedigrees with SCD accumulation were available in 26 genotyped families (48%). We have been analyzed the quantity and nature of the fatal accidents in these families which happened before clinical determination of the diagnosis in probands. Sudden death happened after identification of the inherited arrhythmia at least in 1 member of the family was excluded from analysis. There were 76 cases like referred above (2.9 cases per family on average): seven victims in the age less 1 year old; 34 cases - from 1 to 15 years old; 30 cases - from 15 to 45 years old and five fatal cases - in upwards patients. Pathologoanatomic diagnosis “sudden infant death syndrome” was the most frequent in the infant group. Diagnosis “acute coronary syndrome” was dominating in the other groups. Unexpected death during sleeping took place in 10 individuals, and in 4 cases it was drowning. Nobody was keeping under observation or was treated on account of arrhythmias. Inherited channelopathies are often leaded to SCD accumulation in some generations among relatives before disease could be diagnosed. Despite high cost and long-term duration of the molecular genetics diagnostics we suppose that mutation screening in the genes responsible to primary arrhythmias when post-mortem examination data are not enough for accident explanation. The DNA research methods permit to resolve some diagnostic, ethical and juridical questions and to prevent SCD in relatives.

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KEY WORD INDEX

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