MAYO CLINIC

3rd European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine

FINAL PROGRAM AND ABSTRACTS

THIRD EUROPEAN-AMERICAN SCHOOL IN FORENSIC GENETICS AND MAYO CLINIC COURSE IN ADVANCED MOLECULAR AND CELLULAR MEDICINE

September 1 - 5, 2003

Zagreb
CROATIA

www.european-americangeneticsmeetings.org
www.mayo-zagreb-advancedmedicaleducation.org

Editors
Dragan Primorac
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Stanimir Vuk-Pavloviæ
Moses Schanfield

Prepress and printed by
Studio Hrg, Zagreb

Zagreb, September 2003

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Dear Colleagues,

Welcome to The Third European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine, Zagreb, Croatia, September 1- 5, 2003. The event will provide an opportunity for exchange of the latest information and technology in clinical and forensic genetics as well as cutting-edge advances in cellular and molecular medicine.

The first program in Split in 1997 and the second in Dubrovnik in 2001 were highly successful with the cumulative attendance of more than 800 participants from 62 countries. The program in Zagreb will feature plenary lectures by leaders in the field and poster presentations by participants. Programs in forensic medicine and in cellular and molecular medicine will run in parallel with the introductory and closing sessions held jointly. The Cellular and Molecular Medicine program is co-organized with the Mayo Clinic, Rochester, Minnesota, USA and University of Zagreb Program in Advanced Medical Education and chaired by Mayo's Dr. Stanimir Vuk-Pavlovi}. Special hands-on workshops will be offered by our sponsors Applied Biosystems and Roche Molecular Systems.

We are pleased that the Croatian Society of Human Genetics has decided to hold its Third Congress in conjunction with our program on September 3-4, 2003.

Many plenary talks and selected papers will be published in the Croatian Medical Journal, a publication listed in all major scientific indexes. The attendance of the conference will be accredited by the Croatian Medical Chamber with fifteen credit points. Furthermore, we are happy to offer two Young Investigator Awards (a plaque and US$500 each).
The social program will include a welcome reception for all participants at the Hotel Opera on the 17th floor. A one-day tour after the course will include the visit to the famed Plitvice Lakes National Park, a magical world of lakes, forests and waterfalls, which is under UNESCO protection.

The Croatian capital Zagreb has nine hundred years of documented history with abundant traces of the many civilizations and cultures that have influenced the region. Today it is a city of distinct Central European flavor. Wherever you find yourself in Zagreb, you will be surrounded by historic buildings, concert halls, parks, restaurants, bars, nightclubs and shops.

We are looking forward to sharing this unique scientific and social experience with you.

Sincerely yours,
Program Chairs
Moses Schanfield, Ph.D.
Dragan Primorac, M.D., Ph.D.
Mayo Clinic Course Chair
Stanimir Vuk-Pavloviæ, Ph.D.

CONFERENCE ORGANIZERS

Program Chairs

Moses Schanfield, George Washington University, Washington, DC, USA
Dragan Primorac, Split University School of Medicine, Osijek University School of Medicine and Polyclinic Holy Spirit II, Zagreb, Croatia

Chair, Mayo Clinic Course in Advanced Molecular and Cellular Medicine

Stanimir Vuk-Pavloviæ, Mayo Clinic, Rochester, MN, USA

Chair, The Third Croatian Congress in Human Genetics (September 3 - 5, 2003)

Ana Stavljeniæ-Rukavina, Zagreb University School of Medicine and Clinical Hospital, Zagreb, Croatia

Institutions

Clinical Hospital Split, Croatia
Croatian Society of Human Genetics, Croatia
General Hospital Holy Spirit, Croatia
International Commission for Missing Persons, USA
Mayo Clinic, USA
Polyclinic Holy Spirit II, Croatia
Split University School of Medicine, Croatia
University of Zagreb, Croatia

Standing Committee of the Mayo Clinic-University of Zagreb Program in Advanced Medical Education

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Henry Lee, *Connecticut State Police, Meriden, CT, USA*

Slobodan Macura, *Mayo Clinic, Rochester, MN, USA*
Timothy Palmbach, *Connecticut State Police, Meriden, CT, USA*
Thomas Parsons, *Armed Forces DNA Identification Laboratory, Rockville, MD, USA*
Walter Parson, *Institute of Legal Medicine, Innsbruck, Austria*
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Raj Puri, *Food and Drug Administration, Bethesda, MD, USA*
David Rowe, *University of Connecticut, School of Medicine, Farmington, USA*
Stephen Russell, *Mayo Clinic, Rochester, MN, USA*
John Ryan, *Myriad Genetic Laboratories, Inc., Salt Lake City, UT, USA*
David I. Smith, *Mayo Clinic, Rochester, MN, USA*
Petros Tsipouras, *University of Connecticut, School of Medicine, Farmington, USA*
Richard Weinshilboum, *Mayo Clinic, Rochester, MN, USA*
Patrick Willems, *Synergene, Mechelen, Belgium*

Local Organizing Committee:

Chairperson: **Paola Mariani**
Members: **Šimun Anðelinoviæ, Ivo Bariæ, Ingeborg Barišiæ, Floriana Buliæ-Jakus, Ivana Erceg Ivkošiæ, Aleksandra Fuèiæ, Stjepko Gamulin, Ante Ivkošiæ, Sendi Kuret, Dalibor Marijanoviæ, Krešimir Paveliæ, Damir Primorac, Jadranka Sertiæ, Vedrana Škaro, Ana Stavljeniæ-Rukavina, Elizabeta Topiæ, Renata Zadro, Ljiljana Zergollern Èupak**

Conference secretariat:

Ivana Erceg Ivkošiæ, Ljubica Grbiæ, Ante Ivkošiæ, Marko Maduniæ, Ivana Mikaèiæ, Petra Rožman

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http://www.studiohrg.hr

Scientific Program Information

Cancellations and Refunds

Notice of cancellation has to be made in writing by registered letter or fax to the Conference Office. The policy for refunding registration fees is as follows:

Written cancellation received:

- before July 30, 2003 75% refund
- after July 30, 2003 no refund

The date of the postmark or fax ID was the basis for considering refunds. Refunds will be made after the conference.

Certificate of Attendance

Confirmations of attendance will be issued at the registration desk.

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

The members of the scientific board will review all submitted abstracts and 2 selected authors will receive European-American Intensive Course Investigator Award. Recipients of the award will recieve a check of US$500 as well as a special certificate.

Credits

The Third European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine, Zagreb, Croatia, has been approved for 15 (participants) or 20 (lecturers) points by the Croatian Medical Chamber.

Sponsor Exhibition opening hours

Monday, September 1, 2003	12:05 p.m. - 03:00 p.m.
Tuesday, September 2, 2003	12:05 p.m. - 02:30 p.m.
Wednesday, September 3, 2003	12:05 p.m. - 03:00 p.m.

Language

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

Poster Sessions

All posters are on display on Tuesday and Wednesday, September 2 and 3, 2003, 12:05 p.m. - 3:00 p.m.

Poster Mounting

Tuesday, September 2, 2003	08:30 a.m. - 11:30 a.m.

Poster Removal

Thursday, September 4, 2003	08:00 a.m. - 02:00 p.m.

Poster board numbers can be found in the authors' index. The staff at the Poster Presenter's Desk will help you in finding both the number and location of the board.

Presence at Posters

Forensic poster session	
Tuesday, September 2, 2003	01:00 p.m. - 02:30 p.m.
Molecular poster session	
Wednesday, September 3, 2003	01:00 p.m. - 02:30 p.m.

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

Program Changes

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

Registration Desk Opening Hours

Sunday, August 31, 2003	04:00 p.m. - 08:00 p.m.
Monday, September 1, 2003	07:30 a.m. - 08:00 p.m.
Tuesday, September 2, 2003	08:00 a.m. - 04:00 p.m.
Wednesday, September 3, 2003	08:00 a.m. - 04:00 p.m.
Thursday, September 4, 2003	08:00 a.m. - 03:00 p.m.

Slide and Power Point Preview Room

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

Recipients of the 2001 European-American Intensive Course Awards

Forensic Identity Testing:Frontiers in Molecular and Cellular Medicine:

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

Recipients of The Third European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine Awards

The Third European - American School in Forensic Genetics:
Robert J. Shelton, CO, USA

Mayo Clinic Course in Advanced Molecular and Cellular Medicine:
Chiara Magri, Italy

General Information

Badges

Participants should collect name badges from the conference registration desks. As only registered participants will be permitted to attend the Scientific Sessions, the Exhibition and Poster Areas, you are kindly asked to wear your badge when entering the conference venue. Accompanying persons and exhibitors will also receive badges to allow access to the appropriate areas.
Lost badges can be replaced at the registration desk. However, a handling fee of EURO 10 will be charged.

Bank Services

The official currency in Croatia is the Croatian kuna (HRK). The official exchange rates:
1 EUR = 7.47 HRK* (August 15, 2003)
1 USD = 6.59 HRK* (August 15, 2003)
* Please note that these exchange rates are variable.

Opening hours of official Banks:

Banks and post offices are normally open from 8:00 a.m. to 7:00 p.m., Monday through Friday.

Cash Machines

Large numbers of cash machines accepting bank cards as well as most credit cards are located outside bank buildings throughout Zagreb.

Electricity Supply

220-240 V.

Insurance

In registering for The Third European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine, Zagreb, Croatia participants agree that neither the organizing committee nor the conference office assume any liability whatsoever. Participants are requested to make their own arrangements pertinent to health and travel.

Message Center

A Message Center is available at the registration desk.

Official Carrier

We are pleased to announce that the Croatia Airlines has agreed to be the official carrier of the conference. Croatia Airlines is offering 25% discount to the airfares for the Conference participants and accompanying persons. The discount is applicable on Croatian Airlines only, purchased in Croatia Airlines offices. The discount will be valid for booking 2 months before the conference and applies for traveling one week before, during and one week after the conference.

To receive this special discount you must mention The Third European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine, Zagreb, Croatia. Now, 25% discount voucher is available on line. Please, download and fill the form, than deliver it to yours nearest Croatia Airlines office. Location of the nearest Croatia Airlines office you can find by entering Croatia Airlines link. We wish you a pleasant flight with Croatia Airlines.
http://www.croatiaairlines.hr

Public Transportation in Zagreb

Zagreb offers an efficient system of public transportation with buses and trams. Almost all hotels have easy access to the public transportation system.

Restaurants

Most restaurants in Zagreb are open from 08:00 a.m. - 11:00 p.m. Please note that service charges are included in the price, unless explicitly mentioned. Nevertheless an additional tip of between 5-10% of the bill is expected, if you deem the service good enough. Some restaurants may have a cover charge.

Service Center

The following services are available at cost of the Service Center: photocopying, typing, production of overhead transparencies, computer printouts.

Shops

Shops in Zagreb are usually open from Monday to Saturday, from 08:00 a.m. - 09:00 p.m. Some are open on Sundays. Most shops accept all major credit cards.

Smoking Policy

The Third European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine is officially declared as a "Non-smoking-Conference".

Special requirements

Registrants with special requirements for physical disability or diet should contact us in advance.

Staff

If you should have any questions, the conference staff will be pleased to help you.

Taxi

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

Hotel Information

At Hotel Opera a wide choice of accommodation offers luxury and modern comfort with furnishings that reflect the Croatian tradition of gracious hospitality. The hotel is the perfect choice for both leisure and business travellers.

This five star luxury hotel is located in the heart of the city overlooking major cultural and historical attractions. The hotel is only a short stroll away from elegant shops, delightful parks, tree-lined streets and open spaces, and just five minutes walking distance from the business district and ten minutes from the main train station.

Hotel Features

- 24-hour front desk
- Express check-out
- Separate or early check-in
- Business centre
- Fitness centre/spa
- Indoor swimming pool
- Restaurants/bar/lounge
- 6 meeting rooms
- Beauty salon/Hairdresser
- Concierge
- Car rental
- Shops
- Covered garage: 150 spaces
- Airport transfer by hotel limousine

Transportation to/from Hotel

- Distance from Zagreb intl. airport: 17 km
- Time by taxi: approx. 20 minutes
- Cost by taxi: approx. US$ 20
- Cost by hotel limousine: approx. US$ 40
- Time by bus: approx. 25 minutes
- Cost by bus: approx. US$ 3
- Distance to city centre: 0.5 km
- Car park available for 150 cars

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

Post-congress tour

Friday, September 5, 2003
Plitvice Lakes National Park (Guided tour)
Price per person: $ 20 (bus, guide, entrance fee and lunch)
Duration: full day

Plitvice Lakes National Park, in the heart of Croatia, consists of 16 beautiful blue-green lakes, linked by a series of waterfalls and cascades to form a chain through a wooded valley. Over thousands of years, the waters that flow through this area have passed over limestone and chalk, creating deposits which form natural barriers between the lakes. The lakes range in height from Prošèansko Jezero at 636.6 m (1746 ft) above sea level, to the lowest, Kaluðerovac, at 505.2 m (1386 ft), and in surface area from 81 hectares (33 acres) to one hectare (2.5 acres). Many species of rare birds inhabit the woods around the lakes, as do bears and wolves. Aside from the great beauty of the Plitvice Lakes, which afford many opportunities for hiking, boating and other activities, visitors come here to breathe in the pure mountain air and enjoy local hospitality in the traditional lodges and hotels that lie within the bounds of the park.

INVITED SPEAKERS

The Third European-American School in Forensic Genetics

Antonio Alonso, *National Institute of Toxicology, Madrid, Spain*
Ted Anderson, *Armed Forces DNA Identification Laboratory, Rockville, MD, USA*
Christopher Asplen, *Smith Alling Lane, London, UK*
Suzanne M. Barritt, *Armed Forces DNA Identification Laboratory, Rockville, MD, USA*
Zoran Budimlija, *Office of Chief Medical Examiner, New York, NY, USA*
Cassandra Calloway, *Roche Molecular Systems, Alameda, CA, USA*
Cristian Capelli, *Catholic University of the Sacred Heart, Rome, Italy*
David Coffman, *Florida Department of Law Enforcement, Tallahassee, FL, USA*
Yasser Daoudi, *Applied Biosystems, Foster City, CA, USA*
Pero Dimsoski, *Applied Biosystems, Foster City, CA, USA*
Rebecca Hamm, *Armed Forces DNA Identification Laboratory, Rockville, MD, USA*
Juergen Henke, *Institute fuer Blutgruppenforschung, Cologne, Germany*
Charity Holland, *Bode Technology Group, Springfield, VA, USA*
Mitchell Holland*, *Bode Technology Group, Springfield, VA, USA*
Edwin Huffine, *International Commission on Missing Persons, Sarajevo, Bosnia and Herzegovina*
Marty Johnson, *Applied Biosystems, Foster City, CA, USA*
Greggory LaBerge, *Denver Police Department and University of Colorado Health Sciences Center, Denver, CO, USA*
Carl Ladd, *Connecticut State Police, Meriden, CT, USA*
Henry Lee*, *Connecticut State Police, Meriden, CT, USA*
Jose Lorente, *University of Granada, Granada, Spain*
Stephen Lupton, *Promega Corporation, Madison, WI, USA*
Terry Melton, *Mitotyping Technologies, State College, PA, USA*
Heather Miller Coyle, *Connecticut State Police, Meriden, CT, USA*
Timothy Palmbach*, *Connecticut State Police, Meriden, CT, USA*
Walther Parson, *Institute of Legal Medicine, Innsbruck, Austria*
Haskell Pitluck, *McHenry County Circuit Court, Woodstock, IL, USA*
Tanja Popoviæ, *Centers for Disease Control and Prevention, Atlanta, GA, USA*
Dragan Primorac, *Split University Scool of Medicine, Osijek University School of Medicine and Polyclinic Holy Spirit II, Zagreb, Croatia*
Mechthild Prinz, *Office of Chief Medical Examiner, New York, NY, USA*
Marilyn Raymond, *National Cancer Institute, Frederick, MD, USA*
John Ryan, *Myriad Genetic Laboratories, Salt Lake City, UT, USA*
Antti Sajantila, *University of Helsinki, Helsinki, Finland*
Moses Schanfield*, *George Washington University, Washington, DC, USA*
Peter Underhill*, *Stanford University Medical Center, Stanford, CA, USA*
Erin Williams*, *Foundation for Genetic Medicine, Reston, VA, USA*

* Plenary speaker

Mayo Clinic Course in Advanced Molecular and Cellular Medicine

Gideon Bach, *Hadassah University Hospital, Jerusalem, Israel*
Pavo Barišiæ*, *Institute of Philosophy, Zagreb, Croatia*
Frederick Bieber*, *Harvard Medical School, Boston, MA, USA*
Fiona Brew, *Affymetrix, London, UK*
Phil Danielson, *University of Denver, Denver, CO, USA*
Allan Dietz, *Mayo Clinic, Rochester, MN, USA*
Eithan Galun, *Hadassah University Hospital, Jerusalem, Israel*
Dennis Gastineau, *Mayo Clinic, Rochester, MN, USA*
Francis Glorieux, *McGill University, Shriners Hospital for Children, Montréal, QC, Canada*
Doron Lancet, *Weizmann Institute, Rehovot, Israel*
Slobodan Macura, *Mayo Clinic, Rochester, MN, USA*
Nino Margetiæ, *National Centre for Genotyping, Paris, France*
Tonèi Matuliæ*, *School of Catholic Theology, Zagreb, Croatia*
Dean Nižetiæ, *University of London, London, England*
Krešimir Paveliæ, *Institute Ruðer Boškoviæ, Zagreb, Croatia*
Pier Franco Pignatti, *Institute of Biology and Genetics University of Verona, Verona, Italy*
Doros Platika, *Centagenetix, Cambridge, MA, USA*
Jeffrey Platt*, *Mayo Clinic, Rochester, MN, USA*
Raj Puri, *Food and Drug Administration, Bethesda, MD, USA*
David Rowe, *University of Connecticut School of Medicine, Farmington, CT, USA*
Stephen Russell, *Mayo Clinic, Rochester, MN, USA*
Edgar Schreiber, *Celera Diagnostics, Alameda, CA, USA*
David Smith, *Mayo Clinic, Rochester, MN, USA*
Davor Solter, *Max Planck Institute of Immunobiology, Freiburg, Germany*
Petros Tsipouras, *University of Connecticut School of Medicine, Farmington, CT, USA*
Stanimir Vuk-Pavloviæ, *Mayo Clinic, Rochester, MN, USA*
Richard Weinshilboum*, *Mayo Clinic, Rochester, MN, USA*
Patrick Willems*, *Synergene, Mechelen, Belgium*
Trevor Woodage, *Applied Biosystems, Foster City, CA, USA*
Catherine Wu, *University of Connecticut School of Medicine, Farmington, CT, USA*
George Wu, *University of Connecticut School of Medicine, Farmington, CT, USA*

* Plenary speaker

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

Hotel Opera
September 1 - 5, 2003
Zagreb
Croatia

SCIENTIFIC PROGRAM

THE THIRD EUROPEAN-AMERICAN SCHOOL IN FORENSIC GENETICS

The Third European - American School in Forensic Genetics
Plenary sessions held in conjunction with the Mayo Clinic
Course in Advanced Molecular and Cellular Medicine

Sunday, August 31, 2003

04:00 p.m. - 08:00 p.m. ***Registration (Hotel Opera)***

Monday, September 1, 2003 (day 1)

07:30 a.m. - 08:00 p.m. ***Registration (Hotel Opera)***

Location: CRYSTAL BALLROOM (A and B)

08:30 a.m.	"***Art Treasures of Zagreb***" ***(a documentary film)***
08:50 a.m.	**INTRODUCTION TO THE PROGRAM**
	PLENARY SESSION: FRONTIERS IN GENETICS AND CELLULAR MEDICINE Chairpersons: Richard Weinshilboum and Moses Schanfield
09:00 a.m.	***Remarks from the chair***
09:05 a.m.	**Past and future of DNA typing in forensic sciences** Moses Schanfield, George Washington University, Washington, DC, USA
09:40 a.m.	**Pharmacogenetics and pharmacogenomics: the promise of individualized therapy** Richard Weinshilboum, Mayo Clinic, Rochester, MN, USA
10:15 a.m.	***Coffee break***

Location: CRYSTAL BALLROOM (A and B)

10:30 a.m.	**Inauguration of the 3rd European-American School of Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine**
11:00 a.m.	**The future impact of forensic DNA analysis** Mitchell Holland, Bode Technology Group, Springfield, VA, USA
11:35 a.m.	**Future of organ function replacement** Jeffrey Platt, Mayo Clinic, Rochester, MN, USA

12:10 p.m. **Y chromosome haplotypes: viewing human past trough the lens of the present**
Peter Underhill, Stanford University, Stanford, CA, USA

12:45 p.m. ***GENERAL DISCUSSION***

01:00 p.m. ***ADJOURN***

Location: MAIN WEST HALLWAY

12:05 a.m. - 03:00 p.m. ***Sponsor Exhibition***

Location: ZRINJEVAC / TUŠKANAC SALON

01:00 p.m. **ROCHE MOLECULAR SYSTEMS WORKSHOP**: *Applications of Linear Array mtDNA HVI/HVII Region Sequencing Typing kit (Advance registration required)*

Location: CRYSTAL BALLROOM (B)

INNOVATIONS IN DNA TYPING TECHNOLOGY
Chairpersons: Nicola Oldroyd and Stephen Lupton

04:00 p.m. ***Remarks from the chair***

04:05 p.m. **Development of a male-specific 12-locus fluorescent multiplex**
Stephen Lupton, Promega, Madison, WI, USA

04:35 p.m. **Alternative STR application in human identification**
Pero Dimsoski, Applied Biosystems, Foster City, CA, USA

05:05 p.m. **Development of two new quantitative kits using real-time PCR**
Yasser Daoudi, Applied Biosystems, Foster City, CA, USA

05:35 p.m. ***Coffee break***

05:50 p.m. **Linear array mitochondrial DNA HVI/HVII region-sequence typing kit: applications, methods and interpretation issues**
Cassandra Calloway, Roche Molecular Systems, Alameda, CA, USA

06:20 p.m. **The next step in laboratory automation**
John Ryan, Myriad Genetic Laboratories, Salt Lake City, UT, USA

06:50 p.m. ***GENERAL DISCUSSION***

07:05 p.m. ***ADJOURN***

Tuesday, September 2, 2003 (day 2)

08:00 a.m. - 04:00 p.m. ***Registration (Hotel Opera)***

Location: CRYSTAL BALLROOM (B)

DNA ANALYSIS FROM CHALLENGING SAMPLES
Chairpersons: Jose Lorente and Edwin Huffine

08:00 a.m. ***Remarks from the chair***

08:05 a.m. **Identification of war victims by molecular methods**
Dragan Primorac, Split University Scool of Medicine, Osijek University School of Medicine and Polyclinic Holy Spirit II, Zagreb, Croatia

08:35 a.m. **Ancient DNA and identification of the remains of historical celebrities: the case of Christopher Columbus**
Jose Lorente, University of Granada, Granada, Spain

09:05 a.m. **Quantifying low copy number human genomes in forensic and ancient DNA studies**
Antonio Alonso, National Institute of Toxicology, Madrid, Spain

09:35 a.m. **DNA typing of decomposed bodies and skeletal remains**
Edwin Huffine, International Commission on Missing Persons, Sarajevo, Bosnia and Herzegovina

10:05 a.m. ***Coffee break***

10:20 a.m. **Improved processing of ancient skeletal remains for mtDNA analysis**
Susan Barritt, Armed Forces DNA Identification Laboratory, Rockville, MD, USA

10:50 a.m. **Molecular methods for identification and individualization of marijuana**
Heather Miller Coyle, Connecticut State Police, Meriden, CT, USA

11:20 a.m. **An STR typing system for forensic analysis of domestic cat specimens**
Marilyn Raymond, National Cancer Institute, Frederick, MD, USA

11:50 a.m. ***GENERAL DISCUSSION***

12:05 p.m. ***ADJOURN***

Location: MAIN WEST HALLWAY

12:05 a.m. - 02:30 p.m. ***Sponsor Exhibition***

Location: MAKSIMIR SALON

12:05 p.m. - 03:00 p.m. ***Poster Session***

01:00 p.m. - 02:30 p.m. ***Presence required for poster authors with forensic topics***

Location: CRYSTAL BALLROOM (B)

X CHROMOSOMES, Y CHROMOSOMES AND MITOCHONDRIAL DNA
Chairpersons: Walther Parson and Juergen Henke

02:30 p.m. ***Remarks from the chair***

02:35 p.m. **Inheritance of X-chromosomal STR haplotypes**
Juergen Henke, Institute for Study of Blood Groups, Cologne, Germany

03:05 p.m. **Interpretation of Y STRs mixtures**
Carl Ladd, Connecticut State Police, Meriden, CT, USA

03:35 p.m. **Rapid mtDNA profiling by denaturing high pressure liquid chromotography**
Greggory LaBerge, University of Colorado Health Sciences Center, Denver, CO, USA

04:05 p.m. ***Coffee break***

04:20 p.m. **Analysis of human hair mtDNA: success, size, and site heteroplasmy**
Terry Melton, Mitotyping Technologies, State College, PA, USA

04:50 p.m. **Multiplex SNP panels for increased discrimination in forensic mtDNA testing**
Rebecca Hamm, Armed Forces DNA Identification Laboratory, Rockville, MD, USA

05:20 p.m. **EMPOP: EDNAP mitochondrial DNA population database**
Walther Parson, Institute of Legal Medicine, Innsbruck, Austria

05:50 p.m. ***GENERAL DISCUSSION***

06:05 p.m. ***ADJOURN***

08:30 p.m. ***WELCOME RECEPTION IN THE CRYSTAL BALLROOM OF THE HOTEL "OPERA"***

Wednesday, September 3, 2003 (day 3)

08:00 a.m. - 04:00 p.m. ***Registration (Hotel Opera)***

Location: CRYSTAL BALLROOM (B)

FORENSIC PATHOLOGY, BIOTERRORISM AND DNA IN THE COURTROOM
Chairpersons: Chris Asplen and Tanja Popoviæ

08:00 a.m. ***Remarks from the chair***

08:05 a.m. **Molecular strategies for biothreat agent identification and characterization**
Marty Johnson, Applied Biosystems, Foster City, CA, USA

08:35 a.m. **Laboratory aspects of bioterrorism-related anthrax: from identification to molecular subtyping and microbial forensics**
Tanja Popoviæ, Centers for Disease Control and Prevention, Atlanta, GA, USA

09:05 a.m. **Genetic risk factors for sudden, unexpected death**
Antti Sajantila, University of Helsinki, Helsinki, Finland

09:35 a.m. ***Coffee break***

09:50 a.m. **Forensic DNA legislative update**
Chris Asplen, Smith Alling Lane, London, UK

10:20 a.m. **The use of automation and recidivism research to broaden the scope of Florida's DNA investigative support database**
David Coffman, Florida Department of Law Enforcement, Tallahassee, USA

10:50 a.m. **DNA Innocence Project in the USA**
Chris Asplen, Smith Alling Lane, London, UK

11:20 a.m. **Liability of expert witnesses**
Haskell Pitluck, McHenry County Circuit Court, Woodstock, IL, USA

11:50 a.m. ***GENERAL DISCUSSION***

12:05 p.m. ***ADJOURN***

Location: ZRINJEVAC / TUŠKANAC SALON

12:05 p.m. **APPLIED BIOSYSTEMS WORKSHOP**: *PCR-Based Detection Instruments and Chemistry in Forensic Genetics (Advance registration required)*

Location: MAIN WEST HALLWAY

12:05 a.m. - 03:00 p.m. ***Sponsor Exhibition***

Location: MAKSIMIR SALON

12:05 p.m. - 03:00 p.m. ***Poster Session***

01:00 p.m. - 02:30 p.m. ***Presence required for poster authors with molecular topics***

Location: CRYSTAL BALLROOM (B)

DNA ANALYSIS IN MASS DISASTER
Chairpersons: Mechthild Prinz and Ted Anderson

03:00 p.m. ***Remarks from the chair***

03:05 p.m. **Genetic identification and re-association of human remains from casualty incidents and mass disasters: Recent experience and improvements at the United States Armed Forces DNA Identification Laboratory**
Ted Anderson, Armed Forces DNA Identification Laboratory, Rockville, MD, USA

03:35 p.m. **The World Trade Center mass fatality: special circumstances and lessons learned**
Mechthild Prinz, Office of Chief Medical Examiner, New York, NY, USA

04:05 p.m. ***Coffee break***

04:20 p.m. **Utilizing novel mini-STR multiplexes in the identification of World Trade Center disaster victims**
Charity Holland, Bode Technology Group, Springfield, VA, USA

04:50 p.m. **Experiences with individual body identification cases during the World Trade Center victims ID effort**
Zoran Budimlija, Office of Chief Medical Examiner, New York, NY, USA

05:20 p.m. ***GENERAL DISCUSSION***

05:35 p.m. ***ADJOURN***

Thursday, September 4, 2003 (day 4)

08:00 a.m. - 03:00 p.m. ***Registration (Hotel Opera)***

Location: CRYSTAL BALLROOM (A and B)

PLENARY SESSION: KNOWLEDGE BASES IN GENOMICS, MOLECULAR MEDICINE AND FORENSIC SCIENCE
Chairpersons: Trevor Woodage and Henry Lee

08:00 a.m. ***Remarks from the chair***

08:05 a.m. **DNA and solving cold cases**
Henry Lee, Connecticut State Police, Meriden, CT, USA

08:40 a.m. **Converting genomic data into a knowledge base for genetic studies**
Trevor Woodage, Applied Biosystems, Foster City, CA, USA

09:15 a.m. **Bioinformatics for high-throughput SNP genotyping using MALDI**
Nino Margetic, National Centre for Genotyping, Paris, France

09:45 a.m. ***Coffee break***

10:00 a.m. **Forensic mathematics: mathematics of DNA identification**
Frederick Bieber, Harvard Medical School, Boston, MA, USA

10:35 a.m. **Distribution of Y chromosome haplotypes in humans: forensic implications**
Cristian Capelli, Sacred Heart Catholic University, Rome, Italy

11:10 a.m. **Organizing genetic testing through an international consortium**
Patrick Willems, Synergene, Mechelen, Belgium

11:40 a.m. **Organizing and managing a government DNA testing laboratory**
Timothy Palmbach, Connecticut State Police, Meriden, CT, USA

12:10 p.m. ***GENERAL DISCUSSION***

12:25 p.m. ***ADJOURN***

Location: CRYSTAL BALLROOM (A and B)

PLENARY SESSION: BIOETHICS - ISSUES OF LIFE AND MEDICINE
Chairpersons: Erin Williams and Stanimir Vuk-Pavloviæ

02:00 p.m. ***Remarks from the chair***

02:05 p.m. **Ethical theories of life**
Pavo Barišiæ, Institute of Philosophy, Zagreb, Croatia

02:25 p.m. **Genetics as new creation**
Tonèi Matuliæ, School of Catholic Theology, Zagreb, Croatia

02:45 p.m. **From dust to dust: ethics of identification of remains from mass graves**
Erin Williams, Foundation for Genetic Medicine, Reston, VA, USA

03:15 p.m. **Bioethics Roundtable**: a moderated discussion

CLOSING OF THE 3rd EUROPEAN-AMERICAN SCHOOL IN FORENSIC GENETICS AND THE MAYO CLINIC COURSE IN ADVANCED MOLECULAR AND CELLULAR MEDICINE

Friday, September 5, 2003 (day 5)

08:00 a.m. ***VISIT TO PLITVICE NATIONAL PARK***
Gathering in the main hallway of the Hotel Opera

Joint Programs with "The 3rd European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine"

Monday, September 1, 2003

Location: MAKSIMIR SALON

01:00 p.m. - 04:00 p.m. **Symposium "Advances of Molecular Diagnostics in Infectology and Oncology",** Croatian Society of Medical Biochemists

Wednesday, September 3, 2003

Location: CRYSTAL BALLROOM (A and B)

06:00 p.m. - 07:00 p.m. The Third Croatian Congress of Human Genetic with international participation, (**Opening ceremony)**

Thursday, September 4, 2003

Location: PANORAMA SALON (on the 17th FLOOR of the HOTEL OPERA)

10:00 a.m. The Third Croatian Congress of Human Genetic with international participation, (**Lectures**)

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

Hotel Opera
September 1 - 5, 2003
Zagreb
Croatia

SCIENTIFIC PROGRAM

MAYO CLINIC COURSE IN ADVANCED MOLECULAR AND CELLULAR MEDICINE

Plenary sessions held in conjunction with the Third European-American School in Forensic Genetics

Sunday, August 31, 2003

04:00 p.m. - 08:00 p.m. ***Registration (Hotel Opera)***

Monday, September 1, 2003 (day 1)

07:30 a.m. - 08:00 p.m. ***Registration (Hotel Opera)***

Location: CRYSTAL BALLROOM (A and B)

08:30 a.m.	"***Art Treasures of Zagreb***" ***(a documentary film)***
08:50 a.m.	**INTRODUCTION TO THE PROGRAM**
	PLENARY SESSION: FRONTIERS IN GENETICS AND CELLULAR MEDICINE Chairpersons: Richard Weinshilboum and Moses Schanfield
09:00 a.m.	***Remarks from the chair***
09:05 a.m.	**Past and future of DNA typing in forensic sciences** Moses Schanfield, George Washington University, Washington, DC, USA
09:40 a.m.	**Pharmacogenetics and pharmacogenomics: the promise of individualized therapy** Richard Weinshilboum, Mayo Clinic, Rochester, MN, USA
10:15 a.m.	***Coffee break***

Location: CRYSTAL BALLROOM (A and B)

10:30 a.m.	**Inauguration of the 3rd European-American School of Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine**
11:00 a.m.	**The future impact of forensic DNA analysis** Mitchell Holland, Bode Technology Group, Springfield, VA, USA
11:35 a.m.	**Future of organ function replacement** Jeffrey Platt, Mayo Clinic, Rochester, MN, USA
12:10 p.m.	**Y chromosome haplotypes: viewing human past through the lens of the present** Peter Underhill, Stanford University, Stanford, CA, USA

12:45 p.m. ***GENERAL DISCUSSION***

01:00 p.m. ***ADJOURN***

Location: MAIN WEST HALLWAY

12:05 p.m. - 3:00 p.m. ***Sponsor Exhibition***

Location: CRYSTAL BALLROOM (A)

GENETICS OF DISEASE
Chairpersons: Edgar Schreiber and Frederick Bieber

04:00 p.m. ***Remarks from the chair***

04:05 p.m. **Human Genome Project and cancer genetics**
David Smith, Mayo Clinic, Rochester, MN, USA

04:35 p.m. **Genetic diagnosis of preimplantation embryos**
Frederick Bieber, Harvard Medical School, Boston, MA, USA

05:05 p.m. **A mucolipidosis type IV gene: implications for Ashkenazi Jews**
Gideon Bach, Hadassah University Hospital, Jerusalem, Israel

05:35 p.m. ***Coffee break***

05:50 p.m. **A high throughput diagnostic assay for detection of clinical mutations and SNPs in the human cystic fybrosis gene**
Edgar Schreiber, Celera Diagnostics, Alameda, CA, USA

06:20 p.m. **Genetics of complex diseases**
Pier Franco Pignatti, University of Verona, Verona, Italy

06:50 p.m. **Growth and differentiation of trisomy-21 cells**
Dean Nižetiæ, University of London, London, England

07:20 p.m. ***GENERAL DISCUSSION***

07:35 p.m. ***ADJOURN***

Tuesday, September 2, 2003 (day 2)

08:00 a.m. - 04:00 p.m. ***Registration (Hotel Opera)***

Location: CRYSTAL BALLROOM (A)

PHARMACOGENETICS, PHARMACOGENOMICS AND NEW TECHNIQUES IN MOLECULAR DIAGNOSTICS
Chairpersons: Doros Platika and Krešimir Paveliæ

08:30 a.m. ***Remarks from the chair***

08:35 a.m. **Pharmacogenomics tools: genome-wide expression patterns, automatic data mining and high throughput SNP analyses**
Doron Lancet, Weizmann Institute of Science, Rehovot, Israel

09:05 a.m. **Genetics, stem cells and centenarians: the pathway to longevity therapeutics**
Doros Platika, Centagenetix, Cambridge, MA, USA

09:35 a.m. **Nanotechnology and molecular medicine**
Krešimir Paveliæ, Ruðer Boškoviæ Institute, Zagreb, Croatia

10:05 a.m. ***Coffee break***

10:20 a.m. **Clinical applications of denaturing HPLC DNA profiling**
Phil Danielson, University of Denver, Denver, CO, USA

10:50 a.m. **Affymetrix GeneChip technology: overview and applications**
Fiona Brew, Affymetrix, London, UK

11:20 a.m. ***GENERAL DISCUSSION***

11:35 p.m. ***ADJOURN***

Location: ZRINJEVAC / TUŠKANAC SALON

12:05 p.m. **APPLIED BIOSYSTEMS WORKSHOP**: *PCR-Based Detection Instruments and Chemistry in Molecular Medicine (Advance registration required)*

Location: MAIN WEST HALLWAY

12:05 p.m. - 2:30 p.m. ***Sponsor Exhibition***

Location: MAKSIMIR SALON

12:05 p.m. - 3:00 p.m. ***Poster Session***

01:00 p.m. - 2:30 p.m. ***Presence required for poster authors with forensic topics***

Location: CRYSTAL BALLROOM (A)

GENE THERAPY - GENES FOR TREATMENT OF CANCER AND METABOLIC DISEASES
Chairpersons: Stephen Russell and Francis Glorieux

02:30 p.m. ***Remarks from the chair***

02:35 p.m. **Engineering viruses for cancer therapy**
Stephen Russell, Mayo Clinic, Rochester, MN, USA

03:05 p.m. **Liver directed gene therapy**
Eithan Galun, Hadassah University Hospital, Jerusalem, Israel

03:35 p.m. **Osteogenesis imperfecta: pathophysiology and therapy**
Francis Glorieux, McGill University, Montréal, QC, Canada

04:05 p.m. ***Coffee break***

04:20 p.m. **Use of hammerhead ribozymes in a murine model of osteogenesis imperfecta**
Petros Tsipouras, University of Connecticut School of Medicine, Farmington, CT, USA

04:50 p.m. **Strategies for targeted inhibition of hepatitis viruses**
George Wu, University of Connecticut School of Medicine, Farmington, CT, USA

05:20 p.m. ***GENERAL DISCUSSION***

05:35 p.m. ***ADJOURN***

08:30 p.m. ***WELCOME RECEPTION IN THE CRYSTAL BALLROOM OF THE HOTEL "OPERA"***

Wednesday, September 3, 2003 (day 3)

08:00 a.m. - 04:00 p.m. ***Registration (Hotel Opera)***

Location: CRYSTAL BALLROOM (A)

MOLECULAR AND CELLULAR IMMUNOTHERAPY
Chairpersons: Jeffrey Platt and Raj Puri

08:30 a.m. ***Remarks from the chair***

08:35 a.m. **Modifying cellular immunogenicity**
Jeffrey Platt, Mayo Clinic, Rochester, MN, USA

09:05 a.m. **Introduction to cell graft engineering**
Stanimir Vuk-Pavloviæ, Mayo Clinic, Rochester, MN, USA

09:35 a.m. **Nuclear magnetic resonance in development of cellular therapies**
Slobodan Macura, Mayo Clinic, Rochester, MN, USA

10:05 a.m. ***Coffee break***

10:20 a.m. **IL-13 receptor targeted cancer therapy: from bench to bedside**
Raj Puri, Food and Drug Administration, Bethesda, MD, USA

10:50 a.m. **Liver cell transplantation, distribution and function**
Catherine Wu, University of Connecticut School of Medicine, Farmington, CT, USA

11:20 a.m. ***GENERAL DISCUSSION***

11:35 a.m. ***ADJOURN***

Location: MAIN WEST HALLWAY

12:05 a.m. - 3:00 p.m. ***Sponsor Exhibition***

Location: MAKSIMIR SALON

12:05 p.m. - 3:00 p.m. ***Poster Session***

01:00 p.m. - 2:30 p.m. ***Presence required for poster authors with molecular topics***

Location: CRYSTAL BALLROOM (A)

STEM CELLS, PROGENITORS, TISSUES AND ORGANS
Chairpersons: Davor Solter and Dennis Gastineau

03:00 p.m. ***Remarks from the chair***

03:05 p.m.	**Embryonic stem cells and therapeutic cloning: realities and possibilities** Davor Solter, Max Planck Institute of Immunobiology, Freiburg, Germany
03:35 p.m.	**Bone diseases as afflictions of osteoprogenitor lineage** David Rowe, University of Connecticut School of Medicine, Farmington, CT, USA
04:05 p.m.	***Coffee break***
04:35 p.m.	**Cell therapy from bench to bedside: translational aspects of dendritic cell immunotherapy of cancer** Allan Dietz, Mayo Clinic, Rochester, MN, USA
05:05 p.m.	**Clinical grade cell graft preparation: pharmaceutical plants in medical centers** Dennis Gastineau, Mayo Clinic, Rochester, MN, USA
05:35 p.m.	***GENERAL DISCUSSION***
05:50 p.m.	***ADJOURN***

Thursday, September 4, 2003 (day 4)

08:00 a.m. - 03:00 p.m. ***Registration (Hotel Opera)***

Location: CRYSTAL BALLROOM (A and B)

PLENARY SESSION: KNOWLEDGE BASES IN GENOMICS, MOLECULAR MEDICINE AND FORENSIC SCIENCE
Chairpersons: Trevor Woodage and Henry Lee

08:00 a.m. ***Remarks from the chair***

08:05 a.m. **DNA and solving cold cases**
Henry Lee, Connecticut State Police, Meriden, CT, USA

08:35 a.m. **Converting genomic data into a knowledge base for genetic studies**
Trevor Woodage, Applied Biosystems, Foster City, CA, USA

09:05 a.m. **Bioinformatics for high-throughput SNP genotyping using MALDI**
Nino Margetiæ, National Centre for Genotyping, Paris, France

09:45 a.m. ***Coffee break***

10:00 a.m. **Forensic mathematics: mathematics of DNA identification**
Frederick Bieber, Harvard Medical School, Boston, MA, USA

10:35 a.m. **Distribution of Y chromosome haplotypes in humans: forensic implications**
Cristian Capelli, Sacred Heart Catholic University, Rome, Italy

11:10 a.m. **Organizing genetic testing through an international consortium**
Patrick Willems, Synergene, Mechelen, Belgium

11:40 a.m. **Organizing and managing a government DNA testing laboratory**
Timothy Palmbach, Connecticut State Police, Meriden, CT, USA

12:15 p.m. ***GENERAL DISCUSSION***

12:30 p.m. ***ADJOURN***

Location: CRYSTAL BALLROOM (A and B)

PLENARY SESSION: BIOETHICS - ISSUES OF LIFE AND MEDICINE
Chairpersons: Erin Williams and Stanimir Vuk-Pavloviæ

02:00 p.m. ***Remarks from the chair***

02:05 p.m. **Ethical theories of life**
Pavo Barišiæ, Institute of Philosophy, Zagreb, Croatia

02:25 p.m. **Genetics as new creation**
Tonèi Matuliæ, School of Catholic Theology, Zagreb, Croatia

02:45 p.m. **From dust to dust: ethics of identification of remains from mass graves**
Erin Williams, Foundation for Genetic Medicine, Reston, VA, USA

03:15 p.m. **Bioethics Roundtable:** a moderated discussion

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Friday, September 5, 2003 (day 5)

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Wednesday, September 3, 2003

Location: CRYSTAL BALLROOM (A and B)

06:00 p.m. - 07:00 p.m. The Third Croatian Congress of Human Genetic with international participation, (**Opening ceremony)**

Thursday, September 4, 2003

Location: PANORAMA SALON (on the 17th FLOOR of the HOTEL OPERA)

10:00 a.m. The Third Croatian Congress of Human Genetic with international participation, (**Lectures**)

ABSTRACTS

THE THIRD EUROPEAN-AMERICAN SCHOOL IN FORENSIC GENETICS

Presentation number: F1

OUR EXPERIENCE: TEN YEARS WORK ON IDENTIFICATION OF SKELETAL REMAINS FROM MASS GRAVES

Anðelinoviæ Š, Definis Gojanoviæ M, Sutloviæ D, Drmiæ I, Erceg Ivkošiæ I, Škaro V, Ivkošiæ A,
Paiæ F, Primorac D.
Laboratory for Clinical and Forensic Genetics, Split University Hospital, Split, Croatia
simun.andjelinovic@st.htnet.hr

Significant efforts are currently underway to identify missing individuals discovered in mass graves situated throughout Croatia and southern Bosnia and Herzegovina. During last ten years (1993-2003) more than 900 bodies found in several mass graves have been identified in our Department by standard forensic methods. Unfortunately, standard methods for human identification were not sufficient in approximately 30-35% of all cases and DNA identification was requested. DNA isolation was performed using standard phenol/chloroform/isoamyl alcohol procedure as well as some advanced methods (decalcification with EDTA prior to extraction and NaOH repurification). Recently, new commercial procedures for DNA extraction (DNA IQ System) and DNA quantitation (Alu Quant Human DNA Quantification System) were successfully tested in our laboratory. The quality of isolated DNA varied in different types of bones and due to DNA degradation and contamination. During the last ten years, the following DNA identification systems were used: AmpliType®PM+DQA1 PCR Amplification and Typing Kit, AmpFlSTR Profiler™ PCR Amplification Kit, AmpFlSTR Profiler Plus™ PCR Amplification Kit, PowerPlex™ 16 System, AmpFlSTR Identifiler™ PCR Amplification Kit, immobilized SSO (sequence-specific oligonucleotide) probes for the mitochondrial DNA control region and Y-Plex™6. Up to date we have analyzed 400 samples by DNA methods and obtained full genotypes in 305 samples (76.25%) with DNA matches confirmed in 68 cases.

Presentation number: F2

MATERNITY TESTING IN A LEGAL PRACTICE - CASE REPORT

Anðelinoviæ Š, Mršiæ T[1], Loliæ Ž[1], Jurišiæ Sokiæ M[1], Tonkoviæ S[2], Drmiæ I, Primorac Damir[2].
Laboratory for Clinical and Forensic Genetics, University Hospital Split, Split, Croatia, [1]Law School Split, University of Split, Split, Croatia, [2]Municipal Court Split, Split, Croatia
simun.andjelinovic1@st.hinet.hr

In January of 1994 a twenty day old baby girl was found in the elevator. During the police investigation a twenty-one year old female refugee from Bosnia and Herzegovina confessed that she is the biological mother of the child. She also told investigators that because of her poor economical status she was not able to take care of the child and she hoped that somebody would take care of the baby. She also confessed that several years ago she left another child but that time in Bosnia and Herzegovina. The judge has ordered DNA testing to approve maternity. Surprisingly, the female was excluded as the biological mother in seven DNA loci and police withdrew all charges against her. In this case we would like to underline the importance of complete police, medical and legal investigation as well as collaboration among them. However, many questions such as: "Was the woman really pregnant or has she put to death another child that was never found" still left opened?

Presentation number: F3

MIXED STAINS FROM SEXUAL ASSAULT CASES: AUTOSOMAL STRs OR Y-CHROMOSOME STRs?

Cerri N, Ricci U[1], Sani I[1], Verzeletti A, De Ferrari F.
Institute of Forensic Medicine, University of Brescia, Italy, [1]Hospital "A. Meyer", Genetics and Molecular Medicine Unit, Florence, Italy
deferrar@med.unibs.it

Y-chromosomal STR-loci have been extensively investigated in forensic science for male identification. Y-chromosome analysis can be useful to detect the male DNA fraction from sexual assault stains or rape cases that show a male and female mixture. Normally, the first approach in a DNA analysis of forensic samples of this nature consists in the use of STR systems, because of the fact that these markers give more information and because it's easier to use them. In other occasions the use of STR markers failed to detect the autosomal DNA profile of the semen contributor, so it is necessary to support this information with an Y-STR analysis. The aim of our work is to report our experience in DNA investigation in cases of sexual assault where we only dispose mixed samples from different sources. Analysis of forensic samples was performed by using a panel of autosomal STRs and evaluation of the presence of male contribution examining the Amelogenin locus. Further analysis of samples with a panel of tetrameric Y-STR (DYS19, DYS390, DYS391, DYS392, DYS385, DYS389I and II). In the cases we show that Y-STRs were useful to increase the efficiency of the forensic genetic analyses. It was possible to identify a partial or full Y-profile of the rapists in different mixtures, when autosomal STRs were unable to detect a genetic profile. When DNA from mixed stains produces sufficient male DNA together with female DNA, the analysis of autosomal STRs was sufficient to identify the full profile of the rapist. When the mixture was examined in different male to male proportions, only the full profile of the major component was detected. The main advantage of the Y-STR approach remains the ability to detect the male component in the mixed stains when the DNA of the male contributor is present only in a very small amount.

Presentation number: F4

UNUSUAL FAILURE OF GENDER DETERMINATION USING AMELOGENIN TEST

Drobniè K.
Forensic Lab and Research Centre, Ministry of the Interior, Ljubljana, Slovenia
katja.drobnic@mnz.si

Sex determination can be an important piece of information in various forensic casework analyses and DNA databasing. Because of that gender determination has nowadays become a part of most human identification PCR kits. Although different PCR-based methods are known to identify a sample as originating from a male or a female, the only sex test included in commercially available human identification PCR kits for gender determination is based on the amelogenin sex test described by Sullivan et al. (1993), with primers spanning a part of the first intron, which results in PCR products that vary from each other by 6-bp. Although this method is known as a reliable method for sex determination, the amelogenin gene is exposed to mutation and deletion, like all genetic loci. This poster reports the results of a case where a deletion located on the Amel-Y gene resulted in the detection of only the X-specific PCR product. The same results were obtained by using three different primer sets for the amplification of this region of the amelogenin gene. The presence of Y chromosome was determined by using Y- STR markers. The male phenotype of the individual was additionally confirmed by the amplification of a fragment of the SRY gene. The observed failure rate of the amelogenin sex test was 0.03 %. This observation suggested that genetic determination of sex based only on amelogenin gene marker from crime scene samples, or some other forensic evidence, should not be considered infallible.

Presentation number: F5

ANALYSIS OF THREE-LOCUS STR HAPLOTYPES AS A TOOL TO STUDY INBREEDING EFFECTS

Džijan S, Biruš I, Lauc G.
University of Osijek School of Medicine, DNA Laboratory, Osijek, Croatia
glauc@public.srce.hr

Today it is generally accepted that short tandem repeat (STR) sequences can provide sufficient information to determine identity or paternity in most forensic cases. Elaborate statistical methods have been developed to quantify the significance of genetic matches, but the calculated numbers may not be valid in all situations and have to be used with caution. As a part of the process of identification of war victims in Croatia we have randomly matched hundreds of thousands of genotypes and observed unexpectedly high number of statistically improbable matches between unrelated people. We speculated that this might be a consequence of higher than expected frequency of specific combinations of STR alleles because of local inbreeding. To assess this hypothesis we have developed a novel method that analyses incidence of all possible three-locus haplotype combinations (mini-haplotypes) in a population. After calculating frequencies for all possible mini-haplotypes we confirmed our hypothesis that some combinations are much more frequent than expected. Different combinations were found to be most frequent in Croatian and Hungarian populations supporting the hypothesis that these aberrations from expected values might be population-specific. Probabilities of complete 9-loci haplotypes calculated by combining frequencies of three-locus haplotypes can be significantly different from probabilities calculated using individual alleles, suggesting that currently used statistical methods might not be accurate in genotypes that contain these over-abundant mini-haplotypes.

Presentation number: F6

DNA ANALYSIS OF DAMAGED FORENSIC SAMPLES

Fattorini P, Perossa R, Tomasella F, Grignani P[1], Previderi C[2].
UCO of Legal Medicine, Department of Sciences of Public Health, University of Trieste, Italy, [1]IRCCS Policlinico S. Matteo, Department of Legal Medicine and Public Health, Pavia, Italy, [2]Department of Legal Medicine and Public Health, University of Pavia, Italy
fattorin@univ.trieste.it

The molecular characterization of the DNA recovered from post-mortem specimens is an important topic in forensic medicine, but only few studies have investigated a correlation between the degree of chemical damage of the forensic samples and the reliability of the PCR typing results. DNA damage, with a significant reduction of the four canonical bases, is a common finding in aged forensic samples, as already shown by reverse-phase HPLC/MS and capillary electrophoresis (CE) analysis. A routine use of these methods to check the forensic samples has an important limitation in the amount of DNA, as hundreds nanograms of genetic material are needed. Another important feature of damaged DNA is the low sensibility to human specific DNA probing. For these reason we analysed the DNA extracted from several different forensic specimens (such as bloodstains, skeletal remains, etc) aged up to 30 years by slot-blot hybridization, using a human Alu probe. The same amount of DNA was also amplified by PCR using a commercial kit containing 15 STR markers and the sex determinations marker Amelogenin. Most of the forensic samples with low sensibility to human DNA probing showed high frequency of PCR artefacts (preferential amplification of an allele extra-bans, allelic ladders, etc) or no specific peak for any STR locus. Our results show that the hybridization data can provide useful information on the reliability of the PCR typing results.

Presentation number: F7

POWER PLEX 16 AND IDENTIFILER SYSTEM IN CRIMINALITY IDENTIFICATION

Eminoviæ I, Huffine E[1], Tihiæ N, Hadžiavdiæ V, Hadžiæ N.
DNA laboratory, Department for forensic medicine Tuzla, University Clinical Centre Tuzla, Bosnia and Herzegovina, [1]The International Commission on Missing Persons in the former Yugoslavia (ICMP)
izeteminovic@hotmail.com

The STR analysis is very important in forensic examination criminal cases. We examined the use of PowerPlex 16 (Promega) and Identifiler (Applied Biosystems) system in one crime case. After DNA extraction from the biological trace we made amplification with PowerPlex16 and Identifiler system from the same samples. STR fragments amplified with Identifiler were clear and reportable but PowerPlex 16 were not. In some difficult forensic cases we recommend the use both STR systems to get complete and exact DNA profile.

Presentation number: F8

MODIFIED EXTRACTION NUCLEAR DNA IN SOLVING DIFFICULT CRIMINAL CASES

Eminoviæ I, Huffine E[1], Tihiæ N, Hadžiavdiæ V, Hadžiæ N.
Department for forensic medicine Tuzla, University Clinical Centre Tuzla, Bosnia and Herzegovina,
[1]The International Commission on Missing Persons in the former Yugoslavia (ICMP)
izeteminovic@hotmail.com

We examined combined use in house method and DNA IQ system (Promega) procedure for the extraction DNA from biological trace in order to help the solving some crime cases. This simple and efficient method for biological trace samples included the well washed trousers and the knife without any visual biological trails (from some one hard criminal case). In this method there were used: centrifuge, microwave oven, shaker, chelex, proteinase K, DNA IQ System (Promega). The extraction of DNA could be done within 2.5 hours or over the night. The DNA prepared on this way was good quality and could be used for STR analysis. This combined method could be used for extraction DNA from samples containing a minute amount of the biological trace.

Presentation number: F9

THE ROLE OF DNA ANALYSIS IN WAR VICTIMS IDENTIFICATION IN CROATIA

Furaè I, Karija Vlahoviæ M, Mašiæ M, Marketin S, Raguž I, Kubat M.
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DNA technology has provided powerful methods for forensic application. DNA profiling has rapidly become a routine technique in forensic laboratories worldwide. The same happened in Croatia as well. We perform paternity testing, identity testing, criminal casework and the identification of human remains. The majority of identification cases are those of war victims remains. During the last eight years, after the war ended, 83% of the exhumed war victims remains were identified. Many of these remains have been successfully identified by conventional methods based on medico-legal, anthropological, X-rays and dental examination. However, a number of cases could not be identified by conventional means because either premortal records are missing or remains are badly decomposed. In these cases, DNA analysis has played a significant role especially when reassociation of body was necessary or to exclude possible false identification when presumptive identity of the victim was not correct. As the time goes by, DNA analysis is taking over the most important role in the identification process since bodies exhumed 8-12 years after the death could hardly be identified by any other method. There are still 1275 missing persons in Croatia. In our lab, we usually extract DNA from teeth or bones and then type nuclear STR markers using different multiplex kits. In addition, we use Y chromosome STRs if necessary. When the genomic DNA is present in a low copy number or it is severely degraded, typing of mitochondrial DNA (mtDNA) is the method of choice. In that case, we sequence two hypervariable segments (HV1 and HV2) within the mitochondrial non-coding region. Here, we will report results of the war victims identification done by Zagreb DNA Laboratory and discuss problems that occur in identification process such as degradation, contaminations, mutations, drop-out alleles, statistics and database of relatives of missing persons.

Presentation number: F10

ASSOCIATION ANALYSIS OF SOME MOLECULAR-GENETIC MARKERS WITH CRIMINAL VIOLENCE

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Family, twin and adoption studies have all provided evidence of a genetic component (40-54%) in the origins of criminal behavior. It is known also, that both serotonin and dopamine system dysfunction is associated with criminal violence. The aim of our study was to test contribution of such candidate genes polymorphism as the dopamine D2 receptor (DRD2)(Taq1A - RFLP), the dopamine transporter (SLC6A3) (VNTR), the serotonin 2A receptor (HTR2A) (Msp1 - RFLP), the serotonin transporter (SLC6A4) (VNTR and 5-HTTLPR), catechol-O-methyltransferase (COMT) (Hsp-92II - RFLP), the monoamine oxidase A (EcoRV - RFLP) in criminal violence. 160 violent offenders (convicted of murder or rape) were included in the study. The control group consisted of 301 voluntaries. All subjects were typed for the above-mentioned gene variants using polymerase chain reaction (PCR) technique. No significant differences in allele and genotype frequencies in SLC6A3, HTR2A, SLC6A4, COMT, MAOA were found between violent offenders group and controls. However, significant differences were found in DRD2 genotype frequencies between group of violent offenders, because of significant increase of A2/A2 genotype (χ^2 = 5.25, P = 0.022, OR = 1.64, 95%CI = 1.07 - 2.50) and significant decrease of A1/A2 genotype (χ^2 = 3.89, P = 0.048, OR = 0.65, 95%CI = 0.42 - 0.99), and control group. There were significant differences in allele frequencies in violent offenders group in comparison with controls (χ^2 =5.03, P = 0.03 OR = 1.48, 95%CI = 1.04 - 2.11). So our study supported the involvement of DRD2 polymorphism, but not SLC6A3, HTR2A, SLC6A4, COMT, MAOA polymorphisms, in aggressive behavior.

Presentation number: F11

STR AND HLA ANALYSIS IN PATERNITY TESTING

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STR loci became a valuable tool in paternity testing during the past decade. However, the mutation rate of STR loci is higher than that of conventional genetic markers and for that reason the chance of detecting mutation increases. During the 3-year period, 111 cases of paternity disputes were processed in our laboratory. The analysis was performed on 9 STR (TH01, VWA31, FES/FPS, F13A01, SE33, D1S1656, D12S391, D18S535, and D22S683) loci and one VNTR (D1S80) locus. DNA was isolated from peripheral blood leukocytes by standard salting-out method. STR loci were amplifixed by PCR and the products analyzed by electrophoresis on a polyacrilamide gel in ALFexpress sequencer (Pharmacia Biotech). These cases were also tested for HLA class I and class II alleles by serology. Out of 111 cases, 22 were exclusions. In all cases, exclusion was confirmed on at least 5 loci. STR loci that were informative in majority of cases were SE33 (85.7%) and D12S391 (82.9%). The least informative loci were FES/FPS (55.6%) and F13A01 (57.9%). In 21 cases exclusions were also confirmed by HLA class I, while only in 17 cases HLA class II alleles excluded the alleged father. In three cases we detected a mutation of single STR locus (VWA31, 2xD12S391). In all three cases mutation was a deletion, which occurred at the paternal allele.

Presentation number: F12 (oral presentation)

IDENTIFICATION OF THE MISSING IN THE FORMER YUGOSLAVIA

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During the 1990s armed conflicts that accompanied the breakup of the former Yugoslavia, hundreds of thousands of individuals lost their lives, of which up to 40,000 still remain unaccounted for. Unfortunately, the vast majority of those who remain missing are dead. In order to assist in resolving the fate of those who remain missing, the International Commission on Missing Persons (ICMP) was created as a blue ribbon commission at the G-7 conference in Lyon, France in 1996. Exhumations have been an ongoing process since the war years and several thousand bodies are exhumed annually. Almost all of the bodies being recovered are skeletonized and very few medical or dental records are in existence, making successful large-scale identification by classic, non-DNA techniques virtually impossible. These factors contributed to the ICMP developing a DNA-led identification process. This process involves the collection of blood samples from family relatives, the development and operation of multiple DNA laboratories, and continued support and assistance of the exhumation process. The first DNA assisted identification by the ICMP laboratories occurred on November 16th, 2001. By July of 2003, the ICMP had profiled and databased more than 43,000 blood and 8,000 bone samples. The family reference and bone databases are compared with each other anytime new blood or bone DNA profiles are entered, resulting in 300 - 400 DNA matches being determined per month. The magnitude of success that is being realized by the ICMP demonstrates the potential of a DNA-led process for the identification of thousands of missing.

Presentation number: F13

STUDY OF GENETIC DIVERSITY AMONG FOUR ENDOGAMOUS GROUPS OF PUNJAB (INDIA), USING NUCLEAR AND MITOCHONDRIAL DNA POLYMORPHISMS

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Recent advances in human genetics have revolutionized the field of forensic biology. Indian population is divided into tribes and castes latter; the being further subdivided into endogamous groups based on geographic, linguistic, religious and socio-cultural differences. These subdivisions have accelerated the process of rapid genetic micro-differentiation. Punjab being the northwest frontier of India; all major invasion and migrations into India took place through this region, which led to the mixing of the gene pool. Therefore, for understanding the genetic diversity and relationships among Indian populations the study of micro-evolutionary tendencies among these endogamous population groups of Punjab is highly important. In the present study, eight indels loci, six STR loci, eleven biallelic mtDNA loci, and HVSI region polymorphisms were screened using PCR based techniques to analyze genetic diversity and the micro evolutionary tendencies among the four major endogamous groups of Punjab (viz., Brahmins, Khatris, Jat Sikhs and Scheduled Castes). The forensic usefulness of six STR loci was assessed by calculating Polymorphism Information Content (PIC), Discrimination Power (DP), Power of Exclusion (PE) and Typical Paternity Index (PI) for all the population groups. Further analysis was done using Arlequin and Dispan softwares. All the STR and indel loci except Alu CD4 locus were found to be highly polymorphic in all the groups. The allele frequencies, the heterozygosity and average heterozygosity values for most of the loci were very high. The coefficient of gene differentiation for all the loci when taken together was very low. With respect to 11 biallelic mtDNA polymorphisms, 13 distinct haplotypes were observed. Three main haplogroups M, U and H were observed of which M was the most frequent among these groups. The present study reflected the high level of genetic diversity within all four groups of Punjab but a very low genetic diversity between them. Phylogenetic analysis based on allele and haplogroup frequencies for different genetic markers indicated that all four groups shared closeness with each other, which reflected that the studied populations are homogenous populations owing to their common recent ancestral history.

Presentation number: F14

INCREASING DISCRIMINATORY POWER OF MITOCHONDRIAL DNA TYPING FOR INDIVIDUAL IDENTIFICATION BY USING ND GENES AS ADDITIONAL POLYMORPHIC MARKERS

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Relatively low discriminatory power of mitochondrial genome control region sequence analysis is a kind of a problem for individual identification, hence search for additional marker loci for mtDNA typing is necessary. As a possible candidate for that role we investigated three mitochondrial genes coding for NADH dehydrogenase: ND3, ND4L and ND6. A total of 63 peripheral blood samples taken from unrelated Russians were selected at sample repository at the 124 Central Medical-Forensic Identification Laboratory (Department of Defense, Russia). Total DNA was extracted from blood using Chelex method. Amplification for the mtND genes was done using the following primer sets, that were also used as sequencing primers: for locus ND3: - F10026 5-TTA ACT AGT TTT GAC AAC ATT-3' and R10411: 5'- ATT CGT TTT GTT TAA ACT AT -3'; for locus ND4L: F10451 5-TGA TAA TCA TAT TTA CCA AAT G-3' and R10771 5'-ATA TAA TTG TTG GGA CGA TT-3'; for locus ND6: F14130 5'-CCT ACTC CTA ATC ACA TAA C-3' and F14671 5'-TAG TCC GTG CGA GAA TAA TG-3'. Within the mtDNA loci ND3, ND4L and ND6 we have found 19 polymorphic positions. Polymorphisms found at all three loci were transitions, with one exception for the transversion at position 14484 (ND6). The genetic diversity value for ND4L was found to be 0,208, for ND6 - 0,343 and for ND3 - 0,392. For the ND3-ND4L-ND6 haplotypes the genetic diversity value was found to be 0,672. The data obtained show, that the investigated ND genes reveal much less polymorphism content than that of a mtDNA D-loop region, and therefore they do not seem to be of a value as independent markers for identity testing. However, we have demonstrated that by using ND genes polymorphisms as the additional markers to the mtDNA control region polymorphisms one can essentially increase discriminatory power of mtDNA typing for the purposes of individual identification analysis.

Presentation number: F15 (oral presentation)

DEVELOPMENT OF A MALE-SPECIFIC, 12-LOCUS FLUORESCENT MULTIPLEX

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Learning Objective: to present a new assay for analysis of Y-STR markers to the DNA typing community. Short Tandem Repeat (STR) analysis has become the leading technology for genetic human identification. Frequently, autosomal markers are used for forensic, paternity and anthropological studies. However, some cases can benefit from the analysis of sex-specific Y-STR markers. Y-STR markers consist of polymorphic regions found on the non-recombining region of the Y chromosome. Amplification of these haploid markers occurs only in males and alleles are only inherited through the paternal line. These qualities simplify interpretation of complex male/female mixtures and male kinship studies by removing the female contribution. Several web-based databases of observed Y-STR haplotypes have been initiated (http://www.ystr.org/). These databases include the so-called "Y-STR minimal haplotype", which consists of nine loci: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393. A commercially available, single-amplification assay for these loci has yet to be offered. To this end, a fluorescent multiplex has been developed to include the Y-STR minimal haplotype plus DYS437, DYS438 and DYS439. This new PowerPlex® System uses four-color chemistry allowing analysis on the ABI PRISM® 377 DNA Sequencer, ABI PRISM® 310 Genetic Analyzer and ABI PRISM® 3100 Genetic Analyzer. Amplified samples are labeled with fluorescein, 6-carboxy-4',5'-dichloro-2',7'-dimethoxy-fluorescein (JOE) and carboxy-tetramethylrhodamine (TMR). Fragment sizing is provided by an internal size standard (Internal Lane Standard 600) labeled with carboxy-X-rhodamine (CXR). Color deconvolution can be performed with color matrix kits currently available from Promega Corporation. Allelic ladders have been created, following ISFG recommendations, to increase confidence in allele designation. A PowerTyper™ macro, operating within the Genotyper® software, has been designed to automatically label fragments from GeneScan® data using the supplied allelic ladder and size standard. Primers have been designed to yield amplification products that are less then 350 bp in length. System sensitivity, specificity, robustness and concordance with previously described primer sets will be discussed.

Presentation number: F16 (oral presentation)

HUMAN mtDNA PROFILING BY DENATURING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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The quantity and quality of DNA in biological samples are critical factors in determining which analytical approach to take during forensic investigations. Specimens that are partially or severely degraded may limit the analytical options of the forensic scientist. In cases where nuclear DNA typing by standard Short Tandem Repeat methodologies have failed or would be expected to yield poor results, analysis of two hypervariable regions (HVI and HVII) within the D-loop of mitochondrial DNA can be used to provide information. Mitochondrial DNA analysis in forensic science currently relies on Polymerase Chain Reaction technology, direct DNA sequencing and extensive post-sequencing data analysis by an experienced forensic scientists. Although the demand for mtDNA forensic services continues to grow, few laboratories are prepared to handle this type of analysis. As a result, the cost of mitochondrial DNA typing by commercial labs is extremely expensive and lengthy analytical times often lead to significant delays in the disposition of cases by the courts. Mitochondrial DNA analysis using Denaturing high-performance liquid chromatography (DHPLC) is a novel approach to the detection and classification of sequence variability in the mitochondrial D-loop. The technique, which is widely used in medical research, relies on cross-hybridization of known and questioned samples followed by high-resolution chromatographic separation of the resulting homo- and heteroduplices under partially denaturing conditions. The approach uses PCR primer sets that have been validated and established in forensic science for amplification of the mitochondrial HVI and HVII regions. The resulting chromatographic profiles serve as unique and reproducible means of identifying and cataloging DNA sequence diversity within the forensically relevant hypervariable regions of human mitochondrial DNA. Pair wise comparisons of hair shaft and hair root material from eight unrelated human volunteers has demonstrated the ability to accurately identify sequence differences in all four of the mtDNA fragments in less than four minutes for each comparison. Similarly, DHPLC analysis of related and unrelated individuals has demonstrated the reproducibility of chromatographic profiles for maternally related individuals while analysis of non-maternally related individuals yields a distinctly different chromatogram relative to the maternal mitochondrial genome being examined. By eliminating extensive and laborious sequence analysis associated with existing approaches to mitochondrial DNA profiling, this technology will reduce the time and cost required to obtain conclusive DNA results thereby benefiting criminal investigations, data banking, and mass disaster situations.

Presentation number: F17 (oral presentation)

INTERPRETATION OF DNA MIXTURES IN FORENSIC CASEWORK USING Y-STRs

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Forensic casework often involves the interpretation of DNA mixtures. For example, sexual assault cases routinely require the testing of intimate samples that are a mixture of male and female body fluids. In some cases, only the female profile (or a predominantly female profile) can be detected using standard autosomal STR systems even when sperm/semen are identified by serological or microscopic methods. A Y-chromosome STR system significantly overcomes the problems associated with a large female to male DNA ratio that can lead to incomplete/no amplification of the male DNA. The ability to selectively target the male contributor(s) is a substantial tool for the DNA analyst in these cases. In addition, Y-STR typing can assist in evaluation of mixtures by providing information regarding the number of male contributors. We have recently validated a Y-chromosome STR multiplex (Y-PLEX™6, ReliaGene Technologies, Inc.) for casework. This system amplifies six Y-STR markers (DYS393, DYS19, DYS389, DYS390, DYS391, and DYS385) in a single reaction. The performance of the Y-PLEX 6 kit has many similarities to standard autosomal STRs which may impact mixture interpretation. For example, validation studies demonstrate that the sensitivity of Y-PLEX 6 and its ability to detect the minor contributor(s) in a mixture is comparable to standard STRs (Profiler Plus & COfiler). Y-STR typing can be an important tool in the interpretation of many DNA mixtures. However, the interpretation of DNA mixtures continues to be one of the key issues that is commonly debated in court. Many of the challenges associated with the interpretation of Y-STR results are similar to those for autosomal STRs. They include possible allele sharing among an unknown number of contributors, multiple allele patterns, peak imbalance at DYS385 (and other loci), stochastic fluctuation with low quantity or degraded template, the semi-quantitative activity of Taq polymerase, and microvariants in primer binding sites. These factors can make haplotype assignment of each contributor and subsequent statistical evaluation of the results a more arduous task for the forensic scientist. The use of conservative statistical methods should obviate many court objections associated with mixture interpretation. These considerations and our Y-STR case experiences will be discussed.

Presentation number: F18

COMPARATIVE ANALYSIS OF TWO DIFFERENT APPROACHES FOR BONE POWDERING - OPTIMIZATION OF MOST SENSITIVE STAGE OF DNA IDENTIFICATION OF SKELETAL HUMAN REMAINS

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The process of identifying mortal remains from mass fatalities has advanced rapidly in the past few years. One of the main reasons for this advancement is the use of DNA STR testing as a tool to aid in the identification process. The development of STR profiles from bone samples is not without its limitations because almost all environments cause DNA degradation and over time this degradation makes it increasingly difficult to generate STR profiles. The grinding of bone samples is one of the first steps in the process of DNA testing and at this step it is important to increase the surface area of all the pieces of bone sample while at the same time not causing degradation to the DNA that is contained within the osteocytes. Optimization of all grinding parameters is essential for obtaining results especially when the DNA contained in the bone sample has already been degraded by environmental conditions. Additionally optimizations should also consider costs, the time it takes to grind a sample, and the possibility of contamination by DNA from other sources. After considering a number of different grinding procedures the two that appeared best suited for mass identifications were grinding by Waring® blender and by the use of a Dremel®rotary tool. To test the quality of DNA produced by either procedure twenty bones were ground using each method. The Quality of STR profile obtained using the Promega PowerPlex®16 STR kit were compared on peak heights, peak balance, and the number of detected loci. Obtained results indicate advantage of "blender method" that was chosen for the formal bone powdering procedure in ICMP DNA laboratory.

Presentation number: F19

VICTIMS OF THE SUVA REKA MASSACRE LINKED BY DNA ANALYSIS TO BODIES FOUND IN BATAJNICA. STRATEGIES OF ANALYSIS AND INTERPRETATION OF AUTOSOMAL (STR) AND HAPLOID (mtDNA & Y-STR) DNA DATA

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The prosecutor office of the International Criminal Tribunal for the Former Yugoslavia (ICTY) requested the collaboration of our laboratory to perform DNA testing to identify human remains exhumed in 2001 at a mass grave in the site known as "Batajnica" in Serbia that presumably contained the remains of victims of the 1999 Suva Reka massacre who it is believed were originally dumped in a freezer truck into the Danube. The Institute of Legal Medicine in Belgrade submitted 56 bone samples (including 39 adults, 17 children, under 15 years, and a fetus) with a presumptive identification based mainly on gender and age ranges. This has been inferred by comparing known victims information against the forensic and anthropological findings. To perform comparative genetic analysis we received from the International Commission for Missing Persons 13 reference blood samples from relatives of the Suva Reka victims. We also received four different genealogy trees that included 44 related persons believed to be killed in the Suva Reka massacre. Therefore one of the main challenges of this study was that many of the bodies should be identified through relatives also on the mass grave. DNA was extracted from the bone samples (after initial surface cleaning) by proteinase K digestion, organic extraction and Centricon-100 purification. Human nuclear DNA was quantitated by slot-blot hybridization using Quantiblot (Applied Biosystems) and by a real-time PCR design to target a segment of the X-Y homologous amelogenin (AMG) gene that made possible not only DNA quantitation, but also sex determination. Approximately 30% of bone samples that gave negative or inconclusive Quantiblot results yielded positive real-time PCR results that also allowed identifying 32 male and 24 female bone samples. DNA analysis included sequencing of the hypervariable region I (HV1) of the mitochondrial DNA, autosomal STR profiling (13 CODIS markers) and Y-STR typing (Y-Plex6 & Y-Plex5 from Reliagene). HV1 sequence analysis allowed in a first phase to identify a total of 29 different maternal lineages among the skeletal remains of which 9 haplotypes (from a total of 25 bone samples) matched with the HV1 sequences obtained from reference samples of maternal relatives, suggesting a preliminary identification of several family members from all family groups. STR profiling was successfully performed in all but one bone sample allowing identifying 44 different STR profiles. These results indicated that in some occasions bone remains were sampled in duplicate (8 bodies) or in triplicate (2 bodies). On the other hand, STR profiling allowed identifying 16 bodies by direct or reverse paternity and/or maternity likehood ratio estimations of compatible pair-wise comparisons with both the reference samples from relatives of known identity and with the bodies of presumptive relatives found on the grave. Male bone samples were also subjected to Y-STR analysis. However the information retrieved by the analysis of Y-STR haplotypes was very scarce due to the low haplotype diversity observed among both the reference and the bone samples.

Presentation number: F20 (oral presentation)

THE DEVELOPMENT OF DNA TYPING TECHNIQUES AND MARIJUANA DATABASES FOR FORENSICS

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Amplified fragment length polymorphism (AFLP) analysis is a DNA typing method that can be used for individualizing any single source biological sample. AFLP analysis involves the polymerase chain reaction (PCR) amplification of restriction fragments to generate a band pattern that can be used as an identifier for the sample. The authors are developing an AFLP method for forensic use on marijuana (Cannabis sativa) as a model plant system. AFLP analysis of marijuana seizure samples can determine if seizures from two or more geographic locations share a common profile. Our data show that AFLP profiles can provide genetic evidence of the plant propagation method (seed or cloning) being utilized by the growers of this illicit crop and provide investigative leads for grower and distributor organizations. In order to estimate the random match probability of an AFLP profile from unrelated and nonclonal plants, a large population database needs to be constructed. A Connecticut State and United States National AFLP database containing profiles from marijuana seizure samples is under construction. All AFLP reactions used 20 nanograms of purified plant DNA and the AFLP profiles were normalized prior to scoring and inclusion in the data set. Four selective PCR primer sets were utilized to create a composite AFLP profile per plant sample. A subset of peaks were scored and converted to binary code for ease of comparing profiles. Once candidate matches were identified, the entire AFLP profile was assessed to determine a match. Case applications and the use of short tandem repeat (STRs) loci for screening evidentiary samples will be illustrated as well as our mapping system for assessing whether there is a correlation between geographic origin of the sample and genetic profile.

Presentation number: F21

A COMPARISON OF THE STATISTICAL SIGNIFICANCE IN THE LOCI OF THE PROMEGA POWERPLEX® 16 SYSTEM FROM IDENTIFIED REMAINS

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The ICMP has developed a DNA-led identification effort to assist in the identification of the estimated 30,000 - 40,000 missing persons who remain missing as a result of the breakup of the former Yugoslavia. Due to the conditions of the recovered bodies, coupled with a relative lack of medical and dental records, the vast majority of bodies currently being recovered cannot be identified without the use of DNA testing. By the summer of 2003, more than 8,000 bone samples and 43,000 blood samples had been successfully profiled and databased using the Promega PowerPlex® 16 system. The process of matching blood samples from the family reference database to bone samples in the bone database is challenging, especially considering the large number of samples in the databases. The vast majority of the missing has either a parent or a child as a blood donor. Because of this fact, the ICMP has developed software that performs an initial search based upon half-band sharing patterns. Due to the large size of the database, random half-band sharing matches are relatively common for any given bone sample. In order to determine a random match from a true match, additional factors are addressed. First, blood samples are taken from multiple donors for each missing person, and the DNA profiles from all blood donors must correspond to the potential match with the bone sample. On average the ICMP collects three blood references for each missing person. Once a match is found to exist between a bone sample and multiple donors from the same family, the statistical significance of the match is determined using DNAview software. The DNAview software gives a likelihood ratio (LR) for the significance of the match at each locus. Following the LR analysis, matches that produce a posterior probability of 0.9995 or larger are considered as strong enough to generate a matching report. For the matches where the posterior probability is less than 0.9995, additional family reference samples are sought, which can strengthen or exclude the match. When there are no additional family relatives available to donate, additional loci can be tested. It should be noted that the DNA report does not stand alone in the identification process. After the pathologist in charge of the case receives the DNA report, they combine all other forensic evidence together in order to make the identification. To examine the effectiveness of the alleles in the Promega PowerPlex® 16 system we have looked at the LR produced by each loci in the matching reports generated by ICMP. Initial results show that Penta E produces, on average, the strongest LR while TPOX produced the smallest.

Presentation number: F22

DOUBLE INCOMPATIBILITY AT FGA AND PENTA E LOCI IN A PATERNITY TEST

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In this study we investigated a double inconsistency case of paternity with two mutations at loci FGA (human fibrinogen alpha) and Penta E. The trio (mother, father and daughter) was analysed with a battery of 15 autosomal STR by using a commercially available PowerPlex® 16 System kit, the detection performed with an ultraviolet-automatic sequencer. The paternity was confirmed by twelve additional markers, six ASO markers, one minisatellite and five additional STRs loci. The variation in length of the repeats between the child and the alleged father was confirmed by sequencing. The trio was reanalysed for the same markers with different primers using an infrared automated sequencer, with IR-fluorescent primers. A high paternity index confirmed that the observed inconsistencies were due to double mutation. Sequence analysis at FGA and Penta E showed the probable mechanism. Amplification and detection with an IR-protocol showed consistent results with an UV-protocol and a commercial available kit. This study has been our first case of double mutation at FGA and Penta E in a paternity test. Our data suggest that we can expect to find paternity attribution with one or two inconsistencies when STRs are analysed. In particular, if the two inconsistencies can be explained as single-step mutations or as null alleles, they are unlikely to be a sufficient proof of non-paternity. Conversely, the use of a larger battery of highly polymorphic markers can be useful to reach a high value of paternity index, even in the presence of two mutations. The use of our approach, based on two amplification and detection formats and on the sequence analysis, confirms the observed meiotic paternal mutations.

Presentation number: F23

A CLOSER LOOK AT THE CAUSE OF NON SPECIFIC PRODUCTS AMPLIFIED BY THE PROMEGA POWERPLEX® SYSTEM

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The International Commission on Missing Persons has been charged with the task of identification of approximately 30,000 - 40,000 persons that resulted from the armed conflicts during the breakup of the former Yugoslavia. One of the most challenging parts of this process is the development of STR profiles from 8 - 12 year old bone samples. During the optimization of this part of our process we have found that the Promega PowerPlex® 16 system is significantly more sensitive than other kits used to amplify STR's from bone samples. Although the PowerPlex® 16 system is the most sensitive it still has some limitations for the production of STR profiles. One such limitation is that this kit is more sensitive to the presence of microbial DNA than other kits. The effects of these non specific amplifications appear most strongly in the green dye (joe) of this kit. Five of the six loci, D5S818, D13S317, D16S539, CSF1PO, and Penta D, have been observed to contain non specific products while only D7S820 has not. Positive control DNA, ranging from 50 pg to 1ng total, was amplified with the PowerPlex® 16 kit and all produced signals, although the samples at 50 pg displayed marginal results. This same dilution series was then mixed with another from Morganella morganii that also ranged from 50 pg to 100 ng total. The amplification results on the mixtures revealed that non specific products are amplified when there is less than 100 pg of human DNA and more than 10 ng of microbial DNA. Further tests using PowerPlex®16 mixes with one primer set deleted have shown that the Penta D primers cause most of the amplification of non specific products. The common non specific products will be presented along with the primer set that appear to be causing the amplification.

Presentation number: F24 (oral presentation)

ORGANIZING AND MANAGING A GOVERNMENT DNA TESTING LABORATORY

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The goal of this presentation is to identify issues and challenges associated with integrating a DNA Laboratory within a government laboratory. While methodology and technical procedures are generally uniform for DNA laboratories there are structural, resource, application, and legal ramifications uniquely associated with government laboratories. This presentation will focus on the Connecticut State Forensic Science Laboratory and how and why the DNA unit within that Laboratory was structured so as to best utilize resources and fulfill a comprehensive mission. The mission of Connecticut's Laboratory is to provide high quality examination of physical evidence, conduct relevant research to enhance and develop current methodologies and procedures, provide training to members of the criminal justice system who rely upon the laboratory services, and provide crime scene and reconstruction assistance to investigative personnel within Connecticut. Generally the greatest challenge is to organize and manage the DNA unit is such a manner that there is sufficient autonomy and specialization to address needs and requirements unique to forensic DNA testing, and yet perform this function in collaboration with other laboratory services. This big picture approach is critical if the laboratory is going to use it's resources to solve those difficult cases and problems that are all to often pushed into oblivion, allegedly justified by case backlogs and limited resources. This philosophy will require and develop scientists that maintain their technical specialization while designing case plans and interpreting their results in a generalist manner. Reality in most criminal cases is a need for maximum information from limited and perhaps degradated and/or contaminated specimens. Thus a triage and case flow system must be established to ensure that the most relevant information is obtained from any particular piece of physical evidence. Once these multi-disciplinary issues are identified they can become the basis for future research projects that are designed to address not only the needs and desires of the DNA unit, but essentially all laboratory units as well as the needs of the criminal justice system that rely on the forensic laboratory to objectively provide the necessary information for a just outcome.

Presentation number: F25

OBTENTION OF DNA FROM DENTAL STRUCTURE FOR HUMAN IDENTIFICATION IN FORENSIC CASES

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The present work was carried out in order to determine if the different dental tissues (pulp, dentine and cement) are sources of DNA in forensic samples. A Total of twenty teeth were obtained in 2000 from John Doe's exhumed bodies that had been buried since 1995 at the Cementerio Central de Bogota.The pulp cavity was exposed after cutting each tooth with a high velocity hand piece. The pulp was removed and kept in one 1.5 ml tube. The dentine and cement were obtained by drilling into separate tubes. The DNA was extracted by organic methods after a short decalcification step with EDTA. The tissues from three teeth obtained after surgery were used as controls. The DNA was quantified by hybridization with a probe that recognizes DNA from superior primates and humans. A PCR reaction was carried out for the hipervariable control region between nucleotides 29 and 408 bp of the mtDNA (HV2 region), followed by gel electrophoresis in order to evaluate the amount and efficiency of the amplification. The results showed a stronger amplification signal for pulps, followed by dentine and in a minor degree for the cement. These results suggest that the cementoblast and odontoblast cells that are located within the cement and dentine are embebed within the mineral matrix of the dental structure and therefore are protected for any environmental degradation forces. Thus the DNA found in the different dental structures of forensic samples is suitable for analysis based on DNA methods.

Presentation number: F26 (oral presentation)

LIABILITY OF EXPERT WITNESSES

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The concept of witness immunity stemmed from the old English common law to encourage witnesses to participate in litigation without fear of retaliatory lawsuits from unhappy participants. Expert witnesses have enjoyed almost universal immunity from liability as a result of their court testimony. In the last few years, however, this immunity is becoming eroded to the point that experts should, and rightly so, be cautious, careful and confident when expressing their opinions. Absolute immunity for experts is no longer a sure thing. There are arguments both for and against expert immunity. The primary argument for allowing expert immunity is to encourage witnesses to come forward with candid testimony without fear of reprisal. The primary argument against expert immunity is that the threat of liability will encourage experts to be more careful and accurate in their preparation and testimony. Both arguments are logical. Experts are hired professionals and are paid for their work. They should be held to the same standards as anyone else. If their work is not competent, they should be held responsible. This presentation will discuss the history and background of expert immunity using pertinent cases to explain how it is changing; explain some of the problems, pitfalls and ramifications; and provide some hints how to avoid them.

Presentation number: F27 (oral presentation)

THE WORLD TRADE CENTER MASS FATALITY: SPECIAL CIRCUMSTANCES AND LESSONS LEARNED

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When the two planes hit the World Trade Center towers on September 11, 2001 almost 2800 people died. It took many months to investigate the list of missing individuals to eliminate double entries and fraud attempts. The highly compromised nature and the often very small size of the human remains made the recovery and identification of the victims a special challenge. DNA typing was utilized in combination with non-genetic identification methods and in order to detect a genetic trace of as many victims as possible, testing was attempted on every piece of human origin. To aid in the recovery of small tissue and bone fragments all debris from the disaster site had been transported to a dedicated facility and sorted on conveyor belts. DNA methods used were PCR based STR, mtDNA and SNP typing. Many of the samples were outsourced to commercial laboratories. On July 9th, 2003 1512 individuals have been identified but testing, especially for mtDNA and SNP's is not yet complete. Aside from the poor DNA quality and the resulting partial profiles, problems encountered had to do with incorrect reference sample information and tissue transfer from one body part to another. From the very beginning it had been anticipated that the identification process would be not completely successful and would take a very long time. Therefore the Office of Chief Medical Examiner helped establish an accelerated process to obtain death certificates for missing individuals. The families of the victims turned to the Office for information and explanations of the scientific possibilities and continue to be a presence in the DNA laboratory.

Presentation number: F28

IDENTIFICATION OF FORENSIC SAMPLES USING AN INFRARED-BASED AUTOMATIC DNA SEQUENCER

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The standard for forensic genetics is based on the analysis of a selected panel of STR systems in conjunction with the amelogenin locus for gender determination. We report the use of an IR automated fluorescence monolaser sequencer (LI-COR-4200, Lincoln NE USA) for the analysis of 13 autosomal STR systems (TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317, D16S359, D18S51, D21S11) and the X-Y homologous gene amelogenin system. In order to verify the efficiency of the IR technology in combination with IRDye™800-labeled oligonucleotide, we analysed a large number of various kinds of forensic samples. We based our protocol on four independent multiplex PCR reactions: two tetraplex systems (MU1: and MU2: and two triplex systems (MU3: and MU4:. The forward primer of every primer pair was labeled with a new fluorochrome (IRDye™800, Licor NE USA). A total of 42 DNA samples was examined. Five cigarette butts, six stamps, five hairs, three semen samples and twelve bloodstains were obtained from known donors. A sensitivity study was also performed; we used serial dilutions of K562 control DNA (Promega Corporation WI, USA) or genomic DNA from 10 ng/µl to 5 pg/µl. Finally, a study was performed in several laboratories. Some degraded and undegraded forensic samples were exchanged between laboratories which used the same STR core loci but employed different labelling and detection technologies (UV technology and commercially-available kits). The results obtained were completely in agreement for the undegraded samples and for the particularly difficult forensic samples, there more a few minor differences in the number of successfully typed samples. Our results show the efficiency, reliability and accuracy of the IR system for the analysis of forensic samples. The comparison with the widespread UV-based DNA technology confirms that the two methods are comparably efficient in typing forensic stains. The consistent results observed in this study and in practical casework suggest that it is possible to exchange data between laboratories which use the same core of markers but different technologies.

Presentation number: F29

SINGLE-STRAND CONFORMATION POLYMORPHISM OF HYPER-VARIABLE REGIONS HV1 AND HV2 OF HUMAN MITOCHONDRIAL DNA;DETECTION BY SILVER-STAINING

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Human mitochondrial DNA from 150 individuals, divided in 50 trios consisting of mother (M), child (C) and father (F) was PCR amplified with primers flanking the hyper-variable regions HVR1 and HVR2. The amplified products were then fractionated under non-denaturing conditions in 0.75 mm polyacrylamide gels, silver-stained and compared by single-stranded conformational polymorphism (SSCP). By carefully adjusting the concentrations of the amplimers only a few sharp DNA bands were visualized, which greatly facilitated interpretation. In all but one case, M and C displayed identical patterns, which could be promptly distinguished from that of F. For the remaining cases, either set of primers was sufficient to resolve familial ties. In no instance, M displayed alleles different from those of C within each trio, demonstrating that no false exclusions occurred. The SSCP approach proved to be a cost-effective, robust technique suitable as a preliminary screening prior to DNA sequencing in situations requiring identification of multiple samples. Furthermore, as an extra advantage, the use of 0,75 mm gels obviated the need for gel binders during the silver-staining.

Presentation number: F30

RAPID MITOCHONDRIAL DNA HETEROPLASMY PROFILING BY DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF FORENSIC SAMPLES

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Our aim was to test the sensitivity of Denaturing High Performance Liquid Chromatography (DHPLC) to detect low levels of heteroplasmy in mitochondrial DNA (mtDNA). mtDNA is maternally inherited and is either homo- or heteroplasmic in composition. Heteroplasmy, if reliably detected, can increase the power of discrimination of forensic mtDNA analyses. Human mtDNA was isolated from 3 hair sheath samples using the 10% Chelex® 100 resin (Bio-Rad Laboratories, Hercules, CA) method. mtDNA was also isolated from bloodstains in proficiency test 99-512 from Collaborative Testing Services, Inc. (Sterling, VA) using QIAamp® DNA Blood Mini Kit (Qiagen, Valencia, CA). mtDNA HV1/HV2 regions were amplified by PCR using primers validated for forensic mtDNA analysis. The heteroplasmic DNA mixtures were denatured and reannealed with the resulting homo- and heteroduplicies being evaluated by DHPLC. This approach facilitates the separation of heteroduplices from homoduplices by under partially denaturing conditions. The ability of DHPLC to reliably detect a minority component in a DNA mixture was determined using mixtures of mtDNA amplicons in ratios ranging from 50:50 to 99:1. Sequence-based heteroplasmy was detected in the hair sheath tissue of sibling and maternal samples. DHPLC-based analyses of 4 dried bloodstains from proficiency test 99-512 identified matches between known and questioned DNA samples. These results were consistent with those reported by Collaborative Testing Services, Inc. based on direct sequencing. The current study, however, also revealed that proficiency test standard 99-512 included a heteroplasmic sample that was not detected by dideoxy-sequencing, which is limited to detecting minor components at levels greater than 10% of a total mixture. The greater sensitivity of DHPLC for the detection of heteroplasmy made it possible to accurately and reproducibly detect the minor component in a DNA mixture down to a ratio of 95:5. Heteroplasmy constitutes a mixture of DNA and thus readily forms heteroduplices upon denaturation and renaturation. Temperature-modulated heteroduplex analysis can readily detect basepair substitutions and can separate heteroduplices from homoduplices for subsequent analyses. Thus, DHPLC-based analysis of mtDNA heteroplasmy may serve as a powerful analytical tool to facilitate the expanded use of heteroplasmy as a diagnostic characteristic in forensic mtDNA profiling of individual samples.

Presentation number: F31

METHOD AND ALGORITHM FOR COMPARING LIFE-TIME AND POSTMORTEM PHOTOGRAPHS IN FLOW DATA PROCESSING

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Method and algorithm of the comparison of the life-time and postmortem photographs are presented. The method is intended for the problems of identification a person in the practice of forensic medicine. Algorithms for identifying the most informative regions efface images (facial landmarks), as a concentration of the points of interest are presented. They are based on the neutral network method for extracting local image features and constructing particular and composite feature maps. The algorithms developed demonstrate effective detection of the roost informative regions invariant to changes in image view and size (coefficient of selectivity is greater than 80%). The main application area of the method in forensic medicine can be the task of the primary corpse identification when there, exist a great number of deceased persons, as a result of the natural calamities and catastrophes.

Presentation number: F32

MACEDONIAN HUMAN DNA BANK (HDNAMKD)

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Our aim is to describe establishment of Macedonian Human DNA Bank (hDNAMKD). Blood samples were collected with written consent from the donors, specific protections were provided for vulnerable populations (minors, persons suffering from mental disorders, and adults placed under limited guardianship). DNA was obtained from peripheral blood leukocytes by the phenol extraction method. The MKDSPI is designated laboratory code for the DNA samples stored in hDNAMKD. The samples deposited in hDNAMKD were allocated in one of the three active projects (anthropology, unrelated patients, and related patients project). Depositor of the DNA samples of anthropology project field is IIBHG. The structure and definition of populations in anthropology project are defined according to the personal declaration for nationality, language, and religion. Depositors of the clinical DNA samples (unrelated patients project) are physicians working in cooperation with the IIBHG. There are 10 projects with different clinical diagnosis selected by depositor according to predefined criteria. There are three projects that included family data (related patients project): Related bone marrow transplantation, related renal transplantation, and autism. Depositors are donor's physicians. The samples collected from Macedonian population inside and outside of Macedonia are part of the DNA bank. As of May 2003, a total number of 2092 DNA samples were stored in hDNAMKD. In the anthropology and related patents projects were classified 1186 and 583 samples, respectively. In the unrelated patients project were classified 323 samples. Macedonian Human DNA Bank was created. The DNA bank will provide material for research into the molecular alterations associated with population diversity and genetic disorders. Thus, the response to the needs of individuals and families who are threatened by genetic disease will improve.

Presentation number: F33

THE ICMP INFORMATION WAREHOUSE FOR STORAGE OF INFORMATION NECESSARY TO MATCH BONE SAMPLES STR PROFILES TO FAMILY REFERENCE SAMPLES

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Throughout the former Yugoslavia there are an estimated 35,000 - 40,000 persons missing as a result of the conflicts of the 1990s. The ICMP was created in 1996 at the G-7 Summit in Lyon, France with the purpose of aiding in the resolution of the fate of these missing persons. The vast majority of the bodies currently being exhumed are skeletonized. In addition, very few medical or dental records of these individuals exist. These two facts combine to render classic, non-DNA identification methods virtually useless. Thus, in order to meet its primary mandate, the ICMP has established a DNA led program whereby all bodies are DNA tested and then the STR profiles first matched to family reference samples.

The ICMP currently has four operational DNA laboratories located in Bosnia and Herzegovina (BiH) and Serbia and Montenegro. These four DNA laboratories form an integrated DNA testing system that process between 750 - 1,200 bone samples and 4,000 blood samples per month. To date ICMP has successfully processed over 40,000 family reference samples and 8,000 bone samples. Traditionally DNA testing has been used to confirm an identification that was done by anti mortem and post mortem data. Since this was the first time a DNA-led approach has been used on such a large scale there was a need for development of software to facilitate the process. To meet this need and accelerate the matching of samples the ICMP has developed the ICMPIWH (International Commission on Missing persons Information Warehouse) software. The ICMPIWH software has been designed to house an almost unlimited number of databases, each being capable of holding up to 24 loci per sample and multiple samples per case. The software has been written to import, house, export, match and print STR data. During the Import process the STR data is checked against allelic ladders and any existing anomalies are placed into a report for quality control. Exported STR data is easily read by many popular applications such as Excel or Word. The Matching interface is easy to use and can be customized to search for exact matches of profiles, matches between parents or siblings, and to allow for reduced stringency in the case of mutations or bone profiles that do not contain all alleles. The matching part of the software allows you to compare one or more profiles from a database against one or more from any other database. The profiles that you want to perform search on are cached to RAM on the computer for quick searching and on average one STR profile can be searched against over 40,000 in less than one second. The software also has an internal security controls that allow various levels of access to different components of the software.

The ICMP IWH Software is geared towards storing and searching STR profiles for a High Throughput DNA laboratory.

Presentation number: F34

HEAVY METALS FROM MASS GRAVES BONES AND IDENTIFICATION BY GENOMIC DNA

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The identification process of dead bodies or human remains is being conducted under different circumstances. Exhumation and war victim identification have a special connotation. Different identification methods are used depending on the case circumstance and the state of postmortem body changes. One of the methods is the identification by DNA typing from different biological samples (genotyping). Considering the fact that every person inherits half of the genetic material from the mother and half from the father, DNA typing can verify the relationship between the examined persons. A particular problem is the isolation and DNA typing from human remains found in mass graves, that had undergone the degradation process, as well as postmortem DNA contamination with bacteria, fungi, humic acids, metals etc. This study analyzed the possible influence of metal ions on the successfulness of DNA typing of bone samples from mass graves. The study included 30 bone samples from mass graves and the 5 fresh bone samples. Successful and unsuccessful DNA typing in mass graves bones has been determined by the concentration of iron, copper, lead and cadmium ions and their possible correlation. The influence of single metal ions the and influence of different combinations and concentrations of iron, copper, cadmium and lead ions on DNA typing has been analyzed through in vitro experiments with fresh bone suspensions and metal ions. The results revealed that iron, copper, lead and cadmium ions, if present in bone samples from mass graves, do not inhibit DNA amplification, while they inhibit the DNA amplification only if they are present in the amplification reaction mix.

Presentation number: F35

POSSIBILITIES OF DETERMINATION OF AB0 BLOOD GROUP SYSTEM FROM DEGRADED BLOOD STAINS

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The AB0 blood group system typing remains one of the basic laboratory tasks in a forensic practice. However, problems arise when the analyzed samples are seriously degraded. In our study we tried to compare molecular genetic method with classical serological method for determination of AB0 blood group system. We took blood samples from six volunteers (three men, three women) and made blood stains on pieces of sterile cotton cloth. Bloodstains were incubated at three different temperatures (22 ^{0}C, 37 ^{0}C, 56 ^{0}C) for various periods of time (1 day, 1 week, 14 days, 1 month, 3 months, 6 months, 1 year). For bloodstains degraded at 22 ^{0}C we also analyzed the samples after 3,5 hours of incubation. Moreover, we tried to determine the AB0 blood group system after thermal degradation at high temperature (200 ^{0}C) for 10 min. For the AB0 blood group system typing a Polymerase Chain Reaction (PCR) method was used to amplify glycosyl transferase gene, when DNA had been isolated from artificially created bloodstains, followed by their subsequent artificial thermal degradation. For serological AB0 typing the mixed agglutination and the Therkelsen method were used. The DNA analysis seemed to solve problems with seriously degraded bloodstains but we found out that classical serological methods were even better in some cases.

ABSTRACTS

MAYO CLINIC COURSE IN ADVANCED MOLECULAR AND CELLULAR MEDICINE

Presentation number: M1

RT-PCR BASED ANALYSIS OF DOPAMINE RECEPTOR GENE REARRANGEMENT IN PERIPHERAL BLOOD LYMPHOCYTES IN SCHIZOPHRENIA PATIENTS

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Schizophrenia, commonly developed in adolescents and young adults, is one of the most common mental disorder, but the pathophysiology and etiology of schizophrenia is still obscure. Numerous studies on dopamine and schizophrenia have suggested that the change in the dopamine system is related to schizophrenia, but there is little direct evidence for "dopamine hypothesis in schizophrenia". Changes in the dopamine system are influenced not only by dopamine itself, but also by dopamine receptors. Recent progress in molecular biology reveals the existence of mRNA of D3, D4, and D5 dopamine receptors in peripheral lymphocytes and enabled new insight for schizophrenia research. The purposes of this study were to examine if the mRNA of peripheral dopamine receptors changed in schizophrenia. Thirteen schizophrenic patients and healthy individuals took part in this study. Peripheral blood lymphocytes were separated with Ficoll-Paque gradients and total RNA was extracted. The dopamine receptor D3 and D5 from peripheral lymphocytes was analyzed semi quantitatively and qualitatvely by using PCR amplification of reverse transcribed RNA. Dopamine receptor of three and five genes were not significantly different in treated patients compared to healthy individuals.

Presentation number: M2

PRENATAL AND PREIMPLANTATION DIAGNOSIS OF SEX USING AMELOGENIN GENE BY NESTED-PCR

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Sex determination has many applications in identification, criminology and archaeology. But its most important application is to determine fetal sex in X-linked genetic diseases. The aim of this project in clinical aspects is to establish prenatal and preimplantation diagnosis of fetal sex. We obtained 74 chorionic villus samples (CVS) from pregnant women at 9-12 weeks of gestation, sixteen samples from human embryos which were in different cellular stages (8-16 cells) as well as peripheral blood from thirty male and female blood donors as controls. DNA was extracted from all samples by standard boiling methods. Nested PCR specific for a region of the amelogenin gene was used for sex (X/Y) determination. Results suggest that identification of fetal sex in CVS and single cells is possible. The system sensitivity increased up to amplification of a single cell and it was validated by amplification of DNA from fertile oocytes. We randomly followed up ten families and the sex of all ten newborns agreed with our PCR results. In single cell PCR, to prevent amplification of only a single allele or preferential amplification, we increase the initial temperature of denaturation to prevent the occurrence of allele dropout.

Presentation number: M3

PREMATURE OVARIAN FAILURE IN A MENTALLY RETARDED GIRL WITH EPILEPSY WITH DUPLICATION OF P ARM OF CHROMOSOME 22

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There is increased risk of Premature Ovarian Failure (POF) in women with epilepsy. Premature Ovarian Failure is the premature cessation of menstruation prior to 35 years of age and is associated with elevated gonadotrophins. Generalized tonic clonic seizures are associated with hypothalamic hypogonadism and polycystic ovarian disease. Premature Ovarian Failure (POF) affects 1% of women and is known to be caused by chromosomal abnormalities. Chromosomal rearrangements are also the most frequent cause of mental retardation. We report a case of a 20-year-old mentally retarded girl with POF and history of epilepsy. On cytogenetic analysis of 30 G banded metaphases we found duplication of 22p (46,XX dup22p). The proband had poorly developed secondary sexual characters. The FSH and LH levels were in the normal range and USG showed complete absence of ovarian follicles and the uterus was small in size. The proband was a product of non-consanguineous marriage and there was no family history of delayed menarche or epilepsy. Though it is well known that chromosomal abnormalities lead to POF and mental retardation it is important to understand their correlation and analyze more such cases to understand if genes controlling mental development and ovarian development are linked. Thus understanding the genetic basis of these disorders is important in order to counsel these cases and manage them appropriately.

Presentation number: M4

STUDY OF FOLIC ACID PATHWAY GENES ALTERATION AS MATERNAL CAUSE OF DOWN SYNDROME IN IRANIAN PATIENTS

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Chromosome 21 nondisjunction event is responsible for more than 95% of Down syndrome. The etiology of nondisjunction has not been very well described, however some factors such as folate metabolism is recently considered as an important factor on chromosome nondisjunction event. This study was to evaluate the impact of abnormal folate metabolism induced by folate pathway genes mutation among mother of children with Down syndrome compared to the normal control mother. Common mutations of C677T and A1298C of MTHFR gene are reported to decrease folic acid level in about 30 to 70 percents in heterozygous and homozygous forms respectively in blood samples. To evaluate the impact of these mutations, the parental origin of Down syndrome of all causes were determined at first place and mothers have been categorized into two groups according to the maternal or paternal origin of chromosome 21 trisomy. This study is unique compared to previous studies, paternally originated Down patients were omitted at the first place to increase accuracy of obtaining results. Folic acid gene alterations were studied in 60 families with Down syndrome and 100 normal controls. Our results till now have showed approximately 4 times higher risk of having Down syndrome child among mothers caring these mutations than normal control mothers or cases with paternal origin of Down syndrome in Iranian Down syndrome cases.

Presentation number: M5

EXPRESSION OF THE NM23-H1 GENE AND LOSS OF HETEROZYGOSITY IN MALIGNANT MELANOMA

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Nm23-H1 is metastasis suppressor gene that encodes nucleoside diphosphate kinase (NDPK) which plays an important role in metastasis formation of human tumors. In order to investigate its role in the progression of the malignant melanoma we analyzed 51 primary malignant melanoma and eleven melanoma metastases according to the expression level of nm23-H1 and loss of heterozygosity. Nm23-H1 gene instability of 51 melanoma samples compared with normal tissue samples analysed by PCR loss of heterozygosity assay using specific oligonucleotide primers. Thirty three (64,7%) informative samples were found, among them ten (30,3%) showed LOH regardless of the Clark and Breslow stage of the malignant melanoma. The nm23-H1 protein immunoreactivity was positive in 45 (88,2%) malignant melanoma samples but no statistically significant correlations between the nm23-H1 expression and Clark's and Breslow's stage were found. In this study eleven primary malignant melanoma showed metastasis. One patient was not informative (homozygous), five patients showed the same fragment length (noLOH) in primary and metastatic malignant cells with the same nm23-H1 expression. In two cases primary tumors turned from noLOH into LOH in metastatic tumors and this was followed by a decrease in the nm23-H1 expression. An association between tumor stage according to Clark and Breslow and nm23-H1 protein levels as well as LOH have not been found in malignant melanoma.

Presentation number: M6

SENSORINEURAL DEAFNESS, DYSMORPHIC FACE, OMPHALOCELE, HYPOPLASIA OF THE CORPUS CALLOSUM, SEIZURES AND DEVELOPMENTAL DELAY: A NEW SYNDROME?

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We report on a three and a half-year-old boy with a multiple congenital anomaly-mental retardation syndrome characterised by profound sensorineural hearing loss, severe hypermetropia, omphalocele, bilateral inguinal hernia, hypoplasia of the corpus callosum, seizures and developmental delay. Distinctive facial features include wide forehead, high arched eyebrows, telecanthus, down-slanting palpebral fissures, high and prominent nasal bridge and submucosal cleft. High resolution karyotype and multicolor subtelomeric chromosome screening by fluorescent in situ hybridization showed normal results. A comprehensive review of syndromes with sensorineural deafness yielded no conclusive results, each being excluded on clinical or laboratory or cytogenetic grounds. Our patient shows some overlap with Donnai and Barrow and Malpuech syndromes. However, he is lacking some of the prominent symptoms of both conditions. Therefore we believe that his constellation of anomalies represent a distinct clinical entity.

Presentation number: M7

HAPLOTYPE ANALYSIS OF RELATED ATM MARKERS FACILITATE PRENATAL DIAGNOSIS IN IRANIAN ATAXIA TELANGIECTASIA PATIENTS

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Ataxia Telangiectasia is an autosomal recessive disorder of 1/40000 to 1/100000 in reported populations. There is 25% possibility for having an affected child when parents are carrier for ATM gene mutation. There is no cure available for this disease and prenatal testing is strongly recommended in prevention of this disease. Although preference method is the direct mutation analysis of ATM gene, but large size of the ATM gene with 63 exons and the large number of possible mutation in patients considerably limit the facibility of mutations analysis as a choice in diagnosis. Indirect method is a better tool when parent are not carrier of founder mutation and pass different mutations to their children. Indirect molecular diagnosis using ATM related molecular markers facilitate prenatal diagnosis of AT children. In this study four molecular markers: D11S2179, D11S1787, D11S535, D11S1343 are genotyped in 18 unrelated families from different region of IRAN. Those markers are amplified using extracted sequence primers from Gene Bank with their described PCR conditions. The amplified products were separated using denaturing PAGE gels, and the data were analyzed to detect their pattern of inheritance in each family. In all families segregation of alleles were recording to Mandelian inheritance and affected chromosomes were distinguishable from unaffected ones. All carriers and affected patients were diagnosed accurately. Thus, this method is effectively usable in prenatal diagnosis of ataxia telangiectasia.

Presentation number: M8

MTHFR POLYMORPHISM IN PATIENTS WITH HEAD AND NECK CANCER

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MTHFR is an important enzyme in folate metabolism that converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. A functional polymorphism C677T in MTHFR gene results in the change of an alanine to a valine and thus reduces the activity of the MTHFR enzyme. Altered enzyme activity influences DNA methylation and DNA synthesis that makes MTHFR a candidate for a cancer predisposing gene. In this study we investigated MTHFR polymorphism as a possible genetic risk factor for head and neck cancer. We have studied the possible influence of the MTHFR C677T polymorphism in a case-control study of 120 healthy subjects and 82 patients with head and neck cancer. MTHFR genotypes were determined by PCR-RFLP. A significant decrease of T allele was observed among the cases in male subgroup (27.4% in cases vs. 34.6% in controls, p=0.007) with odds ratio 0.52, 95% confidence interval 0.32-0.80. Men with heterozygous 677CT genotype (59.3% in controls) had decreased risk for head and neck cancer (odds ratio 0.28, 95% confidence interval 0.14-0.54, p<0.001) compared to CC and TT genotypes. Genotype frequencies were in Hardy-Weinberg equilibrium. Further studies are needed on larger number of subjects to confirm the protective role of MTHFR 677T allele.

Presentation number: M9

ASSOCIATION OF THE INTERLEUKIN-1 GENETIC POLYMORPHISM AND MULTIPLE SCLEROSIS RISK

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Multiple sclerosis (MS) is a multifactorial demyelining disorder of central nervous system connected with autoimmune reaction. Cytokine intrleukin-1 (IL-1) is a proinflammatory agent, playing a central role in the beginning and supporting autoimmune process. IL-1 production increases in active demyelinization foci in white substance of brain. IL-1ß gene polymorphism located in the promoter region at position -511 (IL-1ß-511*T/C) correlated with IL-1ß expression. We aimed to investigate if IL-1ß gene polymorphism could be used as marker of susceptibility in MS. In this study, the IL-1ß-511*T/C polymorphism in the IL-1 ß gene was investigated in 100 Tatar and 159 Russian patients with multiple sclerosis from Bashkortostan and Sverdlovsk region. The healthy 167 Tatar and 320 Russian subjects from these areas were selected as controls. Genotyping was done, using polymerase (PCR) amplification. For statistical analysis, allelic and genotype frequency distributions of these polymorphisms in patient and control group were compared by Fisher's exact test. Strength of associations was given as odds ratios (OR). Proportion of -511*C homozygote/heterozygote/-511*T homozygote for IL-1ß promoter in MS patients and controls were 1) 19.0/45.0/36.0% and 2) 32.3/46.7/20.9% in Tatar population and 1) 30.1/40.2/29.5% and 2) 22.5/52.5/25.5%, respectively. In Tatar MS patients we detected a significant decrease of IL-1ß-511*C/C genotype frequency (P = 0.02, OR=0.49), and allele IL-1ß-511*C frequency (41.5% Vs 55.6%; P = 0.002, OR=0.56), whereas the frequency of the IL-1ß-511*T/T genotype is increased (P=0,009, OR=2,12) as well as the frequency of allele IL-1ß-511*T (58.5% Vs 44.3%; P = 0.002, OR=1.77). The frequency of IL-1ß-511*C/T genotype was significantly lower in Russian MS patient than control (P = 0.01, OR=0.6). Our data demonstrated that Tatars and Russian patients with MS are associated with the IL-1ß-511*T/C polymorphism. Tatars IL-1ß-511*T carriers have higher predisposition to MS than IL-1ß-511*C carriers. In Russian population IL-1ß-511*C/T genotype is protective.

Presentation number: M10

GENETIC POLYMORPHISMS OF CYP2C9, CYP2C19 AND MDR1 GENES IN CROATIAN POPULATION AND ITS EFFECT ON THE PHENYTOIN DISPOSITION

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Genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors and other drug targets have been linked to interindividual differences in the efficacy and toxicity of many medications. Anticonvulsant drug phenytoin exhibits nonlinear pharmacokinetics with large interindividual differences. Because of the risk of therapeutic failure or adverse drug effects due to its narrow therapeutic range, there is frequent demand for drug monitoring. Phenytoin is supstrate of the P-450 CYP enzymes 2C9 and 2C19, and also of the P-glycoprotein (Pgp), encoded by the MDR1 gene. In this study we examined the genetic polymorphisms of CYP2C9, CYP2C19 and MDR1 and its effect on the disposition of phenytoin in 64 healthy Croatians. Whether phenytoin metabolic ratio (PMR) defined as the ratio of plasma levels of major phenytoin metabolite 5-(4-hydroxyphenyl)-5- phenylhydantoin (p-HPPH) to phenytoin (PH) correlates with polymorphisms of CYP2C9, CYP2C19 and MDR1. PCR-RFLP were performed for the most frequent alleles: CYP2C9*1,*2,*3, CYP2C19*1*2, *3, and C3435T of MDR1. The 12 h serum concentrations after 300 mg oral dose of phenytoin were used for phenotyping. Phenytoin (PHT) and (p-HPPH) were analyzed by HPLC. Allele frequencies for CYP2C9*1, *2,* 3 were 0.760, 0.135, and 0.095 respectively, for CYP2C19*1,*2, were 0.85, 0.15 respectively. The highest PMR values (mean 0.48) were noted in homozygous subjects for CYP2C9*1 and CYP2C19*1 and lowest PMR values (mean 0.11) in subjects carrying two defective alleles (*2 or*3 or combination), whereas heterozygous subjects had intermediate values (mean 0.22) with major role of polymorphic CYP2C9. Analysis of CYP2C9, CYP2C19 and MDR1 genotype has predictive value for phenytoin disposition in clinical practice.

Presentation number: M11

TRANSPLANTATION OF THE RAT FETAL NEURAL RETINA TO AN ECTOPIC SITE IN VIVO

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Fetal neural retina of the rat can differentiate in vitro in a three-dymensional organ culture model with chemically defined culture medium, although the normal tissue architecture is lost and rosettes are formed . To investigate the developmental potential of the fetal neural retina in an ectopic site in vivo, transplantation under the kidney capsule was performed. Neural retinas were isolated from 20-days-old Fischer rat fetuses under the dissecting microscope. Adult Fischer males were anaesthetized with ether and the skin and muscle èut to approach the kidney. A small DpocketD was done under the kidney capsule to place the transplant. Each rat received one transplant. After 50 days, transplants were fixed and histologically processed. Indirect immunohistochemistry on 5mm thick slides was applied for detection of various antigens. Primary antibodies were: goat polyclonal anti-Sema3A antibody; CS-56, mouse monoclonal IgM anti-chondroitin sulfate; monoclonal anti-PCNA. Biotinilated anti-goat and anti-mouse secondary antibodies for immunoperoxidase staining were used respectively from Vectastain ABC kit following the manufacturer's protocol. 3,3-diaminobenzidine with metal enhancer was used for visualization of positive signals. In transplants, retinal cells forming rosettes were detected. Among them, few cells expressing strongly the proliferating cell nuclear antigen (PCNA) were found. Semaphorin IIIA, a guidance molecule, was scattered throughout the tissue in a punctiforme manner. Sometimes it was found to decorate cells. Chondroitin sulfate was positive in the cell cytoplasm and in the extracellular matrix. Fibronectin was absent from the retina but was found in the kidney capsule. These results show that even after the period of 50 days, in neural retina transplants which have lost their normal cell interactions, some cells are proliferating and therefore still contained in the cycling compartment.

Presentation number: M12

ACUTE LYMPHOBLASTIC LEUKAEMIA PH+ (T 9;22) FOLLOWING LANGERHANS CELL HISTIOCYTOSIS IN CHILD WITH HETEROMORPHYSM ON CHROMOSOME # 9

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Malignancy occurs with more frequency that is expected in patients with Langerhans cell histiocytosis (LCH). Histiocyte Society in 1991 established a registry to collect information on these accidents. Haupt refereed that 92 patients were registered: 71 were children with 27 solid tumors, 21 AnLL, 17 ALL and 6 with lymphoma. Our patient with LCH was treated by 15 cycle of etoposide, followed by maintenance therapy with interferon alpha. The acute lymphoblastic leukemia occurred several months after LCH remission with Ph+ cells in bone marrow samples. He clinically manifested microcephalia, bilateral epicanthal folds, low set ears, low bridged nose, upward slanted, palpebral fissures, high palate, low set umbilicus, four finger line both hands, displastic toes, cryptorchidism and DBP. In peripheral blood and bone marrow karyotype he had heteromorphysm on chromosome # 9: 46,XY, per inv (9)(p11;q11) mat on opposite chromosome (9) than t 9;22 (Ph+). A common genetic predisposition to cancer may be operative in our case. Theoretical possibility of breaking prone regions, virus integration (Haas), methylation and demethylation status diversity can be discussed. Connection with very old repetitive sequences from our mammalian ancestors and condensation state of chromatin may results in the incorporation of critical genes (senescence and tumor suppressor genes) into heterochromatin and the subsequent methylation of this DNA. Silences of the genetic activity might be essential for maintenance of a normal cell. The topological organization of chromosomes 9 and 22 in cell nuclei has a determinative role in the induction of t (9,22) translocations and in the pathogenesis of t (9,22) leukemia.

Presentation number: M13

DE NOVO TRISOMY 8 MOSAICISM: A FIVE-YEARS FOLLOW-UP

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The clinical features of trisomy 8 mosaicism include mental retardation, dysmorphic face, skeletal anomalies (particularly vertebral), congenital hearth defect and kidney malformations. The patients are usually hardly recognizable at the birth due to lack of mayor anomalies, but because of dysmorphism of the face and deep longitudinal palmar and plantar furrows the karyotyping is provided and the diagnosis arise at the birth. Mental retardation, infections, immunodeficiency and malignancies are described. Here we describe a case of de novo trisomy 8 mosaicsm. Correlation between numbers of trisomic cells in different tissue through five years of following up. At the admission the patient karyotype was: 47,XY,+8 (62%)/46,XY (38%) and mosaicism for trisomy 8 was detected in all examined tissue in the same proportions (peripheral blood, bone marrow cells and skin fibroblasts). After five years there was a decrease of proportion (50%) of the trisomic cells in peripheral blood lymphocytes.

Presentation number: M14

MOLECULAR, CYTOGENETIC AND ENDOCRINOLOGICAL ANALYSIS IN HYPOGONADISM

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Klinefelter Syndrome (KFS) is the commonest sex chromosomal abnormality and the commonest cause of male infertility. About 12-15% cases are mosaics with variable phenotype. Most reports do not differentiate KFS from the variants. These variant cases have additional phenotypic anomalies and thus form a distinct entity and therefore were studied in detail. In the present study 145 cases of male infertility were analyzed cytogenetically. Twenty five well spread G banded metaphases were karyotyped using image analyzer (Cytovision, Applied Imaging). In mosaic cases 50 metaphases were analyzed. Six mosaic variant cases with more than one cell line were analyzed at the molecular level by Fluorescence In situ Hybridization (FISH) to detect low level cryptic mosaicism. Semen analysis was done according to the WHO guidelines (1999). We found 11cases with KFS, nine cases were KF mosaics and 5 were mosaic variants. These variant mosaic cases were Case1- 47,XXY (60%)/48,XXYY (16%)/46,XX (20%)/47,XYY (4%), Case 2- 47,XXY (91%)/48,XXYY (3%)/48,XXXY (3%)/46,XY (3%), Case 3- 47,XXY (60%)/48,XXXY (26%)/49,XXXYY (14%), Case 4- 46,XY (50%)/47,XXY (30%)/48,XXYY (20%), Case 5- 46,XY (53%)/48,XXXY (40%)/49,XXXYY (7%) case 6- 47,XXY/48,XXXY. FISH detected an additional cell line of 50,XXXXYY (1%) in case1. These variant cases had additional features than of KFS like mental retardation, difficulty in expressive language, slurred speech, Mitral Valve Prolapse and adjustment problem with peers. FSH and LH were markedly elevated and testosterone levels were low. All cases were azoospermic. FISH as an adjunct to conventional cytogenetics helps in identifying cell lines not identified cytogenetically. Thus variant cases should be considered as a distinct entity as they have additional cardiovascular and other clinical features and should thus be managed accordingly.

Presentation number: M15

PESTICIDES (XENOESTROGENS)-A POTENTIAL HAZARD TO THE MALE REPRODUCTIVE SYSTEM

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The incidence of disorders of the male reproductive system have more than doubled in the past 1 or 2 decades and they reflect adverse effect of environmental factors rather than genetic changes in susceptibility. Therefore the present study was undertaken to understand the etiology in idiopathic cases of infertility. 145 males with idiopathic infertility were referred from the infertility clinic of AIIMS. In each case detailed family, reproductive and occupational history was collected. Semen, cytogenetic and molecular analysis (PCR) to determine Yq microdeletion was done following the standard protocols. Of 145 cases, 36 cases had a numerical or structural chromosomal anomaly and 8 cases had microdeletion of the Azoospermia Factor loci on the long arm of the Y chromosome. Of these 145 men, 16 men and their parents too were engaged in farming thus exposed to DDT and other pesticides. Two of them were oligozoospermic (sperm count of 7.75 million) and 14 were azoospermic. Eight men had small gonads, 3 had normal gonads, 2 had varicocele, 2 had cryptorchidism one of whom had ambiguous genitalia and phallus was absent and one case had hydrocele. Two of these men had AZFb and AZFc deletion respectively and one had 47, XXY chromosomal complement. Rest of the 13 men had no significant exposure to any other etiological factor. Recently clustering of cases of Testicular Dysgenesis Syndrome (TDS) has been reported in people exposed to pesticides. The increasing incidence of reproductive anomalies and decline in male reproductive health may be related to occupational exposure to environmental endocrine disrupters or xenoestrogens or environmental hormones. The results of this preliminary study show that more such cases with pesticide exposure need to be analyzed and should undergo follow up. Awareness of this occupational hazard is developing strategies for prevention to occupationally hazardous insecticides and pesticides.

Presentation number: M16

EPIDEMIOLOGICAL DATA ABOUT BIRTH DEFECTS AND HEREDITARY DISEASES IN URAL'S REGION

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Since 1992 birth defects and hereditary diseases of the populations have been registered in Ural's region. Multiple sources of information are registration forms from maternal houses, child clinics, and pathology departments. Today our register of birth defects and hereditary diseases contains the information on 11286 patients. The structure of diagnoses:

- birth defects: 86% (heart's defects 22%, defects of musculoskeletal system 13%, neural tube defects 12%, defects of urinary system 11%, et others);
- monogenic diseases: 8% (Hemophilia A 108 patients, Hemophilia B 19 patients, Cystic fibrosis 252 patients, Phenylketonuria - 74 patients, Iodide transport defect - 54 patients, Adrenogenital syndrome - 14 patients, Spinal muscular atrophy - 21 patients, et others);
- chromosomal anomalies: 6 % (Down syndrome - 521 patients, Turner syndrome - 58 patients, Klinefelter syndrome - 19 patients, et others);

In 2002 in Ural's region 44003 newborns were borne, and 1006 of them had birth defects. Frequency of birth defects was 22,9 in 1000 births (2,29%). Frequency of some birth defects in 2002: anencephaly 0,34 in 1000, spina bifida 0,95 in 1000, hydrocephaly 0,54 in 1000, cleft lip with or without cleft palate 0,64 in 1000, polydactily 1,27 in 1000, anotia and microtia 0,14 in 1000, total Uml reduction defects 0,25 in 1000, Down syndrome 1,20 in 1000 births. We continue to make the complete register. We hope for cooperation with other research groups.

Presentation number: M17

THE RELATIONSHIP BETWEEN Y CHROMOSOME HAPLOGROUPS AND HAPLOTYPES AND INFERTILITY IN A CROATIAN MALE SAMPLE

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Five biallelic (M9, 92R7, 12f2, SRY1532 and YAP) and six microsatellite (DYS19, DYS385, DYS389II, DYS390, DYS391 and DYS393) Y chromosome polymorphisms were determined in a sample of 75 patients with idiopathic infertility, and in two control groups composed of 100 randomly selected males, and 100 fertile Croatian men. Combination of six biallelic markers yielded six haplogroups (HG) in a random population. Haplogroups 3 (34%) and 2 (28%) were most frequent, whereas haplogroups 2 (58%) and 3 (28%) were most frequent among fertile controls. The remaining haplogroups were not very frequent, while HG 26 was not observed.Among infertile patients, we found that HG 9 is most represented, followed by HG 2, HG 1, HG 3 and HG 21. Haplogroup 26 was observed in eight samples (10,7%). Overall, 79 haplotypes were observed in general population, 72 in fertile men and 63 haplotypes among infertile patients. Differences of microsatellite molecular variance and genetic distance analysis were significant in DYS385, DYS389II, DYS390 and DYS391 loci for all populations. The highest difference was observed between fertile and infertile samples in DYS385, DYS389II, DYS390, DYS391 loci ($p<0,001$). Higher frequencies of hg 9 and 26 suggest that those groups may be at risk for infertility. The test results showed that more stable biallelic markers describe better the differences between infertile men compared with two control populations. Microsatellite markers point out the intra-population differences.

Presentation number: M18

RELATIVE QUANTIFICATION OF mRNA IN OVINE FC RECEPTOR (FCRN) AND POLYMERIC IMMUNOGLOBULIN RECEPTOR (PIGR) USING REAL-TIME REVERSE TRANSCRIPTION PCR (RT-PCR)

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Real-time transcription followed by polymerase chain reaction (RT-PCR) is the most suitable method for the detection and quantification of mRNA. The main objective of this study was to quantify two Ig receptor mRNA expression levels under the influence of mycophenolic acid (MPA). The Fc receptor (FcRn) is responsible for the specific trans-membrane immunoglobulin (IgG) transport while the polymeric Ig receptor (pIgR) recognizes the dimeric IgA and pentameric IgM. Mathematical algorithams are based on RT-PCR efficiency and the crossing point deviations between the sample and control group. A new software REST© (relative expression software tool) for group-wise comparison and statistical analysis of relative expression results in RT-PCR is used. It compares two groups, with up to 100 data points in a sample and 100 in a control group, for reference and up to four target genes. The expression ratio results are tasted for significance by a randomisation test. In this study the influence of a long term MPA treatment (300 mg MPS/day/sheep) on both Ig receptors mRNA expression level in adult ovine tissues (n=9) was investigated. Each tissue exhibited an individual expression pattern of FcRn and pIgR mRNA. Both types were highly expressed in liver > kidney > and gastrointestinal tract. In spleen, thymus and two lymph nodes medium to low expression levels were determined. FcRn mRNA was significantly down-regulated by MPA in liver ($p=0.06$). In ileum ($p=0.05$) and liver ($p=0.05$) a significant up-regulation for pIgR mRNA expression was observed. MPA may have immuno-suppressive effects in liver by low level FcRn expression and therefore a low IgG serum-to-bile transport is expected. But MPA showed stimulatory effects of pIgR expression in liver and ileum, consequently a good IgA and IgM transport in the tissues is given.

Presentation number: M19

IMMUNOGLOBULIN VH GENE MUTATION STATUS AND TELOMERASE ACTIVITY AS PROGNOSTIC MARKERS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western hemisphere. It is characterized by a variable clinical course and heterogeneous prognosis, ranging from a very short to a normal lifespan. Classic prognostic factors, based mainly on clinical parameters, cannot predict which patients will develop progressive disease. More informative appear recently discovered genetic and biologic parameters, such as immunoglobulin (Ig) VH gene mutational status, telomerase activity, ZAP-70 expression and certain cytogenetic abnormalities. Our aim is to investigate the association between Ig VH gene mutational status, telomerase activity and clinical course in patients with CLL. Thirty-seven patients with CLL (19 with progressive and 18 with stable disease) were enrolled in the study. Ig VH gene sequence analysis was performed either by direct sequencing or after cloning the PCR fragments, using the BigDye Terminator DNA Sequencing kit and the ABI 3100 genetic analyzer. Telomerase activity was determined on purified CLL B-cells using the Roche Telomerase PCR ELISA kit. Seventeen of the 19 patients with progressive disease (89%) had an unmutated Ig VH gene, whereas 15 of the 18 patients with stable disease (83%) had a mutated Ig VH gene (P=<0.001). High telomerase activity was also frequently observed among patients with progressive disease (11/19, 58%), whereas it was low or undetectable in most patients with stable disease (16/18, 89%, P=0.008). Unmutated Ig VH genes and high telomerase activity are both associated with progressive disease in CLL. However, the association between clinical course and Ig VH gene mutational status is significantly stronger, indicating that this parameter is the best predictor of prognosis in newly diagnosed patients with CLL.

Presentation number: M20

DETECTION AND CHARACTERIZATION OF MEASLES VIRUS STRAINS IN CASES OF SUBACUTE SCLEROSING PANENCEPHALITIS IN CROATIA

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We describe here the molecular characteristics of two cases of subacute sclerosing panencephalitis (SSPE) in the children from Croatia diagnosed in 2002: a 4-year-old girl and 8-year-old boy who apparently, according to the medical records, both had acute measles at the age of 6 months. However, they were both vaccinated against measles at the age of 12 months. The patients were hospitalized and treated for encephalitis 3,5 and 7 years after the onset of acute measles. Measurement of measles antibody in the serum and cerebrospinal fluid (CSF) was not helpful in the diagnosis of SSPE, as well as RT-PCR used to amplify measles genes directly from blood and CSF. In contrast, RT-PCR used to amplify measles genes directly from brain tissue proved to be useful. The minimum amount of sequence data required for genotyping a clinical specimen is 450 nucleotides that code for the COOH-terminal of the N protein, but complete N, M and H gene sequences were also obtained from the brain tissues. Sequence analysis was done by using a set of designated reference sequences representing different genotypes. Sequence analysis showed the genetic characteristics of wild-type measles virus which could be placed into genotype D6. On the other hand, vaccine virus in use in Croatia belongs to the genotype A. Furthermore, when sequences from these children were compared, we found mutations in analyzed genes.

Presentation number: M21

INCONTINENTIA PIGMENTI AND NEMO GENE DELETION (Δ 4-10) IN A FEMALE INFANT FROM A HEALTHY PARENTS

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Incontinentia pigmenti is a rare, heritable, multisystem disorder that is transmitted as an X-linked dominant trait and is lethal in males. Familial incontinentia pigmenti is caused by mutations in the NEMO gene as IP2, or 'classical' incontinentia pigmenti. Sporadic incontinentia pigmenti, the so-called IP1, which maps to Xp11, is categorized as hypomelanosis of Ito. The paucity of affected males and high frequency of spontaneous abortions in carrier females is the characteristic of the disease. The cutaneous manifestations can be divided into four phases, all of which may not occur in a given patient. Here we present a female infant of healthy parents with clinically evident first phase of IP: erythematous, linear streaks and plaques of vesicles that are most pronounced on the limbs. Patohystologicaly the bigining of the second (verucose) fase of the ilness was evident. By PCR analysis NEMO @ 4-10 deletion was found.

Presentation number: M22

ASSOCIATION ANALYSIS OF POLYMORPHISM OF TAQI A AND NCOI OF D2-RECEPTOR OF DOPAMINE GENE (DRD2) AND OF DOPAMINE TRANSPORTER GENE (DAT1) IN PATIENTS WITH OPIATE ADDICTION

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The aims of the present research were to study TaqI A and NcoI polymorphisms of D2-receptor of dopamine gene (DRD2) and dopamine transporter gene (DAT1) polymorphism in patients belong to two ethnic groups - Russians, regarding to Orient-Slavic ethnic group and Tatars, concerning to Turkic branch of Altaic language family nationalities in general and according to narcotization age onset - 16 years old and younger ("early onset") and over 16 years old ("late onset") and to narcotization experience duration - 4 years and less and more than 4 years with opiate drug addiction. To study the role of the neurotransmitters (dopamine) of the central and peripheral nervous system in the drug addiction development. To search early diagnostic and prophylaxy approaches of the drug addiction. Leukocyte DNA was extracted using standard phenol-chloroform method. The PCR product was electrophoresed on 6% polyacrylamidic gel. For statistical analysis we used a modified a chi-square test using RxC (Rows x Columns) computer program, based on algorithm described by Roff D.A. and Bentzen P. Relative Risk was calculated by Thomson G. Formula (OR - odds ratio). Investigation results of TaqI A polymorphism of DRD2 indicat es that there is no association with opiate addiction neither in Russians nor in Tatars with that polymorphism. It was established that genotype N2/N2 (NcoI) of DRD2 (P=0.0002; OR=0.35) could be considered as an opiate addiction marker of resistance in patients. Genotype N1/N1 (NcoI) of DRD2 (P=0.013; OR=1.41) could be viewed as a marker of risk to opiate addiction in patients. Allele N1 (NcoI) of DRD2 (P=0.027; OR=1.87) associates with "early onset" in Russians. Association of allele N1 (NcoI) of DRD2 with opiate addiction was determined in Tatars in all investigated groups (P=0.004; OR=1.83) and with "late onset" (P=0.003; OR=1.55). It was found that genotype 9/9 of DAT1 could be viewed as an opiate addiction risk marker in "late onset" patients (P=0.016; OR = 5.03). Genotype 9/9 of DAT1 associates with opiate addiction development in Russians in general (P=0.008; OR=16.39) and in Russians with "early onset" (P=0.003; OR=29.38) and with experience duration more than 4 years (P=0.001; OR=36.84). Thus, the results certificate that D2-receptor of dopamine gene (NcoI polymorphism) and dopamine transporter gene (DAT1) are taking part in opiate addiction development.

Presentation number: M23

GENETIC VARIATION OF METHYLEN TETRAHYDROFOLATE REDUCTASE GENE IN IRANIAN PATIENTS WITH CORONARY ARTERY DISEASE

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Several studies showed that elevated plasma homocysteine is a risk factor for coronary artery disease. A common mutation C677T of methylenetetrahydrofolate reductase (MTHFR) gene is reported to be associated with decreased enzyme activities and significant increased level of blood homocysteine. This study was to analyze the frequency of this mutation in 100 patients with CAD compared to the 100 normal controls. It shows the higher prevalence of the tested mutation in CAD patients compared to the normal control in Iranian tested patients. The C677T MTHFR was significantly linked to the CAD, supported by a P value <0.001 and Chi-squere 51.82. Our observation showed the prevalence and significance of this mutation in studied cases compared to the normal control in Iranian cases.

Presentation number: M24

GENE PARK2 ANALYSIS IN PATIENTS WITH PARKINSON'S DISEASE FROM BASHKORTOSTAN

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Parkinson disease (PD) is a neurodegenerative disease, which involves the progressive loss of dopaminergic neurons, primarily in the substantia nigra. The major cases of PD are sporadic and only 5-10% have family history. Autosomal recessive PD (AR-JP) is one of the most frequent form among hereditary forms of the disease. It is caused by mutations in gene PARK2 (6q25.2-27). The range and frequency of PD is differing in different populations. That's why analysis of mutations in regions and ethnic groups is important. The aim of our investigation was studying the genetic component of Parkinson's disease. One of our tasks was analysis of PARK2 gene among patients with PD from Bashkortostan. We have analyzed by SSCP-analysis 12 coding exons of PARK2 gene in 78 patients. They had different forms of Parkinson's disease both hereditary and sporadic. Movement disturbance of single DNA was found out in 1, 2, 3, 4, 6, 7, 8 and 11 exons, 23 patients in all. In the 7th exon we found C to T change in 866 position mRNA, which doesn't result in amino acid exchange. We'll interpretive the rest data by direct sequencing of shifted exons in the index patients. Heterozygous deletion of exon 12 was detected using semiquantitave analysis. We found it out in 2 patients of Russian origin. The deletion wasn't described in literature earlier. Patients bearing the deletion mutation had 65 and 55 age at onset as at sporadic form while patients with juvenile form had age at onset less than 40 years old.The first symptoms of the first patients were pain and weakness of the left arm and the left arm tremor. The second patient had the weakness of the right arm and the right leg shuffling. The corresponding protein, parkin, shows moderate homology to ubiquitin at the N-terminus and contains a RING-finger motif at the C-terminus. Disturbance of PARK2 gene structure can block the biological function of its protein product. So this deletion in heterozygous form may develop Parkinson's disease. Besides, we detected patients from Bashkortostan on Ala53Thr and Ala30Pro mutations in alpha-synuclein gene, but there were no patients with such mutations. It confirms that mutations in alpha-synuclein gene are rare in patients with Parkinson's disease from Bashkortostan.

Presentation number: M25

EFFECT OF CYTOCHROME P450 CYP2C9 GENOTYPE ON WARFARIN SENSIBILITY

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Warfarin is the most commonly used oral anticoagulant for the prevention and treatment of patients with thromboembolic disorders. The dosage required to achieve the optimal therapeutic effect varies up to 120-fold between individuals. Interindividual variability in responses to warfarin therapy is attributed to a multitude of factors, including the genetic polymorphism of the principal enzyme involved in warfarin metabolism, the cytochrome P450 CYP2C9. Besides the wild-type allele CYP2C9*1, two relatively common variant alleles of the CYP2C9 gene with reduced activity have been identified, CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu). The effect of CYP2C9 genotype on warfarin sensibility was studied in 63 warfarin-treated patients. For every patient the weekly dose of warfarin was recorded and prothrombin time was determined. Genotyping for the CYP2C9*2 and CYP2C9*3 was performed by PCR RFLP. The distribution of the CYP2C9 genotypes was as follows: 66.7% for CYP2C9*1/*1, 20.6% for CYP2C9*1/*2, 11.1% for CYP2C9*1/*3 and 1.6% for CYP2C9*2/*3. No patient were homozygous for the CYP2C9*2 or CYP2C9*3 allele. Allele frequencies for CYP2C9*1, CYP2C9*2, and CYP2C9*3 were 0.83, 0.11 and 0.06. Therapeutic INR values (INR=2.0-3.5) were achieved in 30 patients, whereas subtherapeutic INR values (INR=1.5-2.0) were obtained in 19 patients. In 10 patients the INR values were below 1.5, and were above 3.5 in 4 patients. Weekly warfarin doses required to achieve the therapeutic INR range (2.0-3.5) ranged between 9.0-105 mg/week (median: 33.4 mg), and were significantly different in patients with the wild-type CYP2C9*1/*1 (median dose: 40.5 mg/week; range: 23.2-105.0 mg/week) genotype as compared to patients with CYP2C9*1/*2 (median dose: 31.5 mg/week; range: 10.5-46.5 mg/week) and CYP2C9*1/*3 genotype (median dose: 23.6 mg/week; range: 9.0-63.0 mg/week). For patients with subtherapeutic INR values median weekly dose was 40.5 mg in patients with CYP2C9*1/*1 genotype, 30.7 mg in patients with CYP2C9*1/*2 genotype and 16.1 mg in patients with CYP2C9*1/*3 genotype. 9 out of 10 patients with INR values <1.5 were homozygous for the CYP2C9*1 allele, and the median weekly dose was 26.2 mg. As heterozigotes with polymorphic alleles require significantly lower doses to achieve the same anticoagulant effect as wild-type homozygotes, the analysis of CYP2C9 genotype could predict warfarin sensitivity.

Presentation number: M26

SYNDROMA USHER - CASE REPORT

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Thirty years old pregnant woman had diminish of visual acuity. Standard ophthalmology exam was performed and ordered further procedure after parturation- flurescein angiography, visual field, (Goldman and Octopus) visual evoced potencial and electroretinography. We define Retinopathia pigmentosa sine pigmento Fundus examination revealed pallor of optic disc, diffuse retinal blood vessels narrowing, no retinal pigmentation, vitreal liquefaction, macular pseudohole in left eye. Visual field shows narrowing all isopters to 10 degrees. Fluorescein angiography shows window defect and diffuse retinal blood vessels narrowing. Since the hearing device enables the patient normal social contats, hearing disorders originating from childhood have been dicovered later in collecting case-history record. Retinopathia pigmentosa sine pigmento + congenital partial neprogressive deafness + abscence of vestibular symptoms = Usher's Sy type II. We present thirty years old female patient with Usher s syndrome type II. Usher s syndrome is defined as autosomal recessive congenital disease associated with pigmentary retinopathy (our patient has rare form without pigment) with deafness. We present the latest knowledge about Usher s syndrome epidemiology, etiology, patophysiology, mechanism how rhodopsin mutant interferes with normal phototransduction, patohistology retinal changes, differential diagnosis, clinical types, but unfortunately it is still unclear how a cell expressing mutant protein switches on apoptosis.

Presentation number: M27

HYPOPARATHYROIDISM IN A BOY WITH NECK AND THORACAL SKELETAL MALFORMATIONS

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Hypoparathyroidism is etiologically very heterogeneous disease, which can be caused by both congenital and acquired conditions. In our patient it was in association with skeletal malformation, a relation that has not been reported so far. Our patient is 5-year-old boy who was born from normal pregnancy. Birth at term, BW 3580 g, BL 49 cm. Familial history is normal. On age of 13 months chest X rays showed aplasia of second rib and hypoplastic third rib on the left side. Later, hemivertebra Th2 and congenital tortical scoliosis were noted. At the age of 5 year hypocalcaemia was found. He has no history of manifestations of hypocalcaemia. At age of 5 year, when he was hospitalized for the first time in our Department, his height was 108 cm (25 to 50 percen.). He presented short stature, with short neck, pterigium coli, prominent parietal tubera, deformed thorax, lower position of the left scapula, dental caries. The serum calcium levels were 1.92/1.91/1.95/2.08 and the phosphorus 2.13/2.47/2.29/2.14. Levels of PTH were < 0.2 pmol/l (norm. values 1.0 to 6.0) measured two times. Normal was levels of glucose, sodium, potassium, urea, creatinine, total proteins, AST, ALT, ionized Ca, magnesium. Urinary Ca was 0.56 mmol/dU and P 13.20 mmol/dU. Levels of aldosterone, androgens, 17OHP, T3, T4, TSH were normal. His karyotype was normal and FISH analysis for CATCH 22 didn't show microdeletion. Densitometry for lumbal region has showed normal result. Hypocalcaemia normalized following calcitriol therapy. Possible reasons for association of described skeletal dysplasia and hypoparathyoridsim are: coincidental appearance, contiguous gene syndrome that we cannot locate so far, or mutation of a gene important for both parathyroids and bone development. Due to very few X-ray performed in this patient, adverse effect of radiation on parathyroid glands seems highly unlikely.

Presentation number: M28

THE HUMAN CYTOTOXIC T-LYMPHOCIT ANTIGEN 4(CTLA4) GENE POLYMORPHISM IN MYASTHENIA GRAVIS PATIENTS IN BASHKORTOSTAN, RUSSIA

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The autoimmune process in myasthenia gravis results in postsynaptic blockade of neuromuscular junction by autoantibodies directed against the acetylcholine receptor. The production of auto anti-AChR antibodies in myasthenia gravis is dependent on T cell help. Based on function and experimental data, the gene encoding cytotoxic T lymphocyte-associated antigen 4 (CTLA4) has been suggested as a candidate gene for several autoimmune diseases including myasthenia gravis. CTLA4, expressed on activated CD4+ and CD8+ T cells, binds to ligands B7-1 and B7-2 on antigen-presenting cells and functions as a negative regulator of T cell activation. A polymorphism in the CTLA4 gene has been reported to alter T cell activation and may be an important factor in the pathogenesis of autoimmune diseases and confer genetic susceptibility to them. Three CTLA4 polymorphisms have been described: a single nucleotide polymorphism (SNP) in the promoter region (cytosine or thymine) at position -318, a SNP (adenine or guanine) in the coding sequence 1 at position +49 and a repeat length polymorphism (AT)n at position +642 of the 3'-untranslated region. To determine its role in susceptibility to myasthenia gravis we investigated 52 patients and 135 ethnically matched healthy controls for allelic determinants at two polymorphic sites, one in the promoter region by MseI restriction enzyme digest analyses and another in the third exon at position +642 by PCR method. Allele frequencies of C-318T gene polymorphism in patients and in healthy controls for allele C were found to be 0,86 and 0,88 correspondingly and were comparable with the reported data of Swedish Caucasians. As a result of (AT) n polymorphism analyses, 9 and 15 alleles were observed in patients and healthy individuals correspondingly. The length of the amplified fragments varies from 92 to 128pb. The most frequent alleles were 92pb (0,43 for patients and 0,45 for healthy controls) and 112bp (0,27 and 0,19, correspondingly) in both samples. There was no significant difference in the allele frequencies distribution of this locus between patients and healthy controls. Although according to our investigation C-318T and (AT)n CTLA4 gene polymorphisms are not associated with myasthenia gravis in patients of Bashkortostan (Volga-Ural region) the strict stratification of gross sample of patients according to their thymus histopathology and clinical features may reveal associations with these loci.

Presentation number: M29

GENETIC CHARACTERIZATION OF MUMPS VIRUS ISOLATE DURING PASSAGING IN THE AMNIOTIC CAVITY OF EMBRYONATED CHICKEN EGGS

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Due to the inherent genetic instability of RNA viruses, mumps virus contains a heterogeneous mixture of genomic RNA molecules with slightly different nucleotide sequences. Virus growth both in vitro and in vivo results in selection of different subpopulations, depending on growth conditions. In this work we describe the genetic characterization of mumps virus after adaptation of an alleged individual human isolate of mumps virus to the amniotic fluid of embryonated chicken eggs. Sequence analysis of small hydrophobic (SH) gene showed the presence of two dominant virus variants with nucleotide variation of 6% (variant A and variant B). Restriction fragment length polymorphism (RFLP) analysis showed a heterogeneous population of isolated viruses. Using specific primers for virus variant A and variant B, we did not confirm the presence of variant B in samples of variant A and vice versa. The results point out that investigated individual isolate contains two dominant virus variants and few minor substrains. It is very likely that the alleged individual isolate was contaminated early in the attenuation process (2nd or 3rd passage on eggs).

Presentation number: M30

DETECTION OF BCL-6 GENE EXPRESSION IN HUMAN NAIVE AND MEMORY B CELLS WITH REAL-TIME PCR

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B cells are undergoing intensive proliferation after encountering their specific antigen and their cooperating T cells in germinal centres (GC), structures in secondary lymphoid tissues. Consequently, GC B cells differentiate into either memory B cells or plasma cells. BCL-6 is a "gene signature" of GC B cells which blocks terminal differentiation of these cells into plasma cells. The aim of the study was to investigate whether naive and memory B cells possess equal capacity to participate in GC formation. Highly purified human tonsillar naive and memory B cells were stimulated via BCR and/or CD40, surrogate signals for B cells engaged in T-dependent signalling, necessary for GC formation. Semiquantitative multiplex real-time PCR was used to compare changes in BCL-6 mRNA levels between these cells following stimulations. The expression BCL-6 failed to increase under any of the stimulation conditions. Memory B cells down-regulated BCL-6 mRNA in response to BCR and/or CD40 signals, indicating that this subset might have reduced the capacity for GC entry as compared with naive counterparts. Immunohistochemistry analysis at the protein level showed that freshly isolated memory B cells were positive and naive B cells were negative for BCL-6 protein. Furthermore, our results suggested that in vitro conditions did not appear adequate to generate complete GC phenotype in either naive or memory population. It is necessary to identify additional signals in vitro for generating full GC B cell phenotype in vivo.

Presentation number: M31

RELATIONSHIP BETWEEN IMMUNOREACTIVITY AND LOH IN P53 AND NM23 GENES IN OVARIAN CARCINOMA

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In most Western countries, ovarian cancer is the fifth most common cancer in women and the most common cause of cancer death from gynecological cancer. Molecular genetical alterations are important in ovarian tumorigenesis. In this study we analyzed p53 and nm23 genes using immunohistochemistry, and loss of heterosigosity (LOH) in TP53 (17p13) and nm23 (17q21) genetic regions in 48 ovarian tumors (28 serous, 10 mucinous, 10 endometrioid). Immunohistochemicaly 29 (60.4%) tumors were positive for p53 and 29 (60.4%) for nm23 staining. LOH of p53 was identified in 4 of 42 (9.5%) informative ovarian carcinoma, while LOH of nm23 was observed in 14 of 35 (40%) samples. LOH of nm23 showed significant association with immunohistochemical analysis. There were no observed relationship between p53 immunoreactivity and LOH.

Presentation number: M32

FLT3 GENE INTERNAL TANDEM DUPLICATIONS (FLT3/ITD) IN CHRONIC MYELOPROLIFERATIVE DISORDERS AND ACUTE MYELOID LEUKEMIA

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FLT3 is a class III receptor tyrosine kinase expressed in normal stem cells and proportion of blast cells of myeloid leukemia. Internal tandem duplications (ITD) of the FLT3 gene affecting the exon 11 and 12 lead to ligand-independent FLT3 dimerization and constitutive activation. This stimulates proliferation and induces inhibition of apoptosis which contributes to leukemogenesis. Frequency of mutations in acute myeloid leukemia is 15-34% and is associated with poor prognosis or adverse risk factors. We looked for the FLT3/ITD in 99 consecutive patients with the diagnosis of chronic myeloid leukemia (9), CML, or Chronic myeloproliferative syndrome (MPS) in transformation (5), essential thrombocytemia and polycytemia vera (26), myelodisplastic syndrome (3) (MDS), transformed MDS (8), acute myeloid leukemia (42) and six cases of CLL and ALL. Molecular test was FLT3/ITD RT-PCR based assay as described by the EORTC molecular working group (PIMS2). We found FLT3/ITD mutations exclusively in acute myeloid leukemia patients. Six (14,3%) exhibited ITD with various sizes of duplications. Two patients also had characteristic AML-associated genetic mutations t(8;21) and t(15;17), one had complex karyotype that included trisomy 13 and 3 patients had normal karyotype. Interestingly, one of them had two hematological malignancies: CLL and AML. Four of 6 positive patients had also aggressive clinical course with the treatment resistant disease. In conclusion, we found the FLT3/ITD at expected frequency in acute myeloid leukemia cases. This mutation can be expected to accompany other characteristic mutational events e.g. chromosomal translocations but is also associated with normal karyotypes and

can have adverse prognostic significance.

Presentation number: M33

GENTIANELLA GERMANICA DISPLAYS RADIOPROTECTIVE PROPERTIES ON HUMAN LYMPHOCYTES

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The most important chemicals present in plants which are of considerable pharmacological significance are the polyphenols and caffeine. Among poliphenols the flavanols as water soluble substances are the most suitable for in vitro investigations of their effects on human lymphocytes. Since antioxidative nutritienst, particularly polyphenolic compounds and flavonoids recently have been focused as anticancerogens, and antimutagents the purpose of this study was to assess the cytogenetic effects in vitro of a plant extracts of Gentianella germanica, Sanguisorba minor, Cotinus cogyggria, Cornus mass, Crategus monogyna and Anthyllis vulneraria. Peripheral blood used in experiment was obtained from healthy, non-smoking young male donors. Aliquot of heparinized whole blood were irradiated using $^{60}Co\chi$ ray source, dose 2Gy. Micronuclei formation was quantified in cultured peripheral blood lymphocytes employing Cytochalasin-block micronucleus test (CBMN) using concentrations of plant extracts from 2-20μl/ml. All lymphocyte cultures were set up in duplicate. After 72 hours of incubation, parallel cultures were separated on Lymphoprep (Nycomed), collected by centrifugation, washed in physiological saline, and were prepared for measurement of myeloperoxidase activity. Sanguisorba minor, Cotinus cogyggria, Cornus mass, display radioprotective properties: reduce the level of radiation-induced micronuclei in a concentration-dependent manner. The best radioprotective potential was obtained using water solublile extracts of Gentianella germanica: the yield of radiation-induced micronuclei was reduced for 58% at concentrations ranged from 5-10μl/ml. The same concentration range decrease myeleoperoxidase activity. Treatment of irradiated cells with higher concentrations of Gentianella germanica (up to 20μl/ml) leads to increase of the incidence of micronuclei reaching control (untreated) level. Inverse relationship between incidence of micronuclei and myeloperoxidase activity was observed.

Presentation number: M34

THE Y-CHROMOSOME HAPLOGROUP I AS MARKER OF EXPANSIONS AND MIGRATIONS FROM THE BALKANS

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Previous analyses of Y-chromosome haplogroups (Hg) in Europe has suggested a Balkan origin for Hg-I during the Palaeolithic. Hg-I is typical of Western Eurasia and shows its highest frequency in the Balkans with decreasing gradients from that region. To better define the homeland of this haplogroup and the process of its diffusion, we analysed 231 Hg-I Y-chromosomes, found in a total of 2121 male subjects from 24 European and Middle Eastern populations, for 11 (including five new) bi-allelic markers, which are phylogenetically downstream of the diagnostic M170 mutation. Three sub-clades, I-P37, I-M253 and I-M223 were found to account for 95% of Hg-I Y-chromosomes. I-P37 is the most frequent and widespread lineage in Eastern Europe and the Balkans where it reaches 30%. In contrast, it shows low frequencies (0-7%) in Western Europe, where it is further characterised by the mutation M26. The only exception is Sardinia where the high incidence (38%) is most likely due to genetic drift. I-M253 is shared by most populations analysed, but it has appreciable frequencies only in a corridor which connects the Balkans (3-8%) with Scandinavia, where it shows a frequency peak (30%). I-M223 is the rarest sub-clade of Hg-I, accounting only for 8% of it. With the exception of the Dutch population, where it reaches an incidence of 10%, it is scattered at very low frequency (0-4%) in all of Europe and the Middle East. Analyses of preliminary data on the microsatellite variance and the 49a,f-system indicate a complex pattern of diffusion for this lineage that involves both ancient migrations and more recent expansions.

Presentation number: M35

COMPARATIVE POPULATION GENETIC ANALYSIS AT SEVEN STR LOCI IN ISOLATED POPULATIONS OF BOSNIAN MOUNTAIN AREA AND ADRIATIC ISLANDS OF BRAÈ, HVAR AND KORÈULA (CROATIA)

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The significant number of, more or less, isolated local human population interweaves Bosnia and Herzegovina and Croatia. Different levels of isolation, which are presented in different part of this region, could be induced by many factors (geographical, ethnical, ecological, religious, cultural, etc). In last few years DNA technology employing short tandem repeats (STRs) as genetic markers enables extensive research in characterization of isolated human population. Now, for the first time, we have compared several population-genetic parameters between isolated human populations of Bosnian mountain area and Croatian Adriatic islands, using seven highly informative molecular markers (CSF1PO, D13S317, D3S1358, D5S818, D7S820, FGA and TH01). We chose three isolated populations (Bobovica, Dejèiæi and Lukomir - these villages may be considered as "inland islands") from Bjelašnica-Treskavica mountain area, located 40 kilometers southwest from B&H capital Sarajevo, for our research. In addition, data from three Adriatic islands of Braè, Hvar, and Korèula were selected for this comparative study. Distribution of the observed allele frequencies, gene diversity, gene differentiation and genetic distance were examined. Dendrogram based on genetic distance results was constructed. Index and relative genetic specificity based on results of genetic distance analysis was performed as well as index and relative specific heterogeneity based on gene differentiation. The average heterozygosities that reflect within-population heterogeneity are very similar among all examined populations. The constructed dendrogram, based on neighbor-joining method, clearly indicate that Bobovica, Lukomir and Dejèiæi populations are more genetically similar as opposed to Adriatic islands populations, which represents "molecular picture" of real existing relations on the field.

Presentation number: M36

INNATE IMMUNITY TO HANTAVIRUSES

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Hantaviruses cause two severe human diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Monocytes and endothelial cells are considered as the main target cells for hantaviruses. To investigate the role of initial and innate immune response to hantaviruses we infected different cells with hantaviruses. We infected THP-1 (human monocytes line), primary human monocytes, 293HEK (human epithelial kidney line), MRC-5 (fetal lung fibroblasts cell line), and human vein endothelial cells (HUVEC) with Hantaan (HTNV), Sin Nombre (SNV) or Andes (ANDV) viruses. Culture supernatants were collected and assayed by ELISA for the presence mostly of proinflammatory cytokines/chemokines. Total cellular RNA was assayed for the presence of mRNA of cytokine/chemokine receptors and tumor necrosis factor (TNF) superfamily members by using a multi-probe RNAse protection assay. TUNEL assay in conjunction to transmission electron microscopy was used to confirm apoptosis in infected 293HEK cells. Two-color immunofluorescence flow cytometry and IFA were performed to analyze morphological changes of infected monocytes/macrophages. Both, HFRS and HPS viruses induced production of several cytokines/chemokines in infected cells. HTNV seemed to be the main inductor for RANTES in the infected cell lines. The mRNA expression of chemokine receptors CCR1 and CCR5 were 4.8 and 12.6 folds higher in primary monocytes infected with HTNV, than in control cells. However, substantial differences were found between primary monocytes and THP-1 cells. Infected macrophages underwent morphological changes toward dendritic-like cells and increased expression of co-stimulatory molecules: CD40, CD80, CD83 and CD86 was detected by IFA. HTNV was also the main GM-CSF inductor in MRC-5 cells. We found different cytokine/chemokine profile in 293HEK cells infected with HTNV in comparison with HPS viruses. Could it have some influence on kidney immunopathology is still under the question. Additionally, it was observed that hantaviruses cause CPE in 293HEK cells by apoptosis. The elucidation of the pathways involved in innate immunity and factors controlling the transition to adaptive immunity will improve our understanding of the host response to hantaviruses.

Presentation number: M37

EVALUATION OF DIAGNOSTIC RAPID TESTS FOR THE ACUTE INFECTIONS WITH HANTAVIRUSES DURING THE OUTBREAK OF HEMORRHAGIC FEVER WITH RENAL SYNDROME IN CROATIA IN 2002.

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Puumala (PUUV) and Dobrava (DOBV) viruses are causative agents of hemorrhagic fever with renal syndrome (HFRS) in Croatia. Whole Croatia except the coast and islands is HFRS endemic region. Although many diagnostic tests are now developed for the serological confirmation of acute HFRS, there is still need for the accurate rapid test. During the greatest HFRS outbreak in Croatia in 2002, we aimed to evaluate rapid immunochromatographic test for the detection of specific IgM antibodies to PUUV and DOBV. For the preliminary, retrospective evaluation, serum samples of 105 acute HFRS patients were tested with five-minutes, POC PUUMALATM and POC DOBRAVATM point-of-care tests (ERILAB, Finland). HFRS diagnosis has been previously confirmed with one of the standardized techniques like IgM and IgG ELISA tests and IFA test for the detection of the specific antibodies to hantaviruses. Additionally, in some cases diagnosis was confirmed by RT-PCR. Further, both tests were tested for the specificity and sensitivity with the sera of acute HFRS patients infected with PUUV or DOBV. To evaluate the specificity of both rapid tests, we tested sera of patients with confirmed other acute zoonozis in Croatia: leptospirosis, tick borne encephalitis (TBE) and borreliosis. To exclude the subjectivity in the interpretation, several evaluators interpreted the tests and differences among them were statistically tested using c2.test. Retrospective evaluation showed that in about 80% of patients acute HFRS diagnosis was confirmed with the rapid immunochromatographic tests only. There were no statistical differences among the evaluators who interpreted the POC PUUMALATM, although the less subjectivity was found when they interpreted tests one hour after preparation. For the evaluation of POC DOBRAVATM test, additional evaluation is necessary to confirm the specificity and sensitivity of the test when tested in Croatian patients. Our results showed that sera of the patients with acute leptospirosis, TBE or borreliosis did not cross react with POC PUUMALATM POC DOBRAVATM point-of-care tests. The rapid immunochromatographic test is useful tool for the diagnosis of acute HFRS especially during the outbreak. However, the final diagnosis should be confirmed with additional tests.

Presentation number: M38

HUMAN RIGHTS AND TRISOMY 21 PARENTS ORGANIZATION PROJECT

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For persons with genetic diseases and their families the important questions are: How to enhance and protect human rights of people that are not genetically "perfect", informed consent on transplantation, participation in clinical studies and their selfdetermination. Is the primary goal to help in treatment of some lethal diseases: will the "bad" gene carriers be discriminated (job, insurance, reproduction etc). In Split and Dalmatian county area 156 children with syndrome Down are registered in this parents organistaion and their goals are: to assure better socialisation, education of parents, creating a new athmosphere in the public, breaking barrieres that are created by prejudice. The parents organization "Split-21" define their project and it consist of: the diagnosis must be explained to the parents as early as possible (at birth) in presence of baby with the syndrome. All aspects of illness or condition must be explained: causes, development, prognosis, other difficulties, possibilities of inclusion in society (with a positivistic approach). Follow up must be preceded multidisciplinary. In the program they included psychological, defectological and logopedic treatment of DS patients, prenatal diagnosis and genetic testing too. For the children they seeks: right to have their own way of living, adequate education, to be stimulated and encouraged, to be employed!, to be included as equals in society, to express their own opinion, to selfdetermination, to have social security and benefits, to be informed about genetic testing, to be involved in genetic counseling, to be asked about transplantation of organs with explanation suitable for their mental retardation. "Bringing up a disabled child is difficult and can make you feel like a martyr if you let it, but it can also bring out the very best in both yourself and the people around you" (a father of a DS patient).

Presentation number: M39

STUDY OF FOLIC ACID PATHWAY GENES ALTERATION IN FRAGILE X SYNDROME

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Fragile X syndrome is one of the most common causes of inherited mental retardation in males after Down syndrome. Penetrance of fragile X syndrome is approximately 70% in male and 30% in female. It is due to the expansion of CGG repeat region and methylation of FMR1 gene. To date less attention was to study important genetic factors that may play role in neuropathology and severity of physical characteristic of fragile X syndrome. Folic acid, has been prescribed in some fragile X cases for reduction of hyperactivity and attention deficit in prepuberty. Folic acid derivatives have important roles in DNA methylation and neural development and function as well as in synthesis of neurotransmitters and influence divers process in the CNS. Our hypothesis was to study impaired folate metabolism in fragile X patients as the first step to evaluate the linkage between folate metabolism dysfunction and methylation of FMR1 gene. This is the first study to assess this hypothesis by comparing the frequency of three common mutations in C677T, A1298C methylentetra hydrofolate reductase (MTHFR) and A66G methionine synthase reductase (MTRR) genes, key enzymes in folate metabolism, in 34 male fragile X patients and 60 males control. These common mutations are reported to decrease folic acid level and increase plasma homocystein levels less in carriers and more in homozygote forms. All cases have been initially diagnosed with fragile X syndrome by molecular methods. The statistical analysis showed a significant linkage between C677T MTHFR mutations with fragile X syndrome supported with a P value of 0.021 and chi-square of 7.719. However, no significant correlations were obtained for A1298C MTHFR and A66G MTRR mutations. The P value for A66G was stronger 0.148 compared to A1298C 0.241 that is in harmony with the less effect of A1298C mutation in folate metabolism. Significantly high frequency of C677T MTHFR mutation may suggest effect of folate derivatives on neuropathology and methylation process and more investigations to perform on the role of folate metabolism in fragile X syndrome.

Presentation number: M40

GENE TRAP MODIFICATION OF MOUSE GENE NOL1 (NUCLEOLAR PROTEIN 1)

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Important goals in gene analysis are to assess gene function and to determine spatial and temporal expression pattern. Gene trap method is a random approach, which enables molecular tagging of mouse genes with possibility to analyze both, function and expression pattern. Embryonic stem (ES) cells were genetically modified by a nonhomologous DNA vector containing a splice acceptor and fused promoter less lacZ and neoR genes. The vector was integrated randomly within the genome, and the inserted lacZ and neoR genes were active only if the vector was within a transcribed endogenous gene. That modification yielded a few benefits: 1) modified ES cells were resistant to neomycin, and could be selected in vitro, 2) expression of lacZ gene mirrored the expression of endogenous gene mutated by gene trap vector, hence its spatial and temporal expression pattern could be monitored, 3) the gene trap vector tagged the mutated genes, what facilitated their identification by 5' rapid amplification of cDNA ends (RACE), and 4) the insertion was likely to disturb the endogenous gene, that offered possibility to get insight in gene function by analyzing the phenotype of the corresponding mice, obtained from ES cells. Nucleolar protein 1 (Nol1) is a marker of nucleolus in highly proliferated cells. It is present in majority of malignant tissues (lung, breast, prostate, colon, and brain), but also in non-malignant, highly proliferative tissues. High expression of Nol1 in different carcinoma is a marker of poor clinical prognosis. Nol1 expression is regulated during cell cycle: its protein synthesis starts at late G1, and peaks in S phase of cell cycle. We obtained the gene trap insertion within Nol1 gene and we used the mice carrying this mutation to investigate expression and function of Nol1 gene. The expression of Nol1 gene during development was determined by whole mount histochemical staining by beta-galactosidase substrate X-gal. Staining was found all over the embryo and in all investigated developmental stages. The strongest staining was found between stages E8.5 and E12.5. Although all tissues expressed Nol1, it was not present in all cells. At the cellular level, majority of staining was found within the nucleolus. In order to get insight in the function of Nol1, intercross of heterozygous mice carrying gene trap insertion was performed. Using the PCR method we demonstrated that homozygous mice were not present among newborn animals. Therefore we assumed that homozygous phenotype is lethal.

Presentation number: M41

THE CASE OF THE DOWN SYNDROME, MOSAIC FORM WITH BALANSED TRANSLOCATION

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The index patient was an one-month-old boy, born from a 29-year-old mother and a 38-year-old father after six pregnancies (there were four medical abortions and one spontaneous abortion in mother's anamnesis). The latest pregnancy was complicated by the interruption threat at 13-14 weeks of gestation; the ultrasonic research revealed the hydronephrosis of the fetus left kidney at 26 weeks. The childbirth took place at 39-40 weeks of gestation: his weight was 2820g, the length 50cm. The clinical examination of the proband showed some special features, such as the up-slanting of the palpebral fissures, the small rounded helixes, the excess skin on the neck back and the total muscular hypotonia. The child needed taking the chromosome analysis on mild Down Syndrome suspicion. His karyotype was 47,XY+mar. Then both his parents were investigated. His mother's karyotype was interpreted to be 46,XX t(21;22)(q22;q12), his father's analysis was 46,XY. The FISH-method application helped to identify the proband's citogenetic data: 47,XY t(21;22)(q22;q12)mat+21/46,XY t(21;22)(q22;q12)mat. The proband has mosaic form of Down Syndrome and carries his mother's inherited balanced translocation. The characteristic DS features became more evident at the age of 6 months.

Presentation number: M42

ANALYSIS OF GENETIC FINDINGS AMONG 3000 PATIENTS REFERRED TO UREMIA'S CYTOGENESIS CENTER BETWEEN 2001-2002

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Exposure and contact of people with different mutagenic agents such as chemical, ionizing agents, viruses and radiation exposure have been enormously increasing during the last decade. Therefore it is a matter of concern for new couples who are planning to get married or become pregnant. In their search for an answer for their concerns, they are usually referred to their local genetic counseling center for diagnosis or further genetic counseling. But the method of referring patients is not a standard method. Sometime patients have no medical record or even a primary diagnosis. Therefore reviewing records of treated patients in every genetic center is a good opportunity to find out the deficiencies in the referral chain and assessment of the quality of services received by these patients. In addition it would be possible according to these data for the local health policy makers to design a better plan for future of these centers. Standard statistical methods were applied for classifying and analyzing the patients data according to their referral causes such as: consanguineous marriage, monogenic or polygenic disorders, and chromosomal abnormalities. From the 3000 studied patients, 1966 patients were referred to our center for consanguineous marriage and fear of monogenic disorders. 331 couple had repeated abortions, 43 patients had mental retardation problem, 37 patients had ambiguous genetialia, 175 patients had infertility problem, 126 patients checked for tracing B-thalasemia gene mutation, 66 patients checked for blood disorders (hemophilia A), 10 patients were studied for tracing cancer status in them and checking for Philadelphia chromosome, and 112 patients evaluated for body conformation disorders. Analysis of findings suggested a lack of communication and cooperation between referring centers (health centers) and genetic centers in our state. Our results suggest that there is a great need for improvement of our referral method for genetic counseling and diagnosis.

Presentation number: M43 (oral presentation)

NANOTECHNOLOGY AND MOLECULAR MEDICINE

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Nanotechnology is an exciting new field that deals with the design, synthesis and fabrication of structures at nanometer i.e. at the molecular scale. It has the potential to replace tedious, insensitive, and expensive analytical processes. Its application to the practice of medicine is called nanomedicine. Nanomedicine subsumes several overlapping and progressively more powerful molecular technologies: genomics, proteomics, artificial engineered microbes, nanoscale-structured materials, machines and medicinal nanorobots. DNA arrays (DNA-chips) that operate in the micron size range provide the potential to do thousands of experiments simultaneously with very small amounts of material. One of the most important issues in medicine is the proper distribution of drugs and other therapeutic agents within the patient's body, therefore a large amount of research is being undertaken to develop new "smart" materials that can incorporate and release a medicinal substance, e.g.: nanospheres, nanocapsules, and nanopolimers. Besides, new organic-inorganic hybrid nanocomposites are being developed, such as multipurpose carriers prepared by intercalation of biomolecules or pharmaceuticals into layered double hydroxides (LDH). Some interesting possibilities of implementation of natural and synthetic zeolites in pharmaceutical industry and medicine are being investigated. Self-assembly of biomolecules is now emerging as a new route to produce novel materials and to complement others, i.e. ceramics, metals, synthetic polymers and other composite materials. Self-assembling biomolecular materials are of great interest in advanced medicine because they can serve as bioactive extracellular matrices for cell therapies or targeted drug delivery. Nature's nanomachines, such as motors, pumps, and valves, have inspired the design and development of engineered devices. These nanomachines catalyze chemical reactions, transduce information, and transport material within and out of the cell.

Presentation number: M44

A NEW CASE OF PARTIAL TRISOMY 16Q

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Trisomy 16q is a rare condition in livborne infants. Some 28 cases have been reported and most cases are due to unbalanced translocation. Clinical presentation is not well defined because it is usually associated with loss or gain of an other chromosome involved in the rearrangement, while duplications are quite rare. In this report we present the results of clinical and cytogenetic investigation in 10 - years old girl. Clinical examination showed dysmorphic traits including hypoplastic supraorbital ridges, epicantus, dysplastic, low set ears, full cheeks, thin lips, micrognatia. Her somatic and intelectual development were within normal range, although she experianced learning difficulties, mostly due to behavioural problems, aggressiveness and disobedience. Cytogenetic analysis was performed on peripheral blood culture of the patient and her parents. Both parents presented with normal karyotype, while cytogenetic analysis in the proband identified an extra GTG-positive band in the long arm of chromosome 16. FISH with chromosome 16 painting probe stained the aberrant chromosome completely. Analysis thus suggested the duplication of the long arm of chromosome 16 most probably involving 16q13--àq22 region. It has been proposed that the duplication of the distal long arm segment may cause typical features of trisomy 16q, including short survival. On the other hand, the comparison of our patient to other reported cases revealed that trisomy for the proximal segment of the long arm of chromosome 16 may be associated with mild clinical presentation and behavioural problems as a major manifestation.

Presentation number: M45

THE IMPORTANCE OF EARLY MOLECULAR DIAGNOSIS IN THE NEONATES WITH HYPOTONIC SYNDROM; TWO CASES OF CONGENITAL MYOTONIC DYSTROPHY (CMD 1)

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Muscular dystrophies are myopathies with genetic aetiology. The clinical course is progressive with dystrophy of muscles. This is a group of unrelated diseases and each has different way of inheritance and clinical expression. Some manifested in severe forms at the birth with early death, other shows clinically slow progression through the life. Congenital muscular dystrophy or Steinert disease of the adults regarding incidence is the second, after Duchene and Becker muscular dystrophy. Incidence is 1:30 000 births and its way of inheritance is autosomal dominant with different penetration. Different amounts of repetitive triplets of CTG (Cytosine-Timin-Guanin) on the long arm of chromosome 19q13 mapping for miotonine protein kinaze gene (DMPK) are the genetic basis. Here we present two patients with early neonatal form, with various clinical manifestations and molecular findings. First manifested with respiratory distress sindrom (RDS), diaphragmal elevation, severe generalized hypotonia and characteristic phenotype of CMD. The second case had intracranial haemorrhagy, equinovarus deformity and severe generalized hypotonia. The first patient was treated with phenytoine and the muscle tone improved. In other case phenytoin treatment was not necessary. Furthermore, brain MR showed ventriculomegaly and cerebral gliosis, but muscle MR was normal in both patients. Molecular analysis revealed repetitive triplets in protein kinase gene (DMPK) in both cases. In the conclusion the importance of the molecular investigation or even molecular screening, for neonates with hypotonic syndrome is recommended. The problems of prenatal diagnosis in MD are also discussed.

Presentation number: M46

NOVEL TAU POLYMORPHISM IN INTRON 9 ASSOCIATED WITH ALZHEIMER'S DISEASE

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Tau protein is common denominator for a number of neurodegenerative disorders. Tau mutations associated with dementias have been described in the familial cases of various tauopathies. However, no mutations in the tau gene were found in Alzheimer's disease. Therefore we have set up a continous screening for detection of tau mutations in genomic DNA isolated from blood samples of patients diagnosed with probable Alzheimer's disease. All examined samples were from Slovak patients of Caucasian origin. Using sequence analysis of PCR products of relevant parts of tau gene, we have found a novel single nucleotide polymorphism inside of intron 9 of the tau gene. The novel polymorphism G/A was located 176 bp upstream of exon 10 and was found in 57 % of all examined samples.This variation may be important connecting step between nucleotide change, tau pathology and other factors leading to dementias of Alzheimer's type, since single nucleotide polymorphisms found in exons 9, 10, 11, 12 and 13 are linked to frontotemporal dementias with parkinsonism linked to chromosome 17 (FTDP-17) and other tauopaties. The exons 9 to 12 are coding for microtubule binding domains and mutations located in this part of tau gene could modulate the binding ability of tau protein with consequent dementia, since five mutations in FTDP-17, progressive supranuclear palsy (PSP) and corticobasalar degeneration (CBD), located inside of intron 10 in the 5' splice site, modify the ratio between the 3R and 4R tau isoforms.

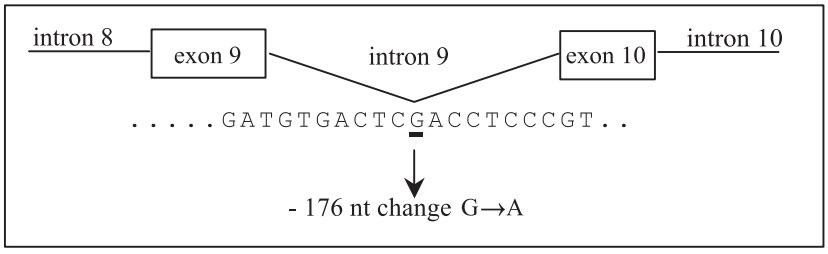

Figure - location of single nucleotide change inside of intron 9

Presentation number: M47

CYTOCHROME P450 - CYP2C9 GENOTYPING IN WARFARIN DRUG THERAPY OPTIMIZATION

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S-warfarin is an anticoagulant therapy drug and its often unpredictable dose response results at least in part due to genetic differences in CYP2C9 enzyme metabolic capacity. Besides wild type- CYP2C9*1, there are mutant alleles CYP2C9*2 and CYP2C9*3 that in homozygotes code enzymes with activities of only 16-20% and 5% of total wild type activity, respectively. Our aim was to investigate the possibility of warfarin dosage prediction by CYP2C9 polymorphism genotyping. We genotyped 181 patients (43.6 % males, mean age 60.2; SD=14.5) by PCR-RFLP method as reported by Nasu K. et al., 1997. Patients were receiving warfarin in doses needed for maintaining prothrombin time within INR range 1.5-2.5. Results showed significantly higher warfarin mean daily dose (DD) among 104 wild type homozygous patients (DD=4.4mg; SD=1.9) compared to 77 patients with at least one mutated allele (DD= 3.7mg; SD 1.7, p=0.010) and 10 patients with both mutant alleles (DD=2.6mg; SD=1.4, p=0.004). Patients were also divided into groups according to warfarin median daily dose: among DD<4.1mg group (90 patients), we found 71% wild type (1*), and 29% mutated (2* and 3*) alleles. These 2* and 3* allelic frequencies were significantly higher (p=0.027) than in DD>4.1mg group (91 patients) (81% wild type (1*), and 19% mutated (2* and 3*) alleles). Genotype frequencies did not differ significantly. Results of our investigation are concordant to other author's findings and suggest a relationship of CYP2C9 defective alleles with lower warfarin doses. Therefore, CYP2C9 genotyping could be of predicting and optimizing importance in anticoagulant drug therapy.

Presentation number: M48

CONDITIONAL DELETION OF S6 RIBOSOMAL PROTEIN GENE BY CD4 CRE TRANSGENE

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To study the relationship between cell growth and cell cycle progression, we interfered with the ribosomal biogenesis by deleting one allele of S6 ribosomal protein gene in thymus by using Cre recombinase under the control of CD4 promoter. In that model we studied thymocyte development and functional properties of mature T cells. Cre/loxP system was used to delete one S6 allele in double positive (CD4+CD8+) cells in thymus. Pups from crossings of homozygous floxed S6 mice with CD4 Cre mouse were screened for Cre transgene with PCR. Deletion of one S6 allele was confirmed by Southern blot analysis and expression of S6 mRNA was measured by Northern blot analysis. Flow cytometry was used to study population of T cells during development and cell size was determined by FSC. Proliferative capacity and cytokine production were analyzed to estimate function of peripheral T cells. In previous studies it has been illustrated that deletion of both alleles completely blocks cell proliferation. Conditional deletion of one S6 allele did not have any consequence on differentiation, size and number of thymocytes, although deletion was 100% effective. However, we observed dramatic effect on number of mature T cells in spleen, whose size was normal. Currently, we are investigating the proliferative capacity and ability of these T cells to secrete cytokines such as IFN-g (interferon gamma) and IL-2. Our goal is to determine the effect of limited ribosome biogenesis on cell growth and cell cycle progression and differentiation of T cells. This study can help us to better understand pathological processes caracterized by impaired ribosome production.

Presentation number: M49

GENETIC COMPARISON OF THE 15 REGIONAL POPULATIONS IN THE PHILIPPINES

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The Philippines archipelago lies in a migratory nexus between mainland Asia and Oceania and is thought to have served a link between these two Pacific Rim regions. Many groups in the Philippines share cultural affinities with both Polynesia and Asia. The pivotal location and geographical barriers inherent in a volcanic island archipelago have given rise to pockets of populations with their own language, culture and religion. Our aim is to study 15 regional populations in the Philippines using five Short Tandem Repeat Markers (STRs) namely HUMvWA, HUMF13A01, HUMFOLP23, HUMDHFRP2 and D8S306, to analyze population relationships and dispersals among groups. Blood or buccal samples (n=1,362) were obtained from 15 urban regional centers and processed as described earlier. Frequencies were determined using the gene count method. Homogeneity test by simple chi-square tests were performed using Popgene software. Co ancestry distance and a dendograms were generated using the GDA software ver 1.1. Co ancestry coefficient values (Fst) range from 0-0.01, indicating low overall genetic diversity amongst urban groups in the Philippines. But chi square tests show that the Philippines is not homogenous in three markers (HUMF13A01, HUMFES and HUMFOLP23). Analysis of 15 regions using UPGMA revealed the presence of two main clusters (A and B), with Cluster B further subdividing into B1 and B2, with one region (Region 6) as an outlier. Clustering of regions is consistent with their geographical location and represented a north to south cline across the entire archipelago. Low Fst values calculated from our study supports the presence of an initial core population that peopled the Philippines. Interactions of this core population with other Asian groups may have resulted in genetic variations leading to a north to south cline, and an overall heterogeneity of modern regional populations. This is particularly important when interpreting data of multi-center studies on relationships and dispersals of Filipinos and different Asian groups. Northern groups have more interactions with Taiwan and China, compared with southern regions that have stronger ties with the Malaysia and Indonesia. Further work to expand the number of STR loci and compare Philippine populations with Asian populations is underway.

Presentation number: M50

MOLECULAR GENETIC DIAGNOSIS OF THE HUNTINGTON DISEASE IN TUZLA REGION POPULATION (BOSNIA AND HERZEGOVINA)

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Huntington's gene, IT15, in chromosome 4p16.3, has 67 axons with 10366 bp coding space and unstable CAG sequence that codes glutamine on 5' terminal. The molecular-genetic analysis of disease determined expansion of nucleotide repeated CAG sequences. In two large Bosnian families with Huntington's disease specific DNA diagnosis of IT15 gene mutation is performed, according to the wishes of two female member with "high genetic risk", that voluntarily accessed to DNA test in order to make plans for own family "without risk" of pathologic gene transmission. Gene detection from blood sample is performed in Center of Medical Genetics, Clinical Center Ljubljana, amplifying DNA sequence in HD gene with CAG tandem repeats, with PCR, methods and gel electrophoresis. Next primers are used: HD3: 5' CCT TCG AGT CCC TCA AGT CCT TC 3', HD5: 5' CGG CTG AGG CAG CAG CGG CTG T 3'. A mutation in IT15 gene (number of CAG tandem repeats 46, size of DNA fragment 165 bp and 245 bp) is detected in DNA of clinically affected patient's brother in first family (Fig. 1A and B), and 170 bp, and 239 bp in clinically affected patient's father in second family. But, results of PCR analysis of DNA sample clinically non affected members showed 23 CAG tandem repeats (fragment size 180 bp) in first and 20 CAG tandem repeats (fragment size 169) in another member that excluded presence of Huntington's disease in both cases. Our example confirms that molecular-genetic analysis of disease is the most powerful tool in solution of dilemmas in non-affected family members, potential carriers of Huntington's gene. We accentuate importance of DNA test in persons with "genetic risk", that are not gene carriers. In that case he or she is able to create own future without fear of pathological gene transmission.

Presentation number: M51

THE INCIDENCE OF FACTOR V LEIDEN AND F II20210A IN PATIENTS WITH ISCHAEMIC STROKE

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Along with heart diseases and malignant tumors, ischemic stroke is the third most frequent cause of death in developed countries. The disease is caused by the damage of the brain due to the occlusion of arteries followed by ischaemia of the part of the body supplied by that blood vessel. 60% of all strokes are caused by atherosclerosis of cerebral arteries, and 25 % of them are caused by the embolism of blood vessels. Polyethiology of the disease varies significantly in different races, nations, as well as gender. Numerous risk factors for stroke influence the incidence of the disease: hypertension, diabetes mellitus, disorder of lipid metabolism, heart diseases, smoking, alcoholism, and obesity. The influence of the mutation in the gene for the coagulational factor V (Factor V Leiden) and in the gene for prothrombin (F II20210A) in the process of the development of stroke is contradictory. The incidence of the just mentioned mutations in different races and nations varies significantly.The research included 88 participants with ischaemic stroke, both male and female, aging up to 65 years. Patients with secondary thrombophilia, patients who were receiving blood transfusion, and in whom Factor VIII of thrombolysis was found, those with infectious conditions during the last two months, as well as those with implanted heart valves were excluded from the research. For the DNA analysis the blood with EDTA as a anticoagulant was used. Genomic DNA was isolated according to the standard procedure using phenol-chloroformextractions. The presence of Factor V Leiden and FII20210A was determined by PCR-RFLP. A 2287-bp fragment encompassing position 1691 of factor V was amplified with primers according to the procedure of Zoller and his associates. Following the digestion with MnlI (MBI Fermentas GmbH, Germany), the normal type allele (1691G) resulted in 157 bp, 93 bp and 37 bp fragments, whereas the mutant allele (1691 A) resulted in 157 bp and 130 bp fragments. Analysis for FII20210A was performed according to the method described by Poort and his associates. After the digestion of amplified 345bp fragments with Hind III (Roche Diagnostics, Meinheim, Germany), the mutant A allele was cleaved in two 322bp and 23bp fragments, whereas the normal G allele remained undigested by the restrictive enzyme. Digested products were separated by electrophoresis in 1,5 % agarose gel (Applied Biosystems, Foster City, CA, USA). The research determined 7 (7,9%) heterozygotes for Factor V Leiden and 5 (5,7%) heterozygotes for FII20210A. Although the frequency of the mentioned mutations in patients with ischaemic stroke increased, there was no statistically significant difference. Possible interactions with other risk factors of cerebrovascular diseases require further research.

Presentation number: M52

THROMBOTIC DIATHESIS AND MUTATION IN THE GENE FOR PROTHROMBIN (FII20210A)- CASE REVIEW

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Causes of thrombotic diathesis (thrombophilia) can be either acquired or inherited. There is a range of well-known inherited causes of diathesis, such as resistence to activated protein C due to the mutation in gene for factor V (FV leiden), deficiency of antithrombin, protein C and protein S, mutations in gene for prothrombin (FII20210A), and infrequent dysfibrinogenemia. A 48-year-old man was hospitalized due to the acute weakness of the left extremities, followed by the lowered sensory powers on the left side of the body and central paresis of the left facial nerve. It has been found out that he was undergoing the treatment for the moderate arterial hypertension for the last 15 years, and that he smokes 25-30 cigarettes a day. Five days after his hospitalization, neurologic deficit developed into hemiplegia. Since the computerized tomography of the brain did not show a pathomorphologic substratum compatible with the newly developed neurologic disorder, magnetic resonance of the brain was also done, which showed hyperintensity of the blurry signal in T2 time in the region of the cranial part of the pons on the left, aqnd hypointensity of the signal in T1 time. After initiating paramagnetic contrast medium the mentioned change looks blotchy along the edges. The described findings correspond to subacute ischaemic insultus, 18×12 in diameter. Duplex echosonography of carotid and vertebral arteries determined fibroid thickening of the walls of the joint and internal carotide arteries on both sides, with uneven inner contures and mildly increased vascular resistence, but without signs of reduction of the inner lumen. Neurologic deficit was only partly reduced during the rehabilitation. A year and a half after the described stroke, the same patient experienced another disorder, intermitent claudication. Since the examination determined obliterating atherosclerosis of terminal aorta and both iliac arteries (Syndroma Lerichae), aorto-bifemoral bypass was placed (Intervascular prothesis, 18×9mm), and thrombendarectomy was done on both sides of the joint and deep femoral arteries. Two and a half years after the first stroke, echosonography discovered progressive changes in carotides - reduction of lumen of the inner carotid arteries on both sides (40% on the right, and 30% on the left). Additional laboratory examination was done. For the DNA analysis whole blood was collected insodium citrate (0,129 mol/l) as anticoagulant. Genomic DNA was isolated according to standard procedures using phenol-chloroform extractions. Analysis of the G20210A mutation in the gene for prothrombin was performed according to the method described by Poort and his associates. After digestion of the amplified 345bp products with Hind III (Roche Diagnostics, Meinheim, Germany), mutated A allele cleaved into two fragments- 23bp and 322bp, whereas the normal G allele remained the same. Result of the digestion by the restrictive enzyme were separated by electrophoresis on 1,5 % agarose gel (Applied Biosystems, Foster City, CA, USA). Analysis determined the presence of heterozygotes for FII20210A. The outbreak of thrombotic diathesis in younger people requires additional diagnostic examination, including genetic testing for possible inherited factors.Incidence of the G20210A mutation in the gene for prothrombin in such patients, as well as frequency of the combination of that mutation with other risk factors, is one of the factors whose role in the development of the thrombotic diathesis should be additionally examined.

Presentation number: M53

C-erbB/2, P53 AND NM23 PROTEIN DETECTED BY IMMUNOHISTOCHEMISTRY AS A PROGNOSTIC FACTORS IN PATIENTS WITH EPITHELIAL OVARIAN CARCINOMA

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Our aim is to demonstrate immunohistochemical expression of p53, c-erbB/2 and nm23 proteins in ovarian cancer and to establish correlations with such predictive factors as clinical stage, grade and vascular invasion. The effect of protein overexpression on patients overall survival was also studied. Immunohistochemical analysis was performed on formalin fixed paraffin-embedded specimens from 80 ovarian carcinomas using the anti-nm23, p53 and c-erbB/2 monoclonal antibodies. All patients were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) staging system as I to IV. Carcinomas were graded as low and high grade according to the modified grading system recommended by Shimatzu and Silverberg . For unvariate analysis, survival time was studied by the Kaplan-Meier method, and the log-rank test was used to analyze differences among groups. For multivariate analysis, Cox proportional hazard regression model was used to examine several parameters simultaneously. Associations of tested parameters were studied by Spearman rank correlation. Advanced clinical stage ($p<0.001$), positive staining for nm23 ($p<0.001$), p53 ($p=0.021$) and c-erbB-2 ($p=0.003$) protein, high histological grade ($p<0.001$) and vascular invasion ($p=0.006$) were connected with shorter overall survival in univariate analysis. In multivariate analysis only clinical stage were separated as an independent prognostic parameter ($p=0,014$), and vascular invasion had marginal statistic significance ($p=0.051$). In early stages multivariate analysis only the presence of vascular invasia was associated significantly with survival ($p=0.008$), whereas none of the analysed factors in advanced stage multivariate analysis showed any independent predictive value for patient prognosis.The results of our study support the view that he presence of p53, nm23 and c-erbB-2 protein overexpression is associated with other parameters characteristic of aggressive tumors and that these overexpression have no independent value for patient prognosis. Although we could not confirm the significance of overexpression of these proteins as a prognostic factors in our ovarian cancer group of patients they should be considered as a potential targets for studies of antibodies directed therapy or gene therapy.

Presentation number: M54

SPECIFIC TRANSGENE EXPRESSION IN MOUSE SPERMATOGONIAL STEM CELLS

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Spermatogonia, especially spermatogonial stem cells, are the cells essential for the continued maintenance of spermatogenesis. Sperm output and the integrity of the male genome rely on their continuous +error free; proliferation and differentiation. Stem cells are defined only in terms of their function. They are self renewing precursor cells that can generate differentiating cell populations to replenish the lost of cells. Stem cells are rare in most systems making it difficult to isolate them and study their function. Estimation is about two stem cells per 104 total testis cells. Many studies of spermatogonia have been performed with morphological methods but the true nature of spermatogonia still remains unknown. Limitation in understanding their control is also the fact that no specific markers are still available for their selection. The aim of this study was to identify a promoter which direct transgene expression to the earlier stages of male germ line differentiation and to use the promoter to drive the expression of a reporter gene and a neutral surface marker. The Stra8 gene expressed in spermatogonia appeared to be hopeful candidate. The 400 pb fragment encompassing the transcription start was sufficient for the expression of a luciferase reporter gene in the testis of Stra8/luc transgenic mice. In two independent families of transgenic mice expressing the reporter, spermatogenesis proceeded normally. Determination of testis section and establishment of developmental patterns of expression in the prepubertal testis indicated that the cloned promoter was active in spermatogonia, but unlike the endogenous gene, only in a fraction of the adult spermatogonia. In order to isolate and identify the cells which express the transgene, Stra8/HAglo transgenic families were generated in which the same promoter drove the expression of a neutral surface marker (HAgloCD4) constituted by two domains of the human CD4 protein fused with the transmembrane and intracytoplasmic regions of the inf!uenza hemagglutinin. Magnetic sorting of CD4+ cells yielded a morphologically homogenous cell fraction which expressed spermatogonial markers: RBM and cyclin A2. All recovered cells were also positive for integrin-b1 and Ep-CAM, which are known as stem cell markers. Isolated cells were negative for c-kit which is expressed in differentiated spermatogonia. Their identity as stem cells was demonstrated by a high efficiency in re-establishing spermatogenesis in a germ cell-depleted testis. The isolation of spermatogonial stem cells is necessary for innovative research into stem cell biology and for the understanding of spermatogonial physiology. Understanding of spermatogonia could have important clinical implications in: infertility treatments, protections of spermatogonial stem cells in oncological patients, development of contraceptive strategies, the field of animal husbandry, generations of transgenic animals, in preservations of valuable animals etc. This work is a contribution to these aims.

Presentation number: M55

ULTRASOUND FINDING OF HYPEREHOGENIC BOWEL AND DILATED BOWEL LOOPS IN A FETUS AFFECTED HOMOZYGOTE FOR CYSTIC FIBROSIS

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Our aim is to present a case of prenatally diagnosed fetus with cystic fibrosis in patient with no family history of this disease. A 23-year-old secundi-gravida was reffered to our center for prenatal diagnosis to evaluate fetal morphology at 17 weeks of gestation. The ultrasound examination revealed hyperechoic bowel of gradus II and dilated fetal bowel loops. Patient's medical history was unremarkable except for a spontaneus misscarriage at 7 weeks of gestation in earlier pregnancy. Amniocentesis revealed a normal male karyotype (46 XY). Cystic fibrosis mutation screening showed that fetus was affected homozygote for most common mutation ΔF 508. Cystic fibrosis mutational analysis of parents showed that they are carriers of the same mutation. Following genetic counceling the parents opted for termination of pregnancy at 20+6 weeks of gestation. A male stillborn, weighing 420g, with normal external appearance, was delivered. Autopsy examination confirmed prenatal findings. Cystic fibrosis screening should include fetal bowel morphology examination in low risk population between 18 and 24 weeks of gestation. The finding of hyperechoic bowel of gradus II should indicate the necessity for prenatal molecular cystic fibrosis testing which is of great importance in low-risk population.

Presentation number: M56

D1S80, 3'APOB, CCR5DELTA32 POLYMORPHISMS IN FIVE ETHNIC GROUPS OF NORTH CAUCASUS

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The minisatellite loci D1S80, 3'ApoB and CCR5delta32 mutation polymorphism were studied in five ethnic groups inhabit Northwestern region of Caucasus. The polymorphism of minisatellite loci D1S80 and 3'ApoB was used due to high capability of these markers to distinguish of ethnic groups. The study encompasses indigenous populations, namely: Adygeis (Shapsugs and Highlanders), Abkhasians, Circassians (all of which belong to Adygei-Abkhazian linguistic family), and Kuban Cossacks (Russians of Krasnodar region, Indo-European linguistic family). Population samples have been collected from healthy unrelated natives under informed consent. Minisatellite loci shown high polymorphism level and average 75 % heterozygosity in all ethnic groups. The data obtained show that the frequency of the CCR5 deletion allele in the Kuban Cossacks does not considerably differ from the frequencies revealed in Europeans. The populations of Adygei-Abkhazian linguistic family, which shows possible linguistic affinity to the Basque, are characterized with different values of the CCR5 delta 32 occurrences. Minisatellite allele frequency distributions in all populations studied were similar to those in European populations ones. We have compared our data with those in other ethnic groups of Eastern Europe: Russians, Byelorussians, Ukrainians, Kalmyks, Yakuts, Komis, Maris, Mordvinians, Baskirs, Tartars. The analysis of genetic variability was made with multidimensional scaling treatment of Nei's original genetic distance matrix. The plot obtained reveals close affinity between Circassians, Abkhazians, Shapsugs and Adygeis. Minisatellite DNA diversity analysis shown that all North Caucasus populations are most closely related with Eastern Slav peoples - Russians, Ukrainians and Byelorussians. The data obtained are in agreement with early settlement of the North Caucasus region and may contribute in evolutionary study of genetic relations between European and Asian populations.

Presentation number: M57

MOLECULAR EPIDEMIOLOGY OF LQTS IN RUSSIAN FAMILIES

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Congenital long QT syndrome (LQTS) is a cardiac disorder with high risk of sudden cardiac death, dominant mode of inheritance and a stable prevalence in different population (1:7000). At least six genes, when mutated, can produce this phenotype. One hundred and forty patients from 67 unrelated Russian families with different types of disease were available for DNA and clinical analysis. Molecular investigation revealed that not less then 20% of the identified mutations arose de novo. We suggested that such considerable level of mutation de novo could support stability of disorder's prevalence. Using PCR-SSCP analysis and DNA sequencing 20% of the coding region of five ion channel genes KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2 "hot parts" of this genes) we identified genetic defects in 33 (~50%) probands. Distribution of mutation among there 5 genes was followed: 27 in KCNQ1 (75%), 6 in KCNH2 (16.7%), 1 in SCN5A (2.8%), 2 in KCNE1 (5.5%) and none in KCNE2. Genotype - phenotype analysis found that clinical traits were gene-specific in large part. Three probands had two different mutations (the one was inherited and second was de novo). In all these cases probands had graver ill than affected parent. We identified 6 polymorphisms in genes KCNQ1 (C513T), KCNH2 (A1692G), KCNE1 (G84A, A112G, and C252T) and KCNE2 (A22G) which prevalence among patients and healthy individuals were the same. We supposed that these substitutions didn't modified clinical phenotype. Polymorphism G253A (D85N) in gene KCNE1 was found in affected group only (0.03). Clinical significance of this substitution feels the need of verification subsequently.

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ACKNOWLEDGMENTS

Under auspices:
Ministry of Health Republic of Croatia
City of Zagreb

We would also like to thank the following institutions and organizations for supporting the Third European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine:

Armed Forces DNA Identification Laboratory, USA
Bode Technology Group, USA
Bridge Foundation, USA
Catholic Medical Foundation, USA
Connecticut State Police, Forensic Science Laboratory, USA
Croatia Airlines, Croatia
Croatian National Tourist Board, Croatia
Croatian Society of Human Genetics, Croatia
City of Zagreb
Foundation for Genetic Medicine, Inc. Manassas, VA, USA
Gendia, Belgium
George Washington University, USA
General Hospital Holy Spirit, Croatia
Government of the Republic of Croatia
Government of the Republic of Croatia, Commission for Detained and Missing Persons
Hadassah University Hospital, Israel
Institute Ruðer Boškoviæ, Croatia
International Commission on Missing Persons, USA
Mayo Clinic, USA
Ministry of Health, Republic of Croatia
Ministry of Science and Technology, Republic of Croatia
Ministry of Tourism, Republic of Croatia
Monroe County Public Safery Laboratory, Rochester, USA
Osijek University School of Medicine, Croatia
Polyclinic Holy Spirit II, Croatia
Split University Hospital, Croatia
Split University School of Medicine, Croatia
Studio Hrg, Croatia
Studio International, Croatia
The Henry C. Lee Institute of Forensic Science, University of New Haven, CT, USA
United States Embassy, Zagreb, Croatia
University of Connecticut Health Center, Department of Genetics and Developmental Biology, USA
University of Split, Croatia
University of Zagreb, Croatia
Zagreb Tourist Board, Croatia
Zagreb University School of Medicine, Croatia

Presentation Number: F1

OUR EXPERIENCE: TEN YEARSORK ON IDENTIFICATION OF SKELETAL REMAINS FROM MASS GRAVES

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Significant efforts are currently underway to identify missing individuals discovered in mass graves situated throughout Croatia and southern Bosnia and Herzegovina. During last ten years (1993-2003) more than 900 bodies found in several mass graves have been identified in our Department by standard forensic methods. Unfortunately, standard methods for human identification were not sufficient in approximately 30-35% of all cases and DNA identification was requested. DNA isolation was performed using standard phenol/chloroform/isoamyl alcohol procedure as well as some advanced methods (decalcification with EDTA prior to extraction and NaOH repurification). Recently, new commercial procedures for DNA extraction (DNA IQ System) and DNA quantitation (Alu Quant Human DNA Quantification System) were successfully tested in our laboratory. The quality of isolated DNA varied in different types of bones and due to DNA degradation and contamination. During the last ten years, the following DNA identification systems were used: AmpliType®PM+DQA1 PCR Amplification and Typing Kit, AmpFlSTR Profiler™ PCR Amplification Kit, AmpFlSTR Profiler Plus™ PCR Amplification Kit, PowerPlex™ 16 System, AmpFlSTR Identifiler™ PCR Amplification Kit, immobilized SSO (sequence-specific oligonucleotide) probes for the mitochondrial DNA control region and Y-Plex™6. Up to date we have analyzed 400 samples by DNA methods and obtained full genotypes in 305 samples (76.25%) with DNA matches confirmed in 68 cases.