FINAL PROGRAM AND ABSTRACTS

THE SECOND EUROPEAN-AMERICAN INTENSIVE COURSE IN CLINICAL AND FORENSIC GENETICS

September 3 - 14, 2001
Dubrovnik
CROATIA
www.european-americangeneticsmeetings.org

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Printing
1000 copies

Zagreb, September 2001

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Dear colleagues,

It is with great enthusiasm that we introduce you to the program for the Second European-American Intensive Course in Clinical and Forensic Genetics to be held September 3 - September 13, 2001 in Dubrovnik, Croatia, one of the most beautiful towns in Europe. Referred to as the "Pearl of the Adriatic", Dubrovnik is situated on the Dalmatian coast, surrounded by mountains and laced with indigo waters of the Adriatic Sea. Dubrovnik's rich one thousand and forty-year history is demonstrated best by the beautiful architecture of the many Gothic, Renaissance and Baroque churches, monasteries, palaces and fountains.

Your interest in, and response to, The First European-American Intensive Course in PCR-Based Clinical and Forensic Genetics held in Split, Croatia during 1997 has been phenomenal. More than 250 delegates from 32 countries participated.

The 2001 meeting is filled with a combination of progressive scientific talks from invited investigators in forensic and clinical medicine, as well as poster presentations of scientific abstracts. We are especially pleased to announce that the Promega Corporation has agreed to be the principal sponsor of the conference.

The first week of the intensive course will address current issues in forensic identity testing. Intensive lectures will include the following topics: crime scene investigation, identification of human remains by nuclear and mitochondrial DNA methods, short tandem repeat analysis, Y-chromosomal testing, and recent technological advancements.

The clinical section of the meeting will begin the following week and will address issues relating to gene therapy of cancer and inherited diseases, microarray and chip technology used in clinical diagnostic and related fields, as well as poster presentations of scientific abstracts. There will also be a comprehensive educational program on the basic science and use of viral and non-viral vectors for gene transfer and expression in animals and patients, sessions on the design and performance of gene therapy clinical trials as well as the session on stem cells manipulation.

Most of the plenary talks will be published in the Croatian Medical Journal, a publication listed in all major scientific indexes. The attendance of the intensive course will be accredited by the Croatian Medical Chamber with fifteen points. Furthermore, we are happy to offer six European-American Intensive Course Awards to emerging scientists to cover travel and lodging expenses for the conference.

The social program at the Second European-American Intensive Course in Clinical and Forensic Genetics will include a welcoming reception for all participants to be held in one of Dubrovnik's most attractive locations. A boat cruise along the Adriatic Coast to the island of Mljet (National Park) will also be included in the events. At least one concert will be organized in the remarkable facility of the Knezev Dvor (Duke's castle) or Sponza. A guided tour will also be arranged for participants to enjoy the amazing 8th - 16th century architecture that makes Dubrovnik so exceptional.

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

Sincerely yours,

Moses S Schanfield, Ph.D.
Dragan Primorac, M.D.,Ph.D.

CONFERENCE ORGANIZERS

Co-Chairman

Moses Schanfield, Monroe County Public Safety Laboratory, Rochester, NY, USA
Dragan Primorac, Split University Hospital, Split, Croatia

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Rebecca Reynolds, Roche Molecular Systems, Inc. USA

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Institutions

Croatian Academy of Medical Sciences, Croatia, **AmeriCares,** USA, **Catholic Medical Foundation,** USA, **Connecticut State Police,** USA, **Croatian Society of Human Genetics,** Croatia, **Foundation for Genetic Medicine, Inc.**, USA, **Institute Ruðer Boškoviæ,** Croatia, **International Center of Croatian Universities,** Croatia, **International Commission on Missing Persons**, USA, **Monroe County Public Safety Laboratory,** Rochester, USA, **University of Connecticut Health Center, Department of Genetics and Developmental Biology,** USA, **Split University Hospital,** Croatia, **University of Split,** Croatia, **University of Split, School of Medicine,** Croatia, **University of Zagreb,** Croatia, **University of Zagreb, School of Medicine,** Croatia

Official Conference Journal

Croatian Medical Journa**l**

Logo design

Boris Ljubièiæ

Web page design

Dalibor Marijanovi}

Photography

Ivo Pervan

Design and print

STUDIO HRG, Zagreb

Scientific Program Information

Cancellations and Refunds
Notice of cancellation had 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, 2001 75% refund
- after July 30, 2001 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.

Coffee Breaks
During the session breaks, refreshments (coffee, tea, water) will be served free of charge to participants wearing name badges. During the morning breaks, coffee will be served in the Foyers. The afternoon breaks take place in the Exhibition Halls.

Credits
The EAICFCG has been approved for 15 (participants) or 20 (lecturers) points by the Croatian Medical Association.

European-American Intensive Course Awards (EAICA)
The members of the scientific board will review all submitted abstracts and 6 selected authors will receive European-American Intensive Course Investigator Award. Recipients of the award will recieve a check of $ 500 as well as a special certificate.

Exhibition opening hours

Monday, September 3, 2001	12:30 p.m. - 02:30 p.m.
Tuesday, September 4, 2001	01:00 p.m. - 03:30 p.m.
Sunday, September 9, 2001	01:00 p.m. - 03:30 p.m.
Monday, September 10, 2001	01:00 p.m. - 03:30 p.m.

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

Poster Sessions
All posters are on display from Monday, September 3, 2001 to Thursday, September 6, 2001 and Sunday, September 9, 2001 to Wednesday, September 12, 2001.

Poster Mounting

Monday, September 3, 2001	07:30 a.m. - 11:30 a.m.
Sunday, September 9, 2001	07:30 a.m. - 11:30 a.m.

Poster Removal

Thursday, September 6, 2001 07:30 a.m. - 11:30 a.m.
Wednesday, September 12, 2001 07:30 a.m. - 11:30 a.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

Monday, September 3, 2001 12:30 p.m. - 02:30 p.m.
Tuesday, September 4, 2001 01:00 p.m. - 03:30 p.m.
Sunday, September 9, 2001 01:00 p.m. - 03:30 p.m.
Monday, September 10, 2001 01:00 p.m. - 03:30 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, September 2, 2001 02:00 p.m. - 08:00 p.m.
Monday, September 3, 2001 08:00 a.m. - 04:00 p.m.
Tuesday, September 4, 2001 08:00 a.m. - 09:00 a.m.
Wednesday, September 5, 2001 07:30 a.m. - 09:00 a.m.
Thursday, September 6, 2001 07:30 a.m. - 09:00 a.m.
Friday, September 7, 2001 02:00 p.m. - 08:00 p.m.
Saturday, September 8, 2001 08:00 a.m. - 04:00 p.m.
Sunday, September 9, 2001 07:30 a.m. - 08:00 p.m.
Monday, September 10, 2001 07:30 a.m. - 09:00 a.m.
Tuesday, September 11, 2001 07:30 a.m. - 09:00 a.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:

Lucia Cifuentes Ovalle, Chile
Katja Drobni}, Slovenia
Tomasz Kupiec, Poland

Frontiers in Molecular and Cellular Medicine:

Rima Dada, India
Anna Gareeva, Russia
Nguyen Hoai Giang, Vietnam

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 = 7.05 HRK* (July 25, 2001)
1 USD = 8.12 HRK* (July 25, 2001)

Opening hours of official Banks:
Banks and post offices are normally open from 7 (7.30) a.m. to 7 (8) p.m., Monday through Friday.

* Please note that these exchange rates are variable.

Cash Machines

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

Electricity Supply

220-240 V.

Insurance

In registering for the The Second European-American Intensive Course in Clinical and Forensic Genetics 2001 participants agree that neither the organising committee nor the conference office assume any liability whatsoever. Participants are requested to make their own arrangements in respect of health and travel.

Message Center

A Message Center is available at 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 Second European-American Intensive Course in Clinical and Forensic Genetics. 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!
www.croatiaairlanes.hr

Public Transportation in Dubrovnik
Dubrovnik offers an efficient system of public transportation with buses. Almost all hotels have easy access to the public transportation system.

Restaurants
Most restaurants in Dubrovnik are open from 08:00 a.m. - 12:00. a.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 Dubrovnik are usually open from Monday to Saturday, from 08:00 a.m. - 21:00 p.m. Some are open on Sundays. Most shops accept all major credit cards.

Smoking Policy
The Second European-American Intensive Course in Clinical and Forensic Genetics 2001 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 Dubrovnik city centre, in front of the hotel. The receptionists will be glad to help you.
Taxi station Pile, Brsalje, Tel: 424-343; *Taxi station Ploèe*, Frana Supila, Tel: 423 164; *Taxi station Gruž*, City harbour, Tel: 418 112; *Bus Station*, *Put Republike* 11, Tel: 357 044.

Hotel Information
Hotel 'Excelsior' is located near the Old City of Dubrovnik. First hotel 'Excelsior' was open on July 10th 1913. Back in those times it was called hotel 'Odak'. In the year of 1965 it was

redesigned and renamed in 'Excelsior' hotel. It was entirely renovated and again redesigned in the year of 1998. The same year hotel 'Excelsior' became a five star hotel.

Distances:

From the Old Town	300 m
From the sea	few meters
From the beach	has its own beach
From the airport	20 km/cca 20 min.
From the bus station	3 km
From the harbor	4 km

The Dubrovnik and Neretva County

The Dubrovnik and Neretva county stretches along a narrow belt along the coast and includes the islands, from the Neretva in the north to Prevlaka in the Croatian Saints Bay (Boka kotorska). The main region is comprised of what used to be the Dubrovnik Republic. The county centre is the best preserved town of museums with its numerous palaces, churches, fortress walls, ancient archives and museums in which are preserved memories of Dubrovnik's citizens which contributed to the world heritage: Ruðer Boškoviæ, Ivan Gunduliæ, Marin Drziæ and others. Dubrovnik was primarily a town and commune annexed to the region from Prevlaka to Ston (Pelješac) and became a Republic under the Libertas flag. The Republic had embassies all over the Mediterranean and even in far away countries. The largest island, Korèula, was the home to the medieval seafarer Marco Polo. The area around Metkoviæ is the site of a Roman settlement and is home to more than 259 bird species. Dubrovnik, Korèula, Cavtat, Ston, Mali Ston and Orebiæ are urban entities registered as monuments of culture. 37 monuments are under protection, the most famous among them being the Mljet National Park, the Neretva Delta and the Trsteno arboretum. The most important event is the Dubrovnik Festival, which was founded in 1950 and which has offered numerous cultural events during the summer months. Science develops at the Dubrovnik Inter-university centre, at the International Centre of Croatian Universities, Institutions and the HAZU Institutes. The American High School for Management and Technology has existed in Dubrovnik since 1997. Diplomatic activities exist in consulates in the UK, The Netherlands and Sweden. Today, the county, and especially its centre, is a famous tourist destination.

For additional information about Dubrovnik please check:
http://dubrovnik.laus.hr
www.dubrovnikportal.com

THE SECOND EUROPEAN-AMERICAN INTENSIVE COURSE IN CLINICAL AND FORENSIC GENETICS

Venue:
Hotel Excelsior
September 3 - 14, 2001
Dubrovnik, Croatia

Forensic Identity Testing
(September 3 - 8, 2001)

SCIENTIFIC PROGRAM

Sunday, September 2nd

02:00 p.m. - 08:00 p.m.	***Registration (Hotel Excelsior)***

Monday, September 3rd (day 1)

08:00 a.m. - 04:00 p.m.	***Registration (Hotel Excelsior)***
08:00 a.m. - 08:15 a.m.	***Opening remarks***
	DNA IN THE COURT ROOM (Chairpersons: Haskell Pitluck and Michael Baden)
08:15 a.m. - 08:45 a.m.	**An introduction to forensic DNA analysis** Moses Schanfield, USA
08:45 a.m. - 09:15 a.m.	**Admissibility of DNA evidence in USA** Haskell Pitluck, USA
09:15 a.m. - 09:45 a.m.	**DNA statistics in the courtroom** Frederick Bieber, USA
09:45 a.m. - 10:15 a.m.	**DNA advisory board update** Arthur Eisenberg, USA
10:15 a.m. - 10:45 a.m.	***Coffee break***
10:45 a.m. - 11:15 a.m.	**Population genetics in forensic medicine** Martin Tracey, USA
11:15 a.m. - 11:45 a.m.	**DNA expert testimony in the courtroom** Linda Kenney, USA
11:45 a.m. - 12:15 p.m.	**DNA and the forensic pathologist** Michael Baden, USA
12: 30 p.m. - 02:30 p.m.	***Poster viewing*** / ***Exhibition session*** *(Vine and cheese reception)*
	IDENTIFICATION (WORKSHOP) (Chairpersons: Mitchell Holland and Thomas Parsons)
02:30 p.m. - 03:00 p.m.	**DNA identification techniques - applications, benefits, pitfalls** Mitchell Holland, USA
03:00 p.m. - 03:30 p.m.	**DNA extraction challenges - identification of Korean war remains** Thomas Parsons, USA

03:30 p.m. - 04:00 p.m.	**High throughput STR analysis of skeletal remains in mass fatality incidents** Charity Holland, USA
04:00 p.m. - 04:30 p.m.	***Coffee break***
04:30 p.m. - 05:00 p.m.	**Developments in obtaining mtDNA sequence data** Thomas Parsons, USA
05:00 p.m. - 05:30 p.m.	**Establishing regional DNA identification capabilities for the identification of human remains** Edwin Huffine, USA
05:30 p.m. - 06:00 p.m.	**Regional DNA identification of skeletal remains using STR techniques** Dragan Primorac, Croatia
06:45 p.m.	***Sightseeing of Dubrovnik***

Tuesday, September 4th (day 2)

08:00 a.m. - 09:00 a.m.	***Registration (Hotel Excelsior)***
09:00 a.m. - 09:30 a.m.	***Opening ceremony***
09:30 a.m. - 10:00 a.m.	**Preservation and collection of DNA evidence** Henry Lee, USA
10:00 a.m. - 10:30 a.m.	**Past and future on DNA typing** Bruce Budowle, USA
10:30 a.m. - 11:00 a.m.	***Coffee break and Press conference***
	IDENTIFICATION OF HUMAN REMAINS BY USE OF NUCLEAR AND MITOCHONDRIAL DNA (Chairpersons: Mitchell Holland and Edwin Huffine)
11:00 a.m. - 11:30 a.m.	**MtDNA and nuclear DNA testing in identifying human remains** Edwin Huffine, USA
11:30 a.m. - 12:00 p.m.	**Mass fatality incidents - An international approach** Frank Ciaccio, Mitchell Holland, USA
12:00 p.m. - 12:30 p.m.	**Disaster mortuary operational response team - a multi-disciplinary approach to the identification of mass fatality victims** Paul Sledzik, Mitchell Holland, USA

12:30 p.m. - 01:00 p.m.	**DNA identification strategies in mass fatality incidents** Mitchell Holland, USA
01:00 p.m. - 03:30 p.m.	***Lunch break*** ***Poster viewing*** / ***Exhibition session***
	Y CHROMOSOME ANALYSIS (Chairpersons: Peter de Knijff and Mechthild Prinz)
03:30 p.m. - 04:00 p.m.	**Application of Y-chromosomal STR haplotypes to forensic genetics** Jurgen Henke, Germany
04:00 p.m. - 04:30 p.m.	**Experiences with using Y chromosome specific STRs in forensic casework** Mechthild Prinz, USA
04:30 p.m. - 05:00 p.m.	**The power of the combined use of chromosome Y microsatellites and unique mutation events** Peter de Knijff, The Netherlands
05:00 p.m. - 05: 15 p.m.	***Coffee break***
05:15 p.m. - 05: 45 p.m.	**Optimized primer and reaction design for Y-STR analysis in forensic casework** Walther Parson, Austria
05:45 p.m. - 06:15 p.m.	**Mutations at Y-chromosomal microsatellite loci** Carsten Hohoff, Germany
06:15 p.m. - 06:45 p.m.	**Development, validation and case work applications of Y-PLEX 6, a multiplexed Y-Chromosome STR genotyping system** Sudhir Sinha, USA
08:30 p.m. - 12:00 p.m.	***WELCOME RECEPTION*** ***(The Dubrovnik Arts Gallery)***

Wednesday, September 5th (day 3)

07:30 a.m. - 09:00 a.m.	***Registration (Hotel Excelsior)***
	INNOVATIONS IN DNA TYPING TECHNOLOGIES (Chairpersons: Rebecca Reynolds and Walther Parson)
08:00 a.m. - 8:30 a.m.	**Genetic analysis: A variety of tools for efficient genotyping** Dennis Reeder, USA

08:30 a.m. - 09:00 a.m.	**Application of AluQuant system** Benjamin Krenke, USA
09:00 a.m. - 09:30 a.m.	**Megaplex PCR amplification and probe hybridization for SNP-based assay** Rebecca Reynolds, USA
09:30 a.m. - 10:00 a.m.	**Immobilized sequence-specific oligonucleotide probe analysis of mtDNA hypervariabile regions I and II** Matthew Gabriel, USA
10:00 a.m. - 11:30 a.m.	***Coffee break***
10:30 a.m. - 11:00 a.m.	**Validation of PowerPlex 16 and Identifier in the USA** Bruce Budowle, USA
11:00 a.m. - 11:30 a.m.	**Integrated solutions for human identification** Nicola Oldroyd, UK
11:30 a.m. - 12:00 p.m.	**"Utility of mtDNA-SNPs in forensics: What can dark quenchers and minor groove binders do for you?"** Walther Parson, Austria
12:00 p.m. - 12:30 p.m.	**Increasing the power of discrimination of mtDNA testing by targetting SNPs throughout the mtGenome** Thomas Parsons, USA
12:30 p.m. - 01:00 p.m.	**Locus specific brackets in STR genotyping** Debang Liu, USA
01:00 p.m. - 02:30 p.m.	**Lunch break**
	PROMEGA'S IDENTIFICATION WORKSHOP (Chairperson: David Phelps)
02:30 p.m. - 03:00 p.m.	**Use of PowerPlex in complex cases** Cecelia Crouse, USA
03:00 p.m. - 03:30 p.m.	**Comparison of PowerPlex 16, Powerplex 1 and 2 and Profiler Plus/Cofiler for forensic use** Christine Tomsey, USA
03:30 p.m. - 04:00 p.m.	**Setting up a large database using STRs** Jeffrey Ban, USA
04:00 p.m. - 04:30 p.m.	**PowerPlex 16 for use in paternity** Arthur Eisenberg, USA
04:30 p.m. - 05:00 p.m.	**Validation of PowerPlex 16** Benjamin Krenke, USA

05:00 p.m. - 05:30 p.m.	***Coffee break***
	PARENTAGE MINISYMPOSIUM (Chairpersons: Arthur Eisenberg and Lotte Henke)
05:30 p.m. - 06:00 p.m.	**Setting up national DNA standards for QC/QA or operating a paternity laboratory** Arthur Eisenberg, USA
06:00 p.m. - 06:30 p.m.	**Contemporary parentage testing** Lotte Henke, Germany
06:30 p.m. - 07:00 p.m..	**Calculation of probability in paternity casework** Martin Tracey, USA
08:30 p.m.	***CONCERT: DUBROVNIK SIMPHONY ORCHESTRA (The Rector's Palace)***

Thursday, September 6th (day 4)

07:30 a.m. - 09:00 a.m.	***Registration (Hotel Excelsior)***
	STR's AND mtDNA ANALYSIS IN FORENSIC CASEWORK (Chairpersons: Peter Gill and Frederick Bieber)
08:00 a.m. - 08:30 a.m.	**Autosomal STR mutations** Bernd Brinkmann, Carsten Hohoff, Germany
08:30 a.m. - 09:00 a.m.	**How CODIS has been used to make some of the hits in Virginia?** Jeffrey Ban, USA
09:00 a.m. - 09:30 a.m.	**Using STRs in forensic casework** Cecelia Crouse, USA
09:30 a.m. - 10:00 a.m.	**Low copy number DNA profiling** Peter Gill, UK
10:00 a.m. - 10:30 a.m.	***Coffee break***
10:30 a.m. - 11:00 a.m.	**Interpretation of complex DNA mixtures** Carll Ladd, USA
11:00 a.m. - 11:30 a.m.	**Case work guidelines and complex mixture interpretation** Christine Tomsey, USA

11:30 a.m. - 12:00 p.m.	**Review of plant DNA typing** Heather Miller Coyle, USA
12:00 p.m. - 12:30 p.m.	**Developments in generation of a DNA typing system in the domestic cat** Marilyn Raymond, USA
12:30 p.m. - 02:30 p.m.	***Lunch break***
	STR's and mtDNA ANALYSIS IN FORENSIC CASEWORK (Chairpersons: Peter Gill and Jeffrey Ban)
02:30 p.m. - 03:00 p.m.	**Recent forensic DNA case work experiences** Theodore Anderson, USA
03:00 p.m. - 03:30 p.m.	**mtDNA testing in identifying human remains from the Bosnia and Herzegovina** Edwin Huffine, USA
03:30 p.m. - 04:00 p.m.	**Characterization of heteroplasmy in hairs and tissues** Casandra Calloway, USA
04:00 p.m. - 04:30 p.m.	**European STRs working group project: identification of skeletal remains by STRs** Antonio Alonso, Spain
04:30 p.m. - 05:00 p.m.	**Missing persons identification: The Spanish "Phoenix" program** Jose Lorente, Spain

Friday, September 7th (day 5)

EXCURSION TO ISLAND MLJET

Excursion includes:

-Bus transportation from Dubrovnik to Ston and from Ston to Trstenik with shellfish and vine degustation.

-Boat cruise from Trstenik to the island of Mljet with visit to National park and seafood lunch at the island.

THE SECOND EUROPEAN-AMERICAN INTENSIVE COURSE IN CLINICAL AND FORENSIC GENETICS

Venue:
Hotel Excelsior
September 3 - 14, 2001
Dubrovnik, Croatia

Frontiers in Molecular and Cellular Medicine
(September 8 - 14, 2001)

SCIENTIFIC PROGRAM

Friday, September 7th

02:00 p.m. - 08:00 p.m.	***Registration (Hotel Excelsior)***

Saturday, September 8th

08:00 a.m. - 04:00 p.m.	***Registration (Hotel Excelsior)***
	HOTEL ALBATROS - CAVTAT MINISYMPOSIUM ON OSTEOGENESIS IMPERFECTA (Preceded by satellite session for parents) (Chairpersons: David Rowe and Dragan Primorac)
02:00 p.m. - 02:30 p.m.	**Mutation detection in the patients with OI** Monica Mottes, Italy
02:30 p.m. - 03:00 p.m.	**Non-collagen forms of OI** Francis Glorieux, Canada
03:00 p.m. - 03:30 p.m.	**Orthopedics issues in treatment of OI** Darko Antièeviæ, Croatia, Boštjan Baebler, Slovenia
03:30 p.m. - 03:45 p.m.	***Coffee break***
03:45 p.m. - 04:15 p.m.	**Surgical treatment of osteogenesis imperfecta in children-our experience from 1975 to 1999** Boštjan Baebler, Oskar Županc, Nataša Berden, Slovenia
04:15 p.m. - 04:45 p.m.	**Bisphosphonates treatment of OI patients** Francis Glorieux, Canada
04:45 p.m. - 05:15 p.m.	**What have we learned from the murine models of OI ?** David Rowe, USA, Ivo Kalajzi}, Croatia
05:15 p.m. - 05:45 p.m.	***Questions and open discussion***
05:45 p.m. - 06:45 p.m.	***CROATIAN ANNUAL OSTEOGENESIS IMPERFECTA SOCIETY MEETING***

Sunday, September 9th (day 1)

07:30 a.m. - 08:00 p.m.	***Registration (Hotel Excelsior)***
08:00 a.m. - 08:15 a.m.	***Opening remarks***
	NEW MOLECULAR TECHNOLOGIES, HUMAN GENETIC HISTORY AND BIOETHICS (Chairpersons: Raimo Tanzi and Chris Tyler Smith)
08:30 a.m. - 09:00 a.m.	**Electrophoresis in DNA analysis** Dean Burgi, USA
09:00 a.m. - 09:30 a.m.	**SNP genotyping using megaplex PCR amplification and linear probe arrays** Rebecca Reynolds, USA
09:30 a.m. - 10:00 a.m.	**READIT™ SNP Genotiping System. A novel enzymatic approach for mutation detection.** Stephen Lupton, France
10:00 a.m. - 10:30 a.m.	**Flow cytometry technology applications in immunology and hematology** Frans Nauwelaers, Belgium
10:30 a.m. - 11:00 p.m.	***Coffee break***
	NEW MOLECULAR TECHNOLOGIES AND BIOETHICS (Chairpersons: Stephen Lupton and Erin Williams)
11:00 a.m. - 11:30 a.m.	**Applications of ABI PRISM technologies for human disease research and molecular diagnostic** Raimo Tanzi, Italy
11:30 a.m. - 12:00 p.m.	**Y-chromosomal markers and human genetic history** Chris Tyler Smith, UK
12:00 p.m. - 12:30 p.m.	**Informed consent in human subjects protocols** Erin Williams, USA
01:00 p.m. - 3:30 p.m.	***Poster viewing*** / ***Exhibition session*** *(Vine and cheese reception)*
06:00 p.m.	***Sightseeing of Dubrovnik***

Monday, September 10th (day 2)

07:30 a.m. - 09:00 a.m.	***Registration (Hotel Excelsior)***
09:00 a.m. - 09:30 a.m.	***Opening ceremony***
09:30 a.m. - 10:00 a.m.	**Genetic basis of autoimmune diseases** Henry Erlich, USA
10:00 a.m. - 10:30 a.m.	**The biology and pathology of twinning in man** Frederick Bieber, USA
10:30 a.m. - 11:00 a.m.	***Coffee break and Press Conference***
	PLENARY SESSION (Chairpersons: Petros Tsipouras and Frederick Bieber)
11:00 a.m. - 11:30 a.m.	**Genetic (in)stability in evolution and disease** Miroslav Radman, France
11:30 a.m. - 12:00 p.m.	**Towards a novel molecular classification of human cancer: From gene expression patterns to antibody based diagnostics** Douglas Ross, USA
12:00 p.m. - 12:30 p.m.	**Molecular diagnostics and prognostic markers in childhood ALL** Andrea Biondi, Italy
12:30 p.m. - 01:00 p.m.	**The new microarray-based approaches to the molecular diagnosis of cancer** Marc Ladanyi, USA
01:00 p.m. - 03:30 p.m.	***Lunch break***
	Poster viewing / ***Exhibition session***
	MOLECULAR DIAGNOSTICS (Chairpersons: Marck Ladanyi and Pier Franco Pignatti)
03:30 p.m. - 04:00 p.m.	**Fluorescence in situ hybridization in modern medical genetics practice** Frederick Bieber, USA
04:00 p.m. - 04:30 p.m.	**Predictive genetic testing - the new possibilities in risk determination in complex diseases** Pier Franco Pignatti, Italy

04:30 p.m. - 05:00 p.m.	**Functional genomics of human chromosome 21 and Down's syndrome** Dean Nižetiæ, UK
08:30 p.m. - 11:30 p.m.	***WELCOME RECEPTION (The Dubrovnik Arts Gallery)***

Tuesday, September 11th (day 3)

07:30 a.m. - 09:00 a.m.	***Registration (Hotel Excelsior)***
	GENE THERAPY (Chairpersons: Marc Ladanyi and Branimir Šikiæ)
08:00 a.m. - 08:30 a.m.	**Oligonucleotide-based gene correction strategies: applications to neuromuscular and cardiovascular disease** George Dickson, UK
08:30 a.m. - 09:00 a.m.	**Hammerhead ribozymes as therapeutic agents in an animal model of Osteogenesis Imprefecta** Petros Tsipouras, USA
09:00 a.m. - 09:30 a.m.	**An integrated strategy for somatic gene therapy for Osteogenesis Imperfecta** David Rowe, USA
09:30 a.m. - 10:00 a.m.	**Retroviral vector design for gene therapy** Alexander Lichtler, USA
10:00 a.m. - 10:30 a.m.	***Coffee break***
10:30 a.m. - 11:00 a.m.	**Genetic modification of human T cells with CD20: a novel suicide gene strategy** Andrea Biondi, Italy
11:00 a.m. - 11:30 a.m.	**Liver cell transplantation - A novel animal model for human hepatic viral infections** Catherine Wu, USA
11:30 a.m. - 12:00 p.m.	**Bone morphogenic proteins in clinical therapy** Slobodan Vukièeviæ, Fran Borove~ki, Croatia
12:00 p.m. - 02:00 p.m.	***Lunch break***

GENE THERAPY: CANCER AND HEPATITIS
(Chairpersons: George Dickson and David Rowe)

02:00 p.m. - 02:30 p.m.	**Antisense oligonucleotide therapies for cancer** Branimir Šikiæ, USA
02:30 p.m. - 03:00 p.m.	**Chromosomal translocations as diagnostic and therapeutic targets in sarcomas** Marc Ladanyi, USA
03:00 p.m. - 03:30 p.m.	**Chemical genetics: An approach to the discovery of new anticancer agents** Antonio Bedalov, USA
03:30 p.m. - 03:45 p.m.	***Coffee break***
03:45 p.m. - 04:15 p.m.	**Targeted polynucleotides for inhibition of hepatitis B and C viruses** George Wu, USA
04:15 p.m. - 04:45 p.m.	**Genes of IGF family and cancer** Krešimir Paveliæ, Croatia
04:45 p.m. - 05:15 p.m.	**Molecular interaction between bone and bone marrow** Ana Marušiæ, Vedran Katavi}, Croatia
08:30 p.m.	***CONCERT: DUBROVNIK SIMPHONY ORCHESTRA*** *(The Rector's Palace)*

Wednesday, September 12th (day 4)

STEM CELL AND PROGENITOR CELL ENGINEERING AND CLINICAL APPLICATION
(Chairpersons: Stanimir Vuk-Pavloviæ and Madhusudan Peshwa)

09:00 a.m. - 09:30 a.m.	**Embryonic stem cells and their possible use, therapeutic cloning, reprogramming of transferred genome** Davor Solter, Germany
09:30 a.m. - 10:00 a.m.	**Biology of dendritic-cell based immunotherapy of cancer** Allan Dietz, USA
10:00 a.m. - 10:30 a.m.	**Mesenchymal stem cells** Alan Smith, USA

10:30 a.m. - 11:00 p.m.	**Following osteoprogenitor lineage with green flourescent protein** David Rowe, USA
11:00 p.m. - 01:30 p.m.	***Lunch break***
	STEM CELL AND PROGENITOR CELL ENGINEERING AND CLINICAL APPLICATION (Chairpersons: Davor Solter and Alan Smith)
01:30 p.m. - 02:00 p.m.	**Cell processing and transplant engineering for human use** Madhusudan Peshwa, USA
02:00 p.m. - 02:30 p.m.	**Interaction of immune cells in neuronal damage repair** Michal Schwartz, Israel
02:30 p.m. - 02:45 p.m.	***Coffee break***
02:45 p.m. - 03:15 p.m.	**Clinical aspects and advances in haploidentical hematopoietic stem cell transplants** Massimo Martelli, Italy
03:15 p.m. - 03:45 p.m.	**Clinical problems and results of dendritic-cell based immunotherapy of cancer** Stanimir Vuk-Pavloviæ, USA
03:45 p.m. - 04:15 p.m.	**Clinical scale magnetic cell separation for stem cell transplantation and cellular immunotherapy** Volker Huppert, Germany

Thursday, September 13th (day 5)

EXCURSION TO ISLAND MLJET

Excursion includes:

-Bus transportation from Dubrovnik to Ston and from Ston to Trstenik with shellfish and vine degustation.

-Boat cruise from Trstenik to the island of Mljet with visit to National park and seafood lunch at the island.

ABSTRACTS

Forensic Identity Testing

Presentation Number: F1

POPULATION DATA OF Y-CHROMOSOMAL STRs IN THE LITHUANIAN POPULATION

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The human Y chromosome is uniparentally inherited and nonrecombining along most of its length. Short tandem repeat (STR) polymorphisms from the male specific part of the Y chromosome have been already recognised to be highly valuable in human evolutionary studies and population genetics. They can also be used in forensic analysis and paternity testing. Present-day Lithuanians (together with Latvians) represent the Baltic branch of the populations speaking languages of the Proto-Indo-European descent. Although Lithuanian population was formed under the pressure of various migration forces, its deep roots preserved the genetic composition of the forebears. Therefore, it is reasonable to analyse genetic differences among the Lithuanian ethnolinguistic groups as well as between Lithuanians and other European populations. We present results of the investigation of genetic structure of Lithuanian population by using highly variable Y chromosomal microsatellite markers. We examined the allele and haplotype frequencies of the Y chromosome-specific short tandem repeat systems (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS385) in 57 males from two main Lithuanian ethnolinguistic groups: Aukstaiciai (A) and Zemaiciai (Z). PCR products were detected using capillary electrophoresis and GenScan Software on the ABI PRISM 310 Genetic Analyzer (ABI/PE). In total 44 different haplotypes were identified as a result of combining the 32 alleles of the 7 Y-linked systems containing 9 loci. 35 haplotypes were seen only once, five - twice, four - three times. The most frequent allele of DYS19 was 15 (52.6%), DYS389-I - 13 (71.9%), DYS-389-II - 30 (57.9%), DYS390 - 25 (49.1%), DYS391 - 11 (54.4%), DYS392 - 11 (59.5%), DYS393 - 14 (36.9%), DYS385 - 11/14 (43.9%). Our results were compared with the data of other European populations.

Presentation number: F2

EXTRACTION AND TYPING OF STR DNA ANALYSIS FROM PAINTED WOODEN SUBSTRATE

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DNA analysis of the Forensic evidences includes extraction of DNA from stains deposited on various substrates which could impart color to the DNA solution. Presence of the color could make DNA Profiling difficult or prove to be inhibitory altogether. Inhibition of the activity of the restriction endonuclease HaeIII by denim dyes is one such example. That study made use of thiopropyl Sepharose 6B to remove co-extracted sulfide dyes. We are reporting thiopropyl Sepharose 6B beads to remove an inhibitor from a wooden painted surface carrying a finger print. DNA was successfully extracted and typed for Profiler plus loci when thiopropyl Sepharose 6B beads treatment was done. We were not able to type untreated DNA.

Presentation Number: F3

ANCIENT DNA OLD AS CITY OF SPLIT

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Ancient DNA is an important tool for diverse disciplines, such as anthropology and archeology. Individual archeological site has been found occasionally, at the northern part of Split city, outside of the Diocletians palace. Extended male skeleton, age 30-40, lying on the back was found in the pit together with ceramics from later Roman period. On the right hand he had a bracelet, made of copper, with open ends. The expected maximum stature based on the length of the long bone was 167 cm (Trotter, Gleser). Estimated time since death was approximately 1760±80 years (by 14 C method). DNA was succesfully extracted by the AFDIL modified method, based on standard proteinase K-phenol extraction, followed by purification with microconcentrators (Alonso A. et al. DNA Typing from Skeletal Remains: Evaluation of Multiplex and Megaplex STR Systems on DNA Isolated from Bone and Teeth Samples. Croatian Medical Journal 2001;42:260-266). We simultaneously amplified nine human short tandem repeats (STR) systems and the amelogenin locus, employing AmpFlSTR Profiler. According to our experience, the high quality of extracted DNA as well as an extremely successful DNA amplification could be due to characteristics found in Split area (clay ground).

Presentation Number: F4

ALLELE AND GENOTYPE FREQUENCIES OF 3 STR LOCI IN TURKISH POPULATION

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Many short tandem repeats (STR) are widely used in the field of forensic medicine. Population surveys of every locus is necessary before introducing it into routine use. Because of this, allele and genotype frequencies of 3 STR loci were carried out on the samples of unrelated Turkish individuals from all geographic areas of Turkey. The STR polymorphisms were analyzed by PCR using, GenePrint STR Systems kit (Promega) according to the recommendations of the manufacturer. HumTHO1, HumTPOX, CSF1PO loci were carried out. No deviations from Hardy-Weinberg equilibrium were found for all three loci examined. The results showed no significant differences between the Turkish and other Caucasian population data. Present study gives the data of highest number of individuals studied for these loci published on Turkish population (n=481 for HumTHO1; n=392 for HumTPOX; n=223 for CSF1PO). Heterozygosity rate (H) and power of discrimination (PD) are as follows: HumTPOX, H: 0.66, PD: 0.81; CSF1PO, H: 0.71, PD:0.88; HumTHO1, H: 0.76, PD: 0.84. These frequency data can be used for DNA based forensic analyses in the Turkish population.

Presentation Number: F5

EXAMINATION OF THE GENETIC DISTANCE BETWEEN A MIXED HUNGARIAN AND TWO ISOLATED SZÉKELY POPULATION BY Y-CHROMOSOME STR SYSTEMS

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Six Y-chromosome (DYS19, DYS389/I, DYS389/II, DYS390, DYS392, DYS393) short tandem repeat (STR) polymorph systems were determined in a mixed Hungarian and two isolated Székely populations. Székely are known as a Hungarian people but the origin of this population is still unknown. We aimed to determine the genetic distance between the Hungarian and the two Székely populations. The bloodstain samples were extracted by Chelex methodology. After the amplification the allele types for the DYS389/I, DYS389/II and DYS392 STR systems were determined with ABI 310 Genetic Analyzer and with horizontal gel electrophoresis and silver staining. Based on these results we suggest that the Székely and Hungarians are not close relatives as it was supposed earlier.

Presentation number: F6

HUMAN IDENTIFICATION BY MULTIPLEX SNP DETECTION

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The validation of multiplex single-nucleotide extension reaction of human genomic DNA for the purpose of human identification and forensic casework is presented. Analyzing the genetic variation caused by single-nucleotide polymorphism is a powerful technique for its discrimination reducing cost, efforts and turnaround time. The distribution of nine known polymorphisms of the 1112 bp PCR product of genomic DNA in a population of healthy Korean was determined. To perform multiplex genotyping, we have designed various length of sequence-specific oligonucleotides for the single-nucleotide extension reaction, and the result was analyzed with ABI 310 Genetic Analyzer. The frequencies of these nine SNP sites were also determined, each using 100 DNA samples extracted from the cord blood. We suggest that by adding more SNP sites, this would be an effective screening test in forensic analysis.

Presentation number: F7

DYS19 MICROSATELLITE IN THE CHILEAN POPULATION

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The current Chilean population was sprung from the admixture between aborigine populations of mongoloid origin (Amerindians) and Spanish conquerors of Caucasian origin. The Spanish conquerors who arrived in the Chilean territory in the early years of the colonization were mainly males. Aborigine males died in the fight; so the unions that gave origin to the Chilean population were mainly between Spanish males and aborigine females, not the opposite. Thus, as result of this asymmetric mating structure it follows that the Y chromosome of the Chilean population must be mainly from Spanish origin, while the other chromosomes must be from mixed—Caucasian and aborigine origin. To prove this hypothesis we studied samples of male blood donors (N = 115) from Santiago, the capital of the country. All samples were analyzed for the ABO and Rh (locus D) blood groups (autosomal genetic markers) and the DYS19 microsatellite (located in the Y chromosome). The aboriginal admixture was estimated for each genetic marker. The percentage of aboriginal admixture was 38.17 % for the ABO system and 31,28 % for the Rh system. Gene frequencies for the DYS19 locus demonstrate that the main alleles are the 14 (B) (as it is often the case with many Caucasian populations) and the 13 (A) allele. The aboriginal admixture for the DYS19 locus was estimated in 21.56 %, which is significantly lower than the values found using autosomal genetic markers. This fact supports the historical background of the population studied. Our study has demonstrated the population genetics consequences of the asymmetric pattern of genome admixture between both ancestral genomes (Caucasian and Amerindian) in the Chilean population.

Presentation number: F8

PRESUMPTIVE CASEWORK AND THE USEFULNESS OF A PRIORI KNOWLEDGE FROM ANTE MORTEM DATA

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The mortal remains recovered from within the former Yugoslavia as a result of conflicts in the 1990's can be categorized as presumptive or non-presumptive cases. Presumptive remains are those in which circumstantial evidence or a priori knowledge of the conditions associated with the loss develops into a presumption of the identity of the individual. A lack, or relative paucity of such information defines non-presumptive cases. Presumptive cases occur in two instances. The first is where documents or other personal possessions are discovered on recovered remains that can be used to make a preliminary identification. Related to this is visual recognition from family members of clothing, jewelry or other personal items. For these cases a bone sample is taken and DNA testing performed to ensure that the presumptive identification is correct. The second instance of presumptive identification occurs when local governmental authorities exhume an individual who had been buried by a relative. In these cases the a priori knowledge is so strong that DNA testing may often not be necessary. However, the ICMP requests that a bone sample still be taken and archived for future reference should a discrepancy arise. It is anticipated that DNA testing will become increasingly common in aiding in the identification of recovered remains. The expectation is that a majority of recovered remains will be non-presumptive and therefore DNA testing will play a key role in many of these cases. In previous mass disasters such as plane and train crashes, ante mortem data has proven useful in the identification of victims. However, the situation in the former Yugoslavia is compounded due to the magnitude of the incidents, the nature of the losses, and the time since the incidents occurred. To date the ICMP has submitted 250 bone samples for DNA testing from presumptive cases and have been notified about the results on 126 of these cases. Seventy-six (60%) cases were matches, forty-six (37%) were not matches (exclusions), 3 produced no DNA profiles and 1 failed to produce consistent results. We are still waiting results on 124 cases. The accuracy rate of presumptive identifications is too low to warrant negating DNA testing in these cases. Nevertheless the a priori knowledge of ante mortem data from presumptive cases does often provide a strong lead in the identification process. For this reason ante mortem data will continue to be part of ICMP's identification method.

Presentation number: F9

ALTAI AND SAYAN REGION AS THE SOURCE AREA FOR ANCESTRAL PALEOINDIANS – AN EVIDENCE FROM MITOCHONDRIAL DNA ANALYSIS

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For Native Americans, extensive RFLP and HVSI sequence analysis has identified four major founding mtDNA haplogroups (A-D), which account together for ~ 97% of modern Native American mtDNAs. It has been shown recently that haplogroup X represents an additional minor founding lineage that is restricted in distribution to northern Amerindian groups. This haplogroup had been found also at low frequencies ~ 4% in Western Eurasian populations, leading to speculation that it originated in West Asia and arrived in the New World by the eastward migration of an ancestral Caucasian population, of which no trace has so far been found in the mtDNA gene pool of modern Siberian/eastern Asian populations. To extend the survey of Asian mtDNAs for the presence of haplogroup X, we used RFLP and sequencing analysis to screen the mtDNAs of a total of 790 Siberians belonging to 10 different populations for the key markers that define this lineage. We have identified haplogroup X mtDNAs only in 7 of 202 Altaians. All Altaian X mtDNAs harbored the consensus haplogroup X motif: -1715 DdeI, +14465 AccI, +16517HaeIII, 16189C, 16223T, 16278T, 73G, 153G, 195C, 263G and differed from each other by length polymorphism mutations at nucleotide positions 16193, 309 and 315. One of these X mtDNAs also harbored a 215G variant which has not been observed in either Native American or European X haplotypes. Median network analysis indicated that Altaian X haplotypes occupy the intermediate position between European and Native American X mtDNA lineages. With this new evidence for haplogroup X being present in Siberia, and in a region where considerable frequencies of the other four founding mtDNA lineages (A-D) in Native Americans occur, it is now possible to think of the Altai and Sayan region as representing the source area for ancestral Paleoindians.

Presentation number: F10

IDENTIFICATION OF FEMALE CELLS IN PENILE SWAB FROM SUSPECT USING MULTIPLEX STR PROFILING

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The aim of this work was to shown the use of STR multiplexes as routine procedure in the purpose of identification of biological stains derived from sexual offences. Although, at times, relatively clean stains are available, but it's far more common for these stains to be composed of a mixture of different cells and physiological fluids. According to this, a differential extraction method has been developed for separated isolation of sperm DNA from other cells DNA presented in the mixed stains. This method is based upon differences in packaging of DNA in the sperm head and in the other cells. But in different body swabs taken from individuals to try to establish which acts took place and links persons together a mixture of same type of the cells coming from different persons is presented (penile or saliva swabs). For above mentioned physical evidence the differential extraction method could not be applied. Results of DNA analyses from these samples shown mixed DNA profiles. In order to establish the identity of individual components in the mixtures, the STR multiplexes are proposed to be used. Some commercial kits are available to allow the simultaneous coamplification of large numbers of highly polymorphic loci, what make them very available for evaluation of these DNA mixtures. This abstract demonstrates a rape case, where no semen, hair or other biological evidence were left by the perpetuator at the crime scene or on the victim. The suspect was arrested soon after committed a crime and a penile swab was taken from him. It's known that cells shed from a female during sexual intercourse can be retrieved from penis of a man over a 1-24 hour postcoital period. DNA from penile swab was extracted by organic method according standard procedure. DNA purification was processed with Centricon™ microconcentrators. DNA was quantified by slot-blotting using Human DNA quantitation kit. DNA analysis was performed using Powerplex 16 System and SGM plus. The likelihood ratio approach was used for representing the strength of the evidence. The interpretation of mixture was enhanced by taking into consideration the area of the peaks. This approach involves the need to be considered on possible artefacts such as stutters or pull-up. The presence of greater than two alleles at some of the loci using Geneprint Powerplex 16 kit obvious indicated on a mixed DNA sample obtained from the suspect's penile swab. According sex identification amelogenin marker it was determined that one component in the mixture was deposited from the female. At a single locus named D21S11 even three alleles were observed which could not belonged to the suspect. This observation was confirmed using another STR multiplex system named SGM Plus system. Comparing these alleles with the victim's STR profile it was evident that she has got "triallelism" at this locus. Identification of the victim DNA was conclusive for all the rest 14 loci when compared to the victim reference DNA. After some investigation it was found out that the victim has Down sindrom what's in concordance with DNA analysis. The presented example indicated a great of usefulness of multiplex PCR technique for identification of forensic mix evidences especially when amount of DNA is low. For this kind of samples the STR multiplex with more polymorphic loci are recommended what makes the interpretation of mixture easier.

Presentation number: F11

POPULATION GENETIC ANALYSIS IN HUNGARIAN POPULATIONS USING THE POWERPLEX™16 SYSTEM

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With the advent of STR profiling it has become possible for forensic scientists to rapidly gain a reliable impression of the scale of population genetic effects caused by several factors such as inbreeding and substructuring. Previous studies have shown that population analysis in Hungarian populations can be of great importance from the viewpoint of the examination of population differentiation. This study provides additional population genetic data of the Hungarian population on the thirteen CODIS core STR loci and the two penta STRs (PentaD, PentaE). Allele frequency and profile databases were generated for four population samples, which had been collected from 223 individuals living in the Budapest area (Central Hungary), from 206 Romanies living in Baranya county (southwestern Hungary), from 116 Romanies living in Hajdú-Bihar and Szabolcs-Szatmár-Bereg counties (eastern Hungary) and from 178 Ashkenazim living in Budapest. In the study the Budapest area sample was used as a reference group for the mixed character of the Hungarian population, because the sample was collected in a blood bank irrespective of ethnic background. Amplification was performed using the *GenePrint*® PowerPlex™ 16 System (Promega) and the ABI Prism™ 310/377 instruments (Applied Biosystems). At the locus FGA one type of intermediate sized alleles were detected in three samples of the eastern Hungarian Romany population. According to sequence results the presence of an additional T in the 5' flanking region leads to an intermediate allele 24.1. The presence of the variant allele in three independent samples with the little evidence for association of alleles between the STR systems indicates possible sampling error or inbreeding in this population. Comparing the allele frequency values by G-statistic, calculating the F_{ST} indices and with the pairwise comparisons of interpopulation molecular variance (AMOVA), the four Hungarian populations could be distinguished using data of fifteen STR loci. The results suggest that the population structure may have an effect on the interpretation of forensic DNA evidence in Hungary.

Presentation number: F12

A ONE STEP SOLUTION FOR IDENTIFICATION OF THE SKELETAL REMAINS

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Various forensic techniques have been applied in our laboratory in an effort to improve identification process of the victims found in several mass graves in Croatia and Bosnia and Herzegovina. In the majority of analyzed bones and teeth samples we observed the presence of minimal amount (pg) of degraded human DNA mixed with high amounts of microbial DNA. Analysis of hundred different samples may be time-consuming processes. Here we described a use of the new IQ DNA isolation system (Promega, USA), which is short and efficient method. Following the DNA isolation method all samples have been analyzed by AluQuant System and amplified with either Power Plex 16 System or AmpFISTR Profiler^{TM} PCR Amplification Kit. Typing of PCR products was performed on ABI Prism 310 Genetic Analyzer.

Presentation number: F13

CROATIAN MITOCHONDRIAL DNA DATABASE

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Mitochondrial DNA (mtDNA) is maternally inherited, shows a rapid rate of evolution and is present in high copy number in each human cell. Those characteristics make analysis of mtDNA an important tool in forensic genetics and population studies. Also, mtDNA analysis has proved to be efficient on samples when genomic DNA is either highly degraded and/or present in a limited amount (e.g. old and badly damaged human remains). After the year 1995 when the war in Croatia was over a lot of war victims' remains were found and needed positive identification. Therefore, in 1997 our laboratory implemented mtDNA sequencing as an alternative to nuclear DNA analysis in cases when genomic DNA analysis was unsuccessful or there were no close relatives available for DNA typing. The aim of this study was to establish mtDNA database for a Croatian population to make possible the interpretation of results from mtDNA sequence analysis in identification cases and casework in Croatia. The polymorphisms of the two hipervariable segments (HV1 and HV2) within the mitochondrial control region were analyzed in a population of 111 unrelated individuals from Croatian ancestry. PCR amplification was carried out in a Perkin Elmer 9600 thermal cycler using already published primer sequences. The consensus nucleotide sequences were obtained by direct cycle sequencing of both DNA strands with the Dye Terminator method and subsequent alignment of the two sequences. Electrophoresis and sequence analysis were performed on 377 automated sequencer (Applied Biosystems). Polymorphisms were compared to previously reported databases of mtDNA sequences. These data provide a basis for the biostatistical evaluation of mtDNA sequencing results and database calculation of base variation and sequence pattern can be carried out.

Presentation number: F14

THE DISTRIBUTION OF D1S80 (PMCT118) AND D17S5 (YNZ22) ALLELES IN A VIETNAMESE POPULATION

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For the first time, Vietnamese population in the North was studied for the D1S80 (pMCT118) locus at a large amount. Blood, bloodstain, hair and saliva samples of 217 individuals were tested by PCR technique. Those samples were extracted by Chelex, then using directly as DNA templates for PCR with the specific primers of D1S80 locus. After amplification, PCR products were separated by 6% polyacrylamide gel electrophoresis (PAGE) and stained by silver staining. From 217 samples amplified on D1S80 locus: 57 different genotypes and 24 alleles were observed. Alleles 15, 35, 37, 40 and 42 had not been found yet. Allele 24 (14,3%) and allele 22 (14,1%) were the most common alleles, whereas alleles 14, 33, 34, 36 and 39 were the less popular ones (0,2%) in Vietnamese population. The D1S80 locus demonstrated a heterozygosity of 80,18%. All mother/child pairs (42 cases) shared at least one D1S80 allele. Similar method was applied for D17S5 locus. The study shows that 12 alleles were observed and 2 ones (B12 and B13) were not found yet in Vietnamese population. B8 was the most spread allele (14,7%). Heterozygosity of D17S5 locus is 83,76%. Those data show that D1S80 and D17S5 loci are highly polymorphic in Vietnamese population and can be used as the important markers for forensics, medical analyses and paternity testing.

Presentation number: F15

NINE STR LOCI MIGHT NOT ALWAYS BE ENOUGH FOR RELIABLE IDENTIFICATION BY REVERSE PATERNITY DETERMINATION

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Since February 2001 the process of DNA identification of war victims in Croatia is supported by the database of nearly 3,000 nine-loci (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820) STR genotypes of relatives of missing persons. Instead of targeted approach to DNA typing, genotype of each analyzed skeletal remain is being compared to all genotypes in the database to identify potential parents and children. Although this approach significantly increased the pace of identification by DNA typing, non-targeted matching of genotypes in a database containing several thousand genotypes is associated with a significant risk of false-positive matches, especially in a situation when identifications are based on reverse paternity determination. Here we are reporting three examples where we observed nine STR loci match between child and spouse of a missing person and skeletal remains that did not originate from that missing person. For two of the three skeletal remains the proposed identity was excluded by analysis of further STR loci (D8S1179, D21S11, and D18S51), while in the third case non-DNA evidence was used. Apparently in a database of relatives containing approximately 3,000 genotypes, nine STR loci covered by AmpFlSTR Profiler kit might not always be sufficient for correct identification through reverse paternity determination. If parents of missing person are not available and the identification is based on the determination of parenthood, multiple children should be analysed whenever possible. In addition, other evidence based on information about time, place and other conditions of disappearance, as well as anthropological and other “classical” forensic data should always be put together and compared before any final conclusion is being made. Introduction of novel procedures that cover more STR loci will definitively decrease the risk of miss-identification, but in situations where large databases of nine-loci genotypes already exist this might not be a simple task.

Presentation number: F16

APPLICATION OF NON-TARGETED STR PROFILING FOR IDENTIFICATION OF VICTIMS FROM A MASS GRAVE IN ÆELIJE

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Five years after the process of DNA identification started in Croatia, a third laboratory dedicated to the identification of war victims was founded at the University of Osijek School of Medicine. After the initial process of education and validation, in spring 2000 DNA laboratory Osijek started to actively participate in the coordinated process of identification of missing persons. At that time, due to limited laboratory capacities, DNA profiling was being performed only on specific "targeted" cases where potential identity was suggested by some other methods. One of the first tasks of DNA laboratory Osijek was analysis of fourteen bodies exhumed from a mass grave in Æelije. Since there were no indications of potential identity, we decided to abandon the established procedure of "targeted" DNA analysis and started the process of compiling a database of all potential relatives. As of September 2000 there were 125 missing persons in the Osijek-Baranja County. We determined 265 individual genotypes of relatives that "covered" approximately 90% of all missing persons. To enable automatic comparison of individual genotypes of unidentified bodies with all relatives in the database we developed and used specific algorithm in Microsoft Excel. From 14 exhumed bodies we managed to obtain full (9 loci) STR genotypes for all but one body. By comparing genotypes of unidentified bodies with genotypes of all potential relatives in the database we managed to determine identity of eight bodies. All eight identities were subsequently confirmed by other forensic evidence and accepted by corresponding families. For the remaining five bodies we were able to show that they do not match any of the missing persons whose relatives provided blood samples for analysis. Identification of victims from a mass grave in Æelije was a first demonstration that DNA profiling can be successfully used for identification of war victims in Croatia without prior determination of potential identities by other methods. This principle is now generally accepted and the central database of all relatives of missing persons coordinated by the Office of Missing and Detained Persons has been formed. The database now contains over 2,500 individual genotypes and has significantly spurred the process of identification of missing persons in Croatia.

Presentation number: F17

MOLECULAR BIOLOGICAL TYPING OF HUMAN REMAINS OF THE AUGUSTINERKIRCHE IN CONSTANCE, GERMANY

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In the course of excavations carried out in the vicinity of the Augustine church in Constance, Germany, a number of human remains were uncovered which can be dated back to the late Middle Ages. A group of four individuals were discovered near the west portal lying strikingly close together. One of the individuals was found to have suffered fight-related injuries, which the person might have sustained during the Appenzell wars (1401-1429). From an archaeological perspective, it is worthwhile finding out whether these people, possibly members of the nobility, are related to each other. The human remains showed different degrees of preservation. A number of the graves were located below the ground water level; in these cases the bones were rather brittle and revealed a brownish colour. In graves that were located above the water table, the skeletal parts were tinted gray and appeared significantly more solid. Several samples were taken from the four individuals found together and also from ten additional individuals so that the results could be compared. After sample preparation which included cleansing, sanding down of the bone surface, UV irradiation, phenol chlorophorm extraction, the amplification was carried out using single (amelogenin, Y-chromosomal STR, mtDNA) and multiplex approaches (Profiler-Plus, MPX). Three to six independent typings were carried out for each individual. The typing of bones which were found above the water table could be reproduced with certainty; occasional allelic dropouts were still observed, but irregular alleles could be observed only in very few cases. Only in a few cases it was possible to type the brownish bones which were exposed to humidity, greatest success being achieved when the amelogenin system was used. The molecular biological diagnosis of gender corresponded in all cases with that obtained by anthropological means. The phalanges, in particular, proved to be a promising substrate. They are usually preserved in toto, can easily be stored and prepared. They are also easier to grind than parts of the femur or humerus.

Presentation number: F18

THE ISOLATION OF LOW NUMBERS OF SPERM FROM CELL MIXTURES USING FLOW CYTOMETRY (FACS): A NEW TOOL FOR THE INVESTIGATION OF RAPE

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The Forensic Science Service (FSS) investigates a large number of rape and other sex-related cases each year. Despite the presence of spermatozoa in the majority of these cases, successful DNA typing of the perpetrator is, on occasion, hampered by the presence of a large number of cells from the victim (mainly epithelial and white blood cells). The method currently employed by the forensic community to separate spermatozoa from female cells prior to DNA typing involves an enzymatic degradation of the female cells to which the spermatozoa are resilient. The 'preferential lysis' method is ineffective however in cases involving very low numbers of spermatozoa, particularly when mixed with a large number of female cells. Despite ultra-sensitive PCR techniques that allow amplification of single sperm, the profile of the rapist is frequently obscured by that of the victim. The aim of this study was to develop a more sensitive method of separating spermatozoa from vaginal cells. A fluorescence-activated cell sorter (flow cytometer or FACS) was used to analise mixtures of sperm and vaginal cells. Our studies have shown that flow cytometry offers a significant improvement over preferential lysis for the analysis of rape cases involving low numbers of sperm. Flow cytometry also provides a highly sensitive and accurate method of recovering a pure sperm population from samples containing single or low numbers of sperm from mixtures of sperm and other material. The use of a sperm-specific antibody in the process also increases the usefulness of results produced, by strengthening the evidence that the DNA profile obtained has originated from the sperm as opposed to another cell type. Flow cytometry therefore represents an opportunity to dramatically increase the effectiveness of DNA typing for a large number of rape and sexual assault cases committed in the UK each year, and therefore a major contribution to forensic science as a whole.

Presentation number: F19 (oral presentation)

INTERNATIONAL COMMMISSION ON MISSING PERSONS: STRUCTURE AND FUNCTION OF THE DNA PROGRAM

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The DNA Program of the International Commission on Missing Persons (ICMP) is charged with implementing cutting edge forensic DNA technology to aid in identifications of the missing from the conflicts that occurred in the former Yugoslavia during the 1990s. The DNA Program is the most recent addition to the various ICMP programs and is part of an integral network of scientific, political and family orientated functions designed to help bring answers to the families of the missing. The DNA Program is structured with a Program Director who is charged with overall Program development and management. In addition, a Deputy Program Director guides the development of DNA laboratories in the region and a Director of the Family Outreach Centers is charged with general oversight of blood collection efforts. Country Directors oversee in-country DNA related activities in Bosnia and Herzegovina (BiH) and the Federal Republic of Yugoslavia (FRY) including blood collection from family members, bone collection from recovered remains, DNA testing, and reporting of DNA testing results. Within BiH three DNA testing laboratories were renovated, supplied and staffed by the ICMP. These staffs are comprised of local hires who were selected and trained by the ICMP. These DNA laboratories are located in Tuzla, Sarajevo, and Banja Luka. The Tuzla facility is responsible for processing most of the blood samples collected from families of the missing, and will employ state-of-the-art robotics. The Sarajevo DNA laboratory processes most of the bone samples collected in BiH and is the largest of the ICMP DNA laboratories. The Banja Luka lab processes both bone and blood samples associated with presumptive cases. Blood collection centers are located in Tuzla, Sarajevo, Banja Luka, Sanski Most and Mostar. On average, these centers collect over 100 blood samples per working day. Within the Republic of Croatia (ROC) three DNA testing laboratories, located in Zagreb, Osijek, and Split, are involved with DNA cases related to missing person cases. All three of these labs are part of a National program and are supported by the Croatian government. The ICMP has supplemented these laboratories with donations of 2 ABI DNA 377 Sequencers, 3 ABI 9700 Thermocylcers, and a limited quantity of DNA kits. The ICMP will hire and fund a contractor to renovate the DNA laboratory in Zagreb. In addition, the ICMP is currently negotiating a contract with the Croatian government whereby the ICMP will provide further financial and material assistance the Croatian DNA effort. Within the FRY, the ICMP sponsored DNA laboratory in Belgrade has been established. This DNA laboratory is able to process both bone and blood samples. In addition, the ICMP operates a series of blood collection centers in the FRY. The ICMP has been charged with the responsibility of DNA testing of all samples from Kosovo. Toward this end, the ICMP has opened two blood collection centers in Kosovo. The samples from Kosovo are being sent out of country for testing. The overall goal of the ICMP is to leave these fully functional and self-sustaining DNA laboratories in place so that additional recovered remains can be identified. Also, in the future, these laboratories will be able to assist local authorities in criminal investigations.

Presentation number: F20

DIFFERENTIAL EXTRACTION OF DNA FROM MIXTURES OF BODY FLUIDS USING MORE STRINGENT CONDITIONS

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A modification to the DNA differential extraction method is proposed which can be applied to separate DNA of vaginal or saliva cells from DNA of spermatozoa. In mixtures with a very high vaginal or saliva cells content from swabs, especially when swabs were taken 1-2 days postmortem, or mixtures of post coital samples from fabrics, without suspects, it is very important to get a male's DNA profile suitable for the DNA data bank searching. Swabs or fabric cuttings were extracted in water for 6 hours at room temperature or overnight at 4^0 C on shaking platform. A part of the stain extract was stained ("Christmas Tree") and the density of sperm heads was quantified a microscopically into 3 groups: 2+ - 4+ with cells: sperm heads ratio 5:1 and 10:1. Lysis buffer and proteinase K were added into a tube with cuttings and pellet and incubated for 60, 90 and 120 min at 56^0 C. Supernatant (cell fraction) was removed, pellet with cuttings washed with PBS, centrifuged, PBS discard, lysis buffer and proteinase K were added like for the first step lysis together with DTT and incubated 120 min at 56 ^{0}C. After the first step lysis a part of the pellets were stained ("Christmas Tree") and after a 90 or 120 min incubation period we could see sperm heads only. Cell fractions as well as sperm DNA fractions were quantified ("QuantiBlot") and multiplex PCR was carried out using AmpFISTR SGMplus kit according to manufacturer's recommendations. Amplified fragments were resolved by capillary electrophoresis using an ABI 310 automated sequencer. Genotype assignment was done by comparison with allelic ladders and allele designation following the DNA recommendations. Pure male's DNA profile suitable for data bank searching was achieved if the first lysis period was conducted for 90 min at 56 ^{0}C (cells: sperm heads ratio 5:1) or 120 min at 56^0 C (cells: sperm heads ratio 10:1).

Presentation number: F21

DNA-IDENTIFICATION OF HUMAN REMAINS RETRIEVED FROM MASS GRAVES IN THE FORMER YUGOSLAVIA

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The main objective of the forensic investigation of mass graves is the positive identification of the human remains that have been exhumed. We performed DNA-analysis on human remains that have been retrieved from a mass grave in the former Yugoslavia. Several alternative DNA-extraction methods have been evaluated to increase the sensitivity of the multiplex PCR-based typing systems. In this study we have also evaluated the efficiency and validity of the SGM plus and Profiler DNA amplification kits using an amplification regime of 34 cycles. It was shown that Low Copy Number (LCN) DNA samples from extracts of bone and teeth that failed to demonstrate typing results using the standard protocol of 28 cycles showed complete DNA-profiles after 34 cycle amplification. We obtained full DNA-profiles from all the remains that were assigned to our laboratory. Furthermore, the development of LCN PCR technology has been of crucial value to the high number of positive identifications that were completed during the course of this study. Comparison the genotypes of the remains to the genotypes of the reference samples from the putative relatives 16 positive identifications were accomplished during the course of this study. For samples that demonstrated a putative kinship, we calculated the likelihood ratio's (LR) to estimate the significance of the presumed biological relationship. The contents of our forensic testimony to the authorities will be discussed. This study has been performed by the NFI under the authority of the International Criminal Tribunal for the former Yugoslavia (ICTY).

Presentation number: F22

USE OF DIFFERENT TYPES OF DNA TESTING IN THE IDENTIFICATION PROCESS

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During the 1990's, more than 250,000 persons died during the breakup of the former Yugoslavia. Today, it is estimated that up to 40,000 persons may still be missing in the countries that comprised the former Yugoslavia. As bodies are recovered, the identification process begins. Due to the nature and number of the losses in Bosnia and Herzegovina (BiH), The International Commission on Missing Persons (ICMP) is developing an advanced DNA testing system within BiH to assist in the identification process. In attempting to use DNA technology to assist in the identification of thousands of recovered sets of mortal remains, it is impossible to establish a uniform, consistent constellation of donors. For some cases it is possible to collect reference samples from the parents of the missing. However, while that would be the most favored scenario, it is often not possible. Therefore, combinations of available donors need to be sought in order to a high match probability. Two major types of DNA are used in creation of databases: mitochondrial and nuclear. Nuclear DNA, involving the amplification of short tandem repeats (STRs) regions located on the introns, or non-coding parts of chromosomes, can be further divided in two subcategories: autosomal STRs and sex-chromosome (in this case Y chromosome) STRs. A primary advantage of using mitochondrial DNA is that it is present in cells in high copy number and is thus more likely to be present in degraded specimens. However, given that it is maternally inherited, and thus shared among many individuals, its usefulness may be somewhat reduced in instances where a significant percentage of the population of a village or town is missing. Autosomal STR typing is far more individually discriminating than mitochondrial DNA. However, since nuclear DNA is present as only one set of chromosomes per cell, it will often degrade past the point of detection long before the accompanying mitochondrial DNA does. In addition, while there may be a large pool of potential mitochondrial donors, some of whom may be many generations removed, it is by far more beneficial if STR donors are close family relatives. Since the Y chromosome is paternally inherited, it will show direct linkage among paternal, male relatives. The drawbacks to this type of testing are somewhat similar to those encountered with mitochondrial testing as far as multiple individuals sharing the same Y chromosome DNA profile. However, by combining classic forensic techniques with DNA testing, this particular problem can usually be overcome. The biggest shortcoming with Y chromosome testing is that it is only applicable to males. Combinations of autosomal STR systems, Y chromosome, and mitochondrial DNA testing can often be used to draw a result from various combinations of donors, even if they may initially seem relatively unconnected. For example, a DNA assisted identification of a person may be possible when the available donors are as diverse as a paternal grandfather and a maternal cousin. Although both Y chromosome and mitochondrial DNA profiles will each be consistent among multiple individuals, the combination of these two tests is very powerful.

Presentation number: F23

Y - CHROMOSOME STR POPULATION STUDY ON SIX LOCI FOR THE TERRITORY OF BOSNIA AND HERZEGOVINA

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Forensic testing laboratories use a variety of different DNA tools and makers to help determine paternity, identify mortal remains, and link suspects to body tissues/fluids found at crime scenes. A number of organizations are working to record, uncover and identify the 40,000 or so missing persons from the recent conflicts in the former Yugoslavia. In many cases families are missing many of their male relatives, including sons, fathers and grandfathers. Due to the lack of suitable donors, in many of these cases nuclear and mitochondrial DNA may not be a completely satisfactory option for identification purposes. In some of these cases, it is expected that Y-chromosome data will prove useful in linking a father to his son, then linking the son to his living maternal relative (mother, grandmother, sibling etc…). Y-chromosome analysis is a relatively new strategy to help clarify DNA testing and is expected to play a significant role in identifying missing individuals from the conflicts within the former Yugoslavia during the 1990's. Data acquired through analysis of the STR's of the Y chromosome is a potentially strong tool for use in determining paternity. In order to properly assess the strength of using Y-chromosome STR data we undertook a population study of 200 unrelated males of different ages. The loci analyzed were DYS 393, DYS 19, DYS 389II, DYS 390, DYS 391, and DYS 385. The STR analysis was performed on the Applied Biosystems 310 genetic analyzer. Data presented here summarize the allele frequency in 200 unrelated males from the former Yugoslavia at the before mentioned loci. The information obtained is an indication of the Y-chromosome diversity within the male population of the former Yugoslavia, and forms an initial database that can be used for calculation of paternity indices.

Presentation number: F24

DETERMINATION OF ABO GENOTYPES OF SPERM STAINS AS TOOL FOR THE EXAMINATION OF RAPE CASES

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We have tested the usefulness of ABO genotyping of sperm stains as the primary tool for the selection of unknown suspects of rape cases for the further identification based on STR analysis. For ABO genotyping the method described by M.Yamada and others (Int.J.Leg.Med.1994 (106): 285-284) for formalin-fixed, paraffin-embedded tissues was modified for use with DNA extracted from sperm cells according to P.S.Walsh and others (BioTechniques 1991 (10) 4:506-513). Briefly, the regions that include nucleotide 258 (a single-base deletion site of the O allele) and nucleotide 700 (a single-base substitution in the A and B alleles) of cDNA at the ABO locus were amplified in two separate reactions. The products of the first reaction were digested with KpnI to distinguish between A/B and O, the products of the second reaction were digested with AluI to determine the presence of allele B and A/O in the examined sample. The enzyme digests were separated on 10% polyacrylamide gels. The suspects having the same ABO genotype as DNA from sperm cells were genotyped using Gene Print Fluorescent STR Systems CTTv, Gamma and FFFL (Promega). The amplification products were analyzed by capillary electrophoresis using fluorescent detection on ABI PRISM 310 (Applied Biosystems). We presented three rape cases, in which this approach has been successfully applied to narrow the spectrum of possible suspects and to speed the process of search for an unknown assailant. In all examined cases, the ABO genotype of the victim has been determined and compared with the genotype, which has been obtained from sperm cells. Simultaneously with ABO genotyping, the DNA samples isolated from sperm cells were STR genotyped to exclude the possibility that the sample contain mixture of DNA from victim and suspect. The suspects selected using this method were identified by autosomal STR analysis. The ABO genotyping allows determination of six genotypes (AA, AO, BB, BO, AB and O) in comparison with serological methods, which can recognize only phenotypes in antigen secreting persons. It has been shown that the practical application of this simple method can simplify the search for unknown suspect for following STR based examination.

Presentation number: F25

IDENTIFICATION OF REMAINS OF SOLDIERS KILLED DURING BATTLE OPERATIONS ON THE TERRITORY OF CHECHEN REPUBLIC FOR PERIOD 1994-2001 YEARS

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The identification of Federal troops' soldiers who were killed during armed conflict in the territory of Chechen Republic is a national problem and the best available solution for it is engaging a modern molecular-biological methods. With support of the administration of the President of Russian Federation in 1998 the DNA Identification Laboratory was formed. This DNA Laboratory solves problems of molecular-genetic identification of the killed person in conditions of mass reception of unidentified bodies. The majority of victims were successfully identified by traditional forensic anthropological and odontological methods. However a number of cases could be identified only by using molecular - genetic methods. For DNA-identification purposes, Applied Biosystems commercial kits were used in our Lab. This DNA-kits are based on the polymerase chain reaction. This DNA-typing systems include the following: HLA DQA1, PolyMarker (PM – LDLR, GYPA, HBGG, D7S8, GC), D1S80, a short-tandem-repeat (Profiler Plus - D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, Amelogenin) and mitochondrial DNA sequencing. With the use of the AmpFlSTR Profiler Plus system we have detected length polymorphisms of repeated DNA sequences. Using mitochondrial DNA sequencing we have observed sequence polymorphisms within two hypervariable regions – HV1 and HV2. Since 1998 we have processed 298 casework samples and more than five hundred reference samples. For 196 samples positive identification was confirmed.

Presentation number: F26

THE MITOCHONDRIAL DNA CONTROL REGION POLYMORPHISM IN THE POLISH POPULATION

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Sequence analysis of the mitochondrial DNA (mtDNA) control region has been applied in forensic science as a method of identifying human biological traces. The use of mtDNA for identification purposes is based on the presence of highly variable regions. Routinely in forensic practice sequence analysis of so-called HV1 and HV2 region is used. Additional polymorphic region HV3, located in the Control Region (CR) of mtDNA molecule has been described recently. The statistical interpretation of results obtained by the sequencing of hypervariable regions depends on the frequency, of the defined type of sequence, in database of mtDNA haplotypes. Therefore it's essential for identification purposes to have a database containing haplotype of mtDNA of a given population. The aim of presented work was to establish a database containing over 150 sequences of HV1 HV2 and HV3 regions, obtained for unrelated people living in southern Poland. Results were compared with data from other populations. The position of polymorhpic sites and frequency of the most common haplotypes do not differ significantly from that in other compared European populations. Additionally a validation study on HV3 region application to the forensic genetics was performed and usefulness of this region to biological trace analysis was shown.

Presentation number: F27

EUROPEAN DNA PROFILING GROUP (EDNAP) HETEROPLASMY STUDY

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This paper describes a collaborative study of heteroplasmy in mitochondrial DNA (mtDNA) undertaken by the European DNA Profiling Group (EDNAP). Heteroplasmy is the co-existence of two or more different mtDNA molecules within a given cell and a greater understanding of heteroplasmy will aid the interpretation of mtDNA results. The objectives of this study were to assess the distribution of heteroplasmy among hairs from a single heteroplasmic individual and to determine whether consistent mtDNA sequencing results are achievable between different laboratories. An individual who displays heteroplasmy in reference samples (blood and buccal cells) was chosen. Fifty-five hairs from this individual were each cut into 2cm sections. These sections were distributed among nine laboratories throughout Europe and one in America. The root sections were retained by the coordinating laboratory. The results of the study have shown that uniformity of sequencing is achievable when different methodologies are used. When heteroplasmy is present in a reference sample it is not always seen in a hair sample and vice versa. At base 16234, where C/T heteroplasmy was observed in the reference samples, nineteen hairs displayed homoplasmy in all sections that were sequenced, nine hairs displayed varying degrees of heteroplasmy in all sections that were sequenced and twenty-seven hairs displayed heteroplasmy in some sections and homoplasmy in other sections. Differences from the reference samples were seen in ten hairs at the poly C regions; at position 16093 thirteen hairs displayed varying degrees of heteroplasmy and in one hair, a complete base change. One hair showed a complete base change at position 16129.

Presentation number: F28 (oral presentation)

LOCUS SPECIFIC BRACKETS IN STR GENOTYPING

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A new type of DNA internal lane standards, locus specific brackets (LSB), have been developed for STR genotyping. LSB are created from their target STR loci by recombinant DNA technology. They have identical flanking region sequences, but fewer or more repeat units than any common allele or known allele of their locus of origin. LSB are applied as internal lane standards in electrophoresis without overlap with true alleles. Because LSB have the same flanking region sequences they are amplified with the same primers used for amplifying true alleles and labeled with the same dye. Therefore, LSB applied as internal lane standards free up the dye employed by conventional heterogeneous internal lane standards for other purposes. A multiplex PCR amplification system, which can amplify all CODIS 13 STR loci and amelogenin in a single PCR and analytical software have also been developed, based on LSB. All together, LSB, the multiplex PCR system, and the analytical software make DNA testing for human identification accurate, simple, efficient, and user friendly.

Presentation number: F29

POPULATION DATA ON THE NINE SHORT TANDEM REPEAT (STR) LOCI IN ROMANIAN PEOPLE, USED IN FORENSIC GENETIC IDENTIFICATION BY DNA TYPING

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The basic function of all forensic testing, whether parentage testing or identification, is to exclude the maximum number of individual possible. This need some statistic calculations that include the population frequencies of alleles determined for forensic identification. We present genotype profile data in Romanian population for the 9 core STR loci: CSF1PO, TH01, TPOX, F13A01, FESFPS, vWA, D16S539, D7S820, and D13S317. Three GenePrintSTR Multiplex Systems provided from Promega (USA) were used. Detection of amplified products after separation by polyacrylamide gel electrophoresis was performed by silver staining method. DNA source was in all of the cases from whole blood samples. Extraction of DNA was performed with Promega's Wizard Genomic DNA Purification Kit. The population studied performed by us was from all of the regions of Romania, randomly sampled. The total number of volunteers from which we obtained blood samples was 190. Successfully processed probes were 160 for loci amplified with CTT Triplex, and Silver STR III Triplex, respectively 184 loci amplified with FFv Triplex. Distribution of samples around Romania that were typed, and allele frequencies for the nine loci amplified, are presented in figures and tables. As a comparison, frequencies show close similarity to those for European populations presented by others.

Presentation number: F30

IMPORTANCE OF CANINE IDENTIFICATION IN THE HUNGARIAN FORENSIC PRACTICE

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The relationship between man and dog is an old, long strong connection with its roots lost in the millenniums. Their common history is more than 700.000 years old. The dog's role was always multiple and of high value, however, this relationship underwent several significant modifications. Nowdays, the number of dogs kept as pets without specific duty (as hunters, guards etc) is increasing and keeps increasing. Cynophilia is a mark of culture, dog breeding as well. The dog is in countless cases a member of the family. It was never in the history so closed to its great friend, man. From the other point of view, the dog's psychology remained the same. The dog accepts the members of the family as members of its pack, and recognizes only one as dominant leader. So, the dog's place in the rank order depends exclusively on the owners. In case of improper raising, the dog becomes the leader of the pack-family and behaving as such one with often tragical results as severe wounding or even lethal attacks, which may engage the forensic practice. Hungary has an old long cynophilic and cynologic tradition, eight recognized (FCI) national breeds and a significantly high canine population, and a well cynologically developed country, however, during the last years several attacks to man were observed. These attacks were in many cases lethal and even had as a victim member of the family. In some cases the human remains on the dog body (e.g. blood) or in the dog's stomach (e.g. bone, tissue) can prove the animal as perpetrator. Lack of these evidences the availability of certain canine specific polymorphic STR loci is a significant tool for identification of canine individuals by their remaining (e.g. hair or saliva) in the victim's clothes or the environment of the attack, resolving of such cases. Ten canine specific STR loci (StockMark Kit Canine I Ver.3, Applied Biosystems, Foster City, CA) were analyzed by fluorescently labeled multiplex PCR using ABI PRISM 310 Genetic Analyzer. The automated data collection was performed and compared applying fluorescent ladder CXR 60-400 (Promega Corp., Madison, Wis.) GS-500 ROX and GS 400HD ROX (Applied Biosystems, Foster City, CA) as internal size standard. The forensic practice requires the availability of sequenized allelic ladders – which are constructed - and data concerning the frequency of the alleles in the local canine population (population studies are examined continuously in mixed- and purebred populations). The results are presented in different cases.

Presentation number: F31

THE SYSTEM OF 9 STR LOCI FOR PATERNITY IN RUSSIA

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The paternity analysis as well as identification of human remains is one of major tasks of forensic genetics. To simplify and cheapen the paternity analysis in Russia we have developed the system of nine STR, 6 of them are dinucleotide (D18S57, D9S290, D1S2667, D3S3694, D8S1769, D11S4127) and other are tetranucleotide (TH01, F13B1, PAH STR). We have performed PCR of each fragment alone and simultaneous electrophoresis of two fragments in polyacrilamide gel stained by ethidium bromide. We have analysed the paternity in more then 600 families from Russia. The alleles distribution in all markers except D3S3694 are according to published in Nature 1996, v.380-14 March in general. In D3S3694 one of rare alleles (with frequency 0.02 in European) encounters with frequency 0.14 in Russian. About 25% of all families had unrelated fathers. (This status was established in the case of alleles incoincidence in three markers as a minimum). The most informative markers for detection of unfiliation are D18S57 and D1S2667 (in 70% cases). The detection of unfiliation for other markers were 65% of cases (for D9S290 and TH01), 64% of cases (for D3S3694), 62% of cases (for D11S4127), 57 and 51% of cases (for D8S1769 and PAH STR corresponding). The least percentage revealing of unfiliation has F13B1 (45%). Also we have detected two mutations in markers F13B1 and PAH STR during the transmitting from parents to child. The systems of STR markers are usefull for detection of paternity and dinucleotide markers are preferable for analysis. Each population has its own alleles frequences for different markers and it should be considered in paternity analysis.

Presentation number: F32

A DNA TEST FOR THE IDENTIFICATION OF TIGER BONE

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The survival of the tiger (*Panthera tigris*) is seriously threatened by poaching to provide raw materials for Traditional Chinese Medicines (TCMs). Most highly prized are the tiger's bones, which are used in combination with other animal and plant derivatives in pills and plasters for the treatment of rheumatism and other ailments. Hundreds of patent remedies have been produced which claim to contain tiger bone, but proof of its presence is needed if legislation prohibiting the trade in endangered species is to be enforced. A highly sensitive tiger-specific real-time PCR assay has been developed to address this problem. The mitochondrial cytochrome b sequence of tigers and other members of the cat family were compared to identify potential primer sites in which the 3' region was unique to tigers. Species-specific amplification of a 165bp region has been reliably achieved from blood, hair and bone as well as from a range of TCMs spiked with 0.5% tiger bone. Purified DNA is firstly amplified on the Roche LightCycler™ and then heated from 60 to 90^{0}C in the presence of SYBR Green, a dye that fluoresces when bound to double stranded DNA. Presence of the tiger cytochrome b amplicon is indicated by a sudden change in fluoresence at 82^{0}C as the product melts, otherwise the peak change in fluoresence occurs at lower temperatures when primer dimers dissociate. Although capable of detecting an 82^{0}C peak produced by the amplification of fewer than 10 substrate molecules, the seven varieties of TCM pills and plasters tested showed no detectable trace of tiger DNA before spiking. Furthermore, sequencing several "tiger bone" fragments seized from TCM shops has shown that they actually originated from cattle and pigs. The potential effects of traditional bone preparation methods, evidence that much lower concentrations are used than alleged on TCM packaging, and substitution of bones from other species all suggest a low likelihood of detecting tiger DNA in patent medicines. Despite this, the basic methods have been thoroughly proven and can be readily applied to derivatives from other CITES protected species, providing a rapid highly sensitive forensic test for species of origin.

Presentation number: F33

DNA TYPING FOR THE IDENTIFICATION OF ANIMALS DNA

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While DNA typing of human samples is performed routinely in many forensic laboratories, handling of animal traces is not that established. Meanwhile DNA marker systems are available, which allow the identification of animal individuals in different species. Several cases will be presented, where animals, who killed or injured people or caused accidents could be identified by DNA typing. In contrast to human marker systems the allelic frequencies are not known to that extent. Therefore, we have to use up to 20 markers to prove the identity between traces and individuals.

Presentation number: F34

DNA ARCHIVING ON FTAR PAPER: PHOTOSENSITIZER INITIATED ATTACKS AS MODELS OF AGING

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Long term aging of dry DNA is thought to be due to the attack of diverse cascades of reactive species with probably, no one single initiator of the cascades explaining all circumstances. Photosensitizer-initiated reactions from hematoporphyrin and riboflavin were used to generate two model systems of reactive species around dry DNA in order to understand such systems and how to block them. Damage was assessed using plasmid DNA as a substrate with an in-situ microgel electrophoretic technique. The DNA damage profiles from the photosensitizer-initiated reactions on dry media are markedly different from that in aqueous systems, so water tension is an important factor in these pathways. Photodynamic hematoporphyrin was oxygen dependent but not that of riboflavin. This indicates that indirect type II pathways, probably via singlet oxygen were more important for hematoporphyrin than riboflavin. The application of liquid paraffin wax, in an attempt to reduce oxygen exposure in hematoporphyrin-initiated attacks did not retard photodynamic damage, but a nitrogen atmosphere does. In both the absence and presence of oxygen, the DNA protection offered by tris-urate (the anti-free radical component of the FTAR matrix) and tris-caffeine (to a certain extent) indicated that most DNA attack was via electrophilic species. Overall, protection of dry archived DNA from spontaneously reactive species such as free radicals appears to be a real issue and, as expected, the predominant species in air appear to involve oxygen but not exclusively or necessarily so and in at least one, probably atypical circumstance, oxygen was protective. This has implications for long-term storage of DNA and for an understanding of the pathways of reactive species attack on DNA.

Presentation number: F35

MULTIPLEX PCR OF STR LOCI AS AN HIGLY EFFICIENT TOOL OF TESTING BLOOD STAIN ARCHIVED FOR A DECADE – THE PATERNITY CASE REPORT

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By performing analysis of DNA polymorphisms (amplified STR and VNTR loci, ASO based systems), there were more than 1 500 cases of disputed paternity conducted at our department since 1993. Resolved among these were also special fatherless cases without direct testing of alleged father (AF) and those, where only archived samples from death AFs were available for analysis. All reports concerning these cases were summarized as inclusion of AF where the W value highly exceeded 99.8% limit or as exclusion confirmed by two DNA polymorphisms at least. Presented herein is the case where the blood sample of AF who died in 1991 was available for testing. It concerned roughly 600 mm^2 large blood spot made in 1989 for clinical reasons and archived without special preservation at the hospital. DNA from the blood spot was extracted by phenol-chloroform extraction, from the samples of mother (M) and child (C) by salting-out method (Miller et al, 1988). Allelotyping was performed by multiplex fluorescent PCR of STR loci using Promega kits PowerPlex 16, PowerPlex 2.1, Gamma STR, and FFFL and AmpFlSTR SGM Plus (PE Biosystems). Thus, a majority of STRs were tested repeatedly. Amplifications were followed by fragmentation analysis in the Genetic Analyzer ABI PRISM 310 using software GeneScan. Due to degraded DNA in eleven year old sample, no readable result was obtained in four out of twenty one tested loci - F13AO1, FES/FPS, F13B, and Penta D. However, allelotyping of the trio M-C-AF in seventeen STR loci (THO1, TPOX, CSF1PO, vWA, D5S818, D13S317, D7S820, D16S539, D3S1358, D8S1179, D18S51, D21S11, LPL, FGA, Penta E, D2S133, D19S433) has provided more than sufficient evidence to resolve this case in favor of true paternity of death AF. The values of indices estimated on the basis of allele frequencies of Czech population were as follows: P_e = 99.9999%, PI (L) = 51 096 478, and W = 99.9999% under assumption of 50 % prior probability.

Presentation number: F36 (oral presentation)

DEVELOPMENT, VALIDATION AND CASE WORK APPLICATIONS OF Y-PLEX™6, A MULTIPLEXED Y-CHROMOSOME STR GENOTYPING SYSTEM

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The presentation will describe the design, development and validation of the Y-PLEX™6 STR kit. This kit is a multiplex system that analyzes six tetranucleotide STR loci providing information for seven alleles. The Y-STR markers included in the Y-PLEX™6 kit are DYS393, DYS19, DYS389II, DYS390, DYS391, and DYS385. An allelic ladder has also been developed that allows for accurate and reproducible allele designations. The Y-STR loci are detected via fluorescent dye labeling which makes it suitable for use of either the Applied Biosystems 310 Genetic Analyzer or 377 DNA Sequencer. Analysis and identification of Y-chromosome based genetic marker has a limited but highly useful role in DNA based human identification. A number of Y-chromosome specific STR markers are now available to the academic and forensic community. The availability of these markers provides an opportunity to be able to type male DNA samples. Specifically, in a crime setting with mixed DNA samples from multiple males, it is now possible to discriminate between the suspect and the evidence samples on the basis of the haplotypes that can be generated using the Y-specific markers. A series of experiments have been performed in order to assess the validity of this system as per DAB guidelines for use in forensic casework analysis. The standard deviation for the migration of all alleles in the allelic ladder was less than 0.1 base pair. Different combinations of male and female DNA mixtures were analyzed. The presence of female DNA did not interfere with the identification of male DNA up to a ratio of 1:2 male to female, DNA from twenty-four animal species was amplified using the Y-PLEX™6 STR kit; none of the DNA sample resulted in amplified product. Blood and semen samples were spotted on a 100% cotton cloth exposed to soil, liquid soap, gasoline, 10% bleach, and *E. coli*. The DNA samples exposed to soil and liquid soap did not amplify. The sensitivity studies suggest that the male DNA quantity between 1-2ng give good amplification, with RFU's more that 200 for all alleles. A haplotype databases of Caucasians, African American and Native American populations will be presented.

Presentation number: F37

TYPING OF CELLS RECOVERED FROM HEARING AID FOR DEOXYRIBONUCLEIC ACID (DNA) FOR THE STR USING PROFILER PLUS/KIT

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In Forensic Laboratories, a wide range of evidence is received such as bedding, clothings, under garments, shoes, hair, etc. A hearing aid was received as a piece of evidences in a case which was recovered from the suspect. A procedure is described here to remove the cells from Wax deposited on the hearing aid successfully. The Extracted DNA was quantitated using the Quanti blot. The DNA was subjected to Profiler Plus and Cofiler loci. All the 13 loci were successfully typed and linked to the one of the victims.

Presentation number: F38

CASEWORK EXPERIENCE: IDENTIFICATION OF HUMAN SKELETON REMAINS BY STR-MULTIPLEX ANALYSIS

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The aim of this study is to evaluate the efficiency of the two multiplex PCR kits: AmpFlSTR Profiler and AmpFlSTR SGM Plus in forensic casework. The SGM Plus kit is composed of Amelogenin and ten STR markers: D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01 and FGA. The Profiler kit is composed of Amelogenin and nine STR markers: D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820. DNA amplification was performed according to the manufacturer's protocol. The amplified samples were analysed on an ABI Prism 310 Genetic analyser. The bio statistical evaluation was performed by DNA-view (C. Brenner, USA). The DNA was extracted using proteinase K and SDS, purification with phenol-isoamylalcohol-chloroform and washed and concentrated using Centrex-30 micro concentrator devices and finally, in all cases purified using Qiaquick columns (Qiagen). A critical point when working with small amounts of DNA is the possibility of contamination with exogenous DNA. To avoid contamination very strict technical precautions were taken during all steps of the process. In about 40 % of our analysed bone samples there were one or more drop off markers. Usually, there is a good correlation between dropping off percent and length of the molecule but that is not always the case. In one forensic case nuclear STR markers could be analysed from 30-35 years old skeleton remains. In conclusion, STR multiplex analysis using AmpFlSTR SGM Plus and AmpFlSTR Profiler is sufficient to identify old human remains.

Presentation number: F39

GENETIC ANALYSIS OF THE POLYMORPHISM ON 8 SHORT TANDEM REPEAT (STR) LOCI IN THE CROATIAN POPULATION

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The aim of this study was to obtain further DNA data on the Croatian population but also to establish a STR system for forensic and clinical purposes. Loci included in an ideal system should be highly informative and polymorphic, amplified under same conditions during PCR reaction and run under same conditions of electrophoresis. A study investigating 8 STR loci (TH01, VWA31, FES/FPS, F13A01, D1S1656, D12S391, D18S535, and D22S683) was performed on a representative sample of the Croatian population (citizens of the Croatia's capital Zagreb). Genomic DNA was extracted from peripheral blood of 164 healthy, unrelated individuals using salting out method. STR loci were amplified using PCR reaction with locus specific primers and two different PCR conditions (1. TH01, VWA31, FES/FPS, and F13A01 loci; 2. D1S1656, D12S391, D18S535, and D22S683 loci). Samples were loaded on 6% poliacrilamide gel, followed by electrophoresis and detection in automated laser fluorescence sequencer (ALFexpres, Pharmacia). The determination of allele sizes was performed using Fragment Manager software (Pharmacia). Allele and genotype frequencies, expected and actual heterozigosity, power of exclusion, matching probability and polymorphism information content (PIC) were calculated for each locus. Based on the PIC value and the number of alleles, loci could be divided in two groups. First group included loci with a lower PIC value and less than 15 observed alleles (TH01: PIC=0.8135; VWA31: PIC=0.8099; FES/FPS: PIC=0.7435; F13A01: PIC=0.8099 and D18S535: PIC=0.78309). The second group contained loci with high PIC value and more than 19 observed alleles (D1S1656: PIC=0.8972; D12S391: PIC=0.9085 and D22S683: PIC=0.9082). All loci confirmed with Hardy-Wienberg equilibrium. Total power of exclusion was calculated for each combination of loci. In conclusion, our results showed that the highest power of exclusion was obtained for combination D1S1656-D12S391-D22S683, therefore these loci can be considered as good markers for paternity testing as well as for follow up chimerism after allogenic bone marrow transplantation.

Presentation number: F40

EGYPTIAN DATA BASE FOR Y-CHROMOSOME POLYMORPHISM

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One hundred thirty five samples were collected from Tanta area males in Egypt. The DNA was quantitated using Quanti blot and 2-5 ng of the DNA used to amplify b Y-chromosome markers using Relia gene Kit. The Data was tested for the Hardy Weinberg equilibrium. The Y- Chromosome loci tested for this study are DYS 19, DYS 385, DYS 389, DYS 390, DYS 391 and DYS 393 in a single PCR reaction.

Presentation number: F41 (oral presentation)

CASEWORK GUIDELINES AND COMPLEX MIXTURE INTERPRETATION

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Interpretation guidelines for Short Tandem Repeat (STR) casework analysis are reviewed. The general guidelines will encompass any detection format used such as the ABI Prism™ 310 Genetic Analyzer, the ABI Prism™ 377 Genetic Sequencer or the Hitachi FMBIO™ Fluorescent Scanner. The talk will follow the guidelines published by the Scientific Working Group for DNA Analysis Methods (SWGDAM), a working group of DNA forensic experts in the United States. The use of preliminary data, the designation of alleles and loci, the interpretation of peak heights and band intensities, conclusions and statistical interpretation are all part of forming the final report. The rule “the interpretation of results in casework is a matter of professional judgement and expertise. Not every situation can or should be covered by a preset rule” is stressed. Development of minimum and maximum threshold values, heterozygote ratios, stochastic limits and determination of major and minor components based on validation studies will be discussed. The talk travels through setting criteria to evaluate internal lane standards, positive and negative controls, blind controls, and for the assessment of amplification and electrophoresis. It continues with establishing ranges for interpretation, designating alleles in accordance with the United States Combined DNA Index System (CODIS), defining true alleles versus stutter, bleed through (pull up), spikes, (n+4), “-A”peaks and other anomalies and defining conditions for determining that the source is a single contributor versus multiple contributors. Guidelines for partial profiles are also outlined. Examples of a variety of profiles are given and the potential interpretation, using signal intensities and genetics. Patterns interpretations based on one, two, three, four and more alleles are made. In addition, report writing strategies and wording routinely used by the Pennsylvania State Police DNA Laboratory system are given.

Presentation number: F42 (oral presentation)

COMPARISON OF POWER PLEX™ 16, POWER PLEX™ 1.1 AND 2.1 AND AMPFISTR™ COFILER/PROFILER PLUS FOR FORENSIC USE

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The Pennsylvania State Police DNA Laboratory employs the use of several detection formats and manufacturer's kits to analyze forensic casework. Why was this complicated path of procedures chosen? The author will review the problems and the perks associated with using multiple formats. The validation process and the training program developed to certify each scientist in three detection formats ABI Prism® 310 Genetic Analyzer and 377 Genetic Sequencer and the Hitachi FMBio®II Fluorescent Scanner and three amplification systems (Power Plex 16™, Power Plex™1.1 and 2.1 and AmpFISTR™ Profiler Plus and Cofiler is outlined). Writing protocols and establishing interpretation guidelines for multiple formats is easier then initially realized. What appears as something too complex, in reality, allows for flexibility, cross checks and confirmation of data generated on complex mixtures and degraded samples. A general protocol is used for interpretation of data, statistics, and report writing. The multiple formats allow the scientist the ability to analyze complex cases in all formats, each format exhibiting its own sensitivity and primer selection. Rare microvariants and potential internal primer mutations giving rise to a homozygote pattern rather then the true heterozygote pattern can be checked using different primer systems. Stochastic effects in one system may not be a problem in another thus assisting in obtaining results in a poor sample. Difficult calls based on heterozygote ratios are checked. Alleles falling above or below the ladders of a locus make it difficult to assign the allele to a particular locus. However, it can be rerun in another amplification system that utilizes different primers and electrophoretic mobility to determine its true locus assignment. Loci exhibiting tri alleles can be verified. Mixture ratios can be checked and an advantage can be taken of the sensitivity of each system. Back orders do not cripple the laboratory nor do instrument failures. The validation results on each of the three amplification systems (Power Plex 16™, Power Plex™ 1.1/ 2.1 and AmpFISTR™ Profiler Plus/ Cofiler) are compared.

Presentation number: F43

ALUQUANT™ HUMAN DNA QUANTITATION SYSTEM IN THE FLUOROSCAN ASCENT FL LUMINOMETER

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The AluQuant™ Human DNA Quantitation System uses DNA probes that are specific to repetitive human elements allowing quantitation without PCR amplification. Quantitation of human DNA by the AluQuant™ Human DNA Quantitation System is provided by a series of reactions. Following an initial denaturation, DNA samples are incubated with the READase™ Polymerase, READase™ Kinase and human-specific probe. This coupled enzymatic reaction produces ATP relative to the amount of human DNA present. In a second incubation, ATP produced in the first reaction is used by Luciferase to produce a proportional and measurable amount of light. Background noise is determined by concurrent analysis of the sample without the human-specific probe. The quantity of DNA can then be calculated through comparison of the signal to standards of known DNA quantity. In order to achieve the best result with the AluQuant™ we decided to use for the measurement of the light output the Fluoroscan Ascent FL luminometer. This instrument offers us the possibility to measure all samples under unchanged conditions due to the use of the buil-up incubator, shaker and dispenser. We followed the AluQuant™ manufacturer protocol with the exception of Luciferase/Luciferin reagent transfer to the 96 luminometer plate. The reactions were transferred to the luminometer plate first and the dispenser automatically injected the Luciferase/Luciferin reagent. The use of dispenser slightly increases the Luciferase/Luciferin reagent volume needed for the assay. The use of the dispenser, shaker and temperature control speeds up the whole process but keeps the reaction conditions constant for all samples. The use of the Fluoroscan Ascent FL luminometer for the AluQuant™ Human DNA Quantitation System ensures that the reaction conditions are kept constant for all samples. The use of dispenser slightly increases the Luciferase/Luciferin reagent volume needed for the assay.

Presentation number: F44

GENEPRINT POWERPLEX16 THERMAL CYCLING PROTOCOL FOR THE HYBAID MULTIBLOCK SYSTEM

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DNA typing is a useful and well-established tool in forensic casework but there is still a lot of space for improvements and technological innovations of the process. This paper presents 3 protocols, which can help the forensic laboratories to set up a better standard of laboratory practice. The protocols described below make use of the instrumentation of the Thermo Electron Bioscience Division – Hybaid MultiBlock System, KingFisher magnetic particle processor and Fluoroscan Ascent FL luminometer. We want to demonstrate the usefulness of the presented protocols for the crucial steps of forensic DNA testing – extraction, quantitation and PCR. MultiBlock System (MBS) is based on the concept of a central computer that executes programs and monitors the performance of a number of satellite blocks. Accurate sample temperature control is achieved by Active Tube Control software. This type of control uses the remote thermistor probe mounted in an appropriate tube. The tube termistor probe monitors the temperature within the dummy sample tube and this information is fed back to precisely control the block temperature to achieve the optimum cycling profile. In order to transfer the PCR protocol for the GenePrint PowerPlex 16 System (Promega Corporation, Madison, WI) from the Perkin-Elmer GeneAmp PCR System 9700 Thermal Cycler we used the thermistor lead extension from a block control machine (P-E 9700) to tube control. During the transfer of the protocol we used the control tube of the MBS as a temperature probe in the block control machine. The final protocol is shown in the table bellow.

PP16_ MBS	Stage 1	Stage 2	Stage 3			Stage 4			Stage 5	Stage 6
	1 cycle	1 cycle	10 cycles			22 cycles			1 cycle	1 cycle
Temp °C	95,0	96,0	94,0	60,0	70,0	90,0	60,0	70,0	60,0	4,0
Time	00:11:00	00:01:00	00:00:30	00:00:30	00:00:45	00:00:30	00:00:30	00:00:45	00:30:00	HOLD
Gradient	0	0	0	0	0	0	0	0	0	
Ramp °C/sec	MAX	MAX	1,32	0,60	0,25	1,50	0,60	0,25	MAX	
Temp Inc	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
Time Inc	0	0	0	0	0	0	0	0	0	

The protocol PP16 MBS is optimized for the use of PowerPlex 16 with the AmpliTaq Gold DNA polymerase accordingly to the manufacturer recommendations. The protocol PP16 MBS gives on the Hybaid MultiBlock System exactly the same DNA profile, including the peak height, sister peak misbalance and background noise if compared to the data obtained with the block control machine Perkin-Elmer GeneAmp PCR System 9700 Thermal Cycler.

Presentation number: F45

KINGFISHER GENOMIC DNA PURIFICATION PROTOCOL

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Labsystems KingFisher™ magnetic particle processor is designed for the automated transfer and processing of magnetic particles in a microwell scale. The principle of the KingFisher system is based on the use of magnetic rods covered with disposable, specially designed tip combs and microstrips. The instrument functions without any sucking or aspiration parts or devices. Genomic DNA isolation kit (Labsystems, Finnland) is based on paramagnetic particles and can be used with KingFisher magnetic particle processor to automate genomic DNA purification. The kit is designed for isolation of DNA for several downstream applications including PCR and restriction analysis.
The following protocol uses 40ml of blood as a starting material:
The sample is incubated with particles in well A for 3 minutes
The sample is incubated with particles in well B for 3 minutes
The sample is incubated with particles in well C for 3 minutes
The sample is incubated with particles in well D for 3 minutes
The particles with bound DNA are washed in well E (washing buffer)
The particles are washed in well F (70% EtOH)
The particles are washed in well F (70% EtOH)
The purified DNA is released into distilled water in well H
The particles are removed and returned into well F.
The genomic DNA purification protocol takes only 29 minutes. KingFisher enables simultaneous processing and purification of up to 24 samples in one run. We observed no cross-contamination between wells. We obtained good quality DNA even from badly decomposed blood samples.

Presentation number: F46

THE FIRST CASE OF CONSANGUINITY TESTING BY DNA TYPING IN ARMENIA

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On May 15, 2001 a child was born in one of the maternity homes of Yerevan City, and died about 36 hours later. On May 22, 2001 the parents filed a lawsuit against maternity home declaring that the dead child was supposititious and their actual child could be alive. The aim of this work was to establish whether the dead child was born of these parents or it was supposititious. Blood from the child's corpse was dried on filter paper. About 25 mm^2 piece of bloodstained filter paper was incubated in the SV RNA Lysis Buffer for 5 min with occasional vortexing. DNA was further isolated using SV Total RNA Purification kit (Promega). Parent's DNA was isolated from 300 ul of fresh blood collected into the EDTA containing tubes using Wizard Genomic DNA purification kit (Promega). DNA amplification was performed using GenePrint STR System (CTT, FFv and SilverSTR III Multiplex) (Promega). Amplification results were read on 0,4 mm thick sequencing size polyacrilamide gel (0,4% for CTT and FFv, and 6% for SilverSTR III). Gels were silver stained using Silver Sequence DNA Staining Reagents (Promega). Consanguinity index and probability of consanguinity were calculated using population data on STR allele frequencies for Caucasian-Americans provided by Lins et al. (1998). The STR alleles detected in the samples studied were as follows.

Sample	CSF1P0	TPOX	TH01	F13A01	FESFPS	vWA	D16S539	D7S820	D13S317
Child	11,11	8,11	6,10	7,7	11,11	14,16	11,13	10,10	9,11
Mother	11,12	8,11	6,7	7,12	11,12	14,17	10,11	8,10	9,11
Father	11,11	8,8	7,10	4,7	11,12	16,18	12,13	10,12	11,11

Consanguinity index was 41.811.288, and probability of consanguinity was more than 99,999% (50% prior probability). Based on this evidence the child was proven to be born of the parents tested. This is the first case of litigious consanguinity testing by DNA typing in Armenia. Further development of forensic DNA testing technologies in Armenia is of crucial importance.

Presentation number: F47

EAST SLAVS POPULATION DATA ON FORENSIC MINISATELLITE LOCI

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Allele polymorphism for D1S80 (pMCT118) and 3'ApoB minisatellite loci have been explored. All East Slavs ethnosis have been studied, namely: Russians, Byelorussians and Ukrainians. This study encompassed 12 population samples collected on the base of the individual's informed consent from unrelated slavonic individuals living at least on three generations in areas studied. Allele frequency distributions for the loci were obtained. The typing utilised PCR technique for human identification in forensic and paternity testing. For precisely determinate allele variants specially designed allelic ladders were used. 25 alleles of the D1S80 locus and 23 alleles of the 3'ApoB locus were detected. The most common alleles of the D1S80 locus were 18, 24 and 31, for the 3'ApoB locus 34, 36 and 48 alleles (according to Ludwig) respectively. Observed heterozygosity levels have appeared to be high and ranged from 76% to 82%. No deviations from Hardy-Weinberg expectations were observed. Genetic distances were calculated according to Nei. The analysis of genetic distances as well multidimensional statistical treatment of data obtained indicated certain differentiation between East Slavs ethnosis. The Gst values for both loci were found to be four times less than Gst for classical immunobiochemical markers. First principal component of variability maps were constructed using reliability theory to evaluate the degree of accuracy. East-West direction was noted as basic pattern of East Slavs gene pool.

Presentation number: F48

F13A01 IN STR POLYMORPHISM FFV - DETECTION OF A NEW ALLELE

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In our Laboratory of Forensic Genetics we routinely use STR system FFv (made by Promega Corporation) for paternity testing. Recently by chance we have detected a new allele in F13A01 locus that was not published anywhere so far. That is why we would like to acquaint the public with this specific allele.

Presentation number: F49

IDENTIFICATION OF THREE INDIVIDUALS FROM MEROVINGIAN TOMB BY USE OF GENOMIC AND MITOCHONDRIAL ANCIENT DNA

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In many instances related to forensic questions, determination of sex and relationship of soil-stored skeletal remains is desirable. With our powerful extraction method we are able to extract successfully ancient DNA molecules from ancient skeletal remains (from 1400 years up to 1600 years). The ability to establish the sex and relationship of such skeletal remains contributes important information also to archaeological and anthropological analyses. Ancient DNA undergoes fragmentation during preservation, resulting in short DNA molecules. The analysis of genomic STR (short tandem repeat) markers and the examination of sequence polymorphisms in D-loop region of mitochondrial DNA are likely to be successful with old or degraded material. We carried out analyses on skeletal remains of three noble individuals („a, b, c“) from merovingian tomb in Niederstotzingen (Germany). The sex determination was made by means of amelogenin and DYS390. The results were not congruent with morphological data (three males). One heavily armed lady was evident buried with two warriors. An interesting relationship was proved in the tomb examined. The mtDNA sequence comparison and Y-marker results proved that both males („a, b“) are not related, whilst „b“ and „c“ share the same mtDNA polymorphic sites. The man „b“ can be cousin or uncle mother´s side of the woman „c“. STR results show that the man „b“ is obvious not father of the woman „c“. The young man „a“ can have very probably the same father as the young woman „c“ (illegitimate sister and brother). Our results of these experiments appeared correct since they were consistent and repeatable and the relevant extraction and PCR controls were clean. Such molecular data surely will aid in improved understanding of human remains supporting various anthropological disciplines. Such data bear important implication also for evolutionary genetics and forensic sciences.

Presentation number: F50

DNA FINGERPRINTING OF PLANTS

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The DNA itself seems to be the most powerful candidate for providing discriminatory polymorphisms. The principal objective of our investigation was to determine, whether plant individuals are distinguishable for forensic purposes. An analysis of DNA polymorphisms were carried out on Fagus sylvatica and on Acer platanoides from South Germany. The samples were collected in 24 different areas. Our study involves one individual from each area. Twelve specimens of Fagus sylvatica and twelve specimens of Acer platanoides were studied. The CTAB (hexadecyltrimethylammonium bromide) method was used to extract total DNA. The DNA extracts from Fagus sylvatica was treated with Rnase. The concentration and the quality of the extracted DNA was checked on an agarose gel. Various methods from molecular biology reveal DNA sequence polymorphisms that can be used as highly informative markers at the level of populations and individuals. The isolated DNA was submitted to the RAPD-PCR. The RAPD (random amplified polymorphic DNA) method is finding increasing application in such fields as epidemiology, molecular genetics, microbial ecology, molecular evolution, and taxonomy. Twenty arbitrary 10-mer primers were tested. PCR products were run on non-denaturing polyacrylamide gels. DNA fragment sizes were compared with a length marker. The repeatability of DNA bands assignments was assessed in five independent extractions and PCR amplifications. Only bands reproducible in the independent amplification reactions were included in the data analyses. Negative controls were always kept to check microbial or other contaminations. The RAPD data analyses revealed not only interspecific variation between Acer platanoides and Fagus sylvatica with species-specific DNA fragments. We have found successful primer sequences, which generate individual, reproducible, high polymorphic characters by all specimens included in this study. Each plant individual of our study have a unique genotype. These results are of great importance for the forensic sciences.

Presentation number: F51

MICROSATELLITE AND GENDER DNA TESTING IN NEOLITHIC HUMAN SCULL BONE

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The well preserved skeleton of man from a Funeral Baker culture in a Neolithic cemetery (dated 2300-5000 BC) were found at Karmanowice (South-West Poland), 80 cm under ground. The exhumed fragment of scull bone, after 11 years of room temperature storing, was taken for investigation. Despite of hard environmental factors, still was possible to obtain nuclear DNA (Human Quantitation Kit QuantiBlot - Applied Biosystems). The nuclear DNA was degraded and a performation of shortening of fragments chosen for investigation allowed the positive typing. The analysis of HUM TH01 STR was performed when extremely flanked fragment (69-85bp) with new designed primers were taken for amplification. The gender was estimated using a fragment of amelogenin gene (78bp, exon 6) with two ways detection and confirmed by amplification of the fragment of 93bp of SRY gene. Additionally, the mtDNA HV2A region was amplified also. The techniques for degraded DNA investigation are still being sought in our laboratory.

ABSTRACTS

Frontiers in Molecular and Cellular Medicine

Presentation number: C1

A PARTIAL TRISOMY OF 10q IN CHROMOSOME 5 FOUND IN TWO CHILDREN

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A Syrian family of mother carrier of a translocation (5;10) and two children with the derivate of this translocation (chromosome 5 with genetic material of chromosome 10) were found. The malformation and the karyotypes associated to these cases will be presented.

Presentation number: C2

AL-AQEEL SEWAIRI SYNDROME, A NEW AUTOSOMAL RECESSIVE DISORDER WITH MULTICENTRIC OSTEOLYSIS AND ARTHRITIS WITH A NOVEL MUTATION OF MATRIX METALLOPROTEINASE 2 GENE (MMP-2)

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We reported an autosomal recessive multicentric osteolysis in a Saudi Arabian family with distal arthropathy of the metacarpal, metatarsal and interphalangeal joints, which eventually progressed to the proximal joints and resulted in ankylosis and generalized osteopenia. In addition, they had large, painful to touch palmar and plantar pads and mild dysmorphic facial features including proptosis, a narrow nasal bridge, bulbous nose and micrognathia. Using a genome-wide search for microsatellite markers from 6 members of this family, localized the disease gene to chromosome 16q12-21 with a LOD score of 4.59. Haplotype analysis with additional markers narrowed the critical region to 1.2 cM between markers D16S3032 and D16S3140 and identified the matrix metalloproteinase 2 (MMP-2 , gelatinase A, collagenase type IV, EC 3.4.24.24) gene as a disease candidate. All affected individuals were homoallelic for a nonsense mutation (TCA>TAA) in codon 244 of exon 5, predicting the replacement of a tyrosine residue by a stop codon in the first fibronectin type II domain (Y244X) leading to no MMP-2 enzyme activity in serum and/or fibroblast of affected individuals. The discovery that deficiency of this well characterized gelatinase/collagenase results in an inherited form of an osteolytic and arthritic disorder provides invaluable insights for the understanding of osteolysis and arthritis and the in vivo function of MMP-2.

Presentation number: C3

MOLECULAR CHARACTERIZATION OF INBORN ERRORS OF METABOLISM

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Inherited genetic disorders are quite common in our Saudi community because of the high degree of consanguineous marriages. Biopterin dependent phenylketonuria is a special form of PKU which is quite common in Saudi Arabia. We studied 25 patients with this disorder and we found various mutations leading to variable phenotypes. Vitamin D dependent Ricket's type II is another unique disorder. The c-DNAs from two families were sequenced, it was found that each cell line contained a nucleotide substitution resulting in a stop codon, causing truncated receptor protein of 148 and 291 amino acids which accounts for the severe resistant phenotype. Wilson's disease is another common disease. DNA haplotypes of dinucleotide repeat polymorphism (CA) repeat in the Wilson's disease gene have helped us in confirming the diagnosis and in finding heterozygote carriers in one family. We found a unique syndrome, with unique dysmorphic features, bone and joint abnormalities. We mapped the gene for this disorder to 16q11.2-21. In conclusion our Saudi community is quite unique. Molecular genetic studies of these diseases have helped us in further understanding of these diseases in their clinical management and in finding a unique molecular genetic defect at the DNA and protein (enzyme) level.

Presentation number: C4

SCREENING FOR THE MOST PREVALENT HFE MUTATIONS (C282Y AND H63D) IN THE GENERAL POPULATION IN REPUBLIC OF MACEDONIA

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Hereditary hemochromatosis is a condition of abnormal iron metabolism (iron overload), biochemically characterized by increased transferin saturation (>40%) and increased level of serum ferritin. Clinically, the disease is presented with early multisystemic and non-specific signs such as weakness, malaise, fatigue, impotence, abdominal pain, joint pain, and late sequelae including dark (bronze) skin pigmentation, diabetes mellitus, hepatic cirrhosis, primary liver cancer, hypogonadotrophic hypogonadism, cardiomyopathy, arthropathy. The mutations in the HFE gene responsible for hemochromatosis (C282Y and H63D) were proven to be the most prevalent ones in the white population, making hereditary hemochromatosis the most common genetic disease in the whites, far exceeding the combined incidence of cystic fibrosis, phenylketonuria and muscular dystrophies. The prevalence of C282Y mutation in Europe is estimated to be 1 in 8-10 (heterozygotes) and 1 in 200-400 (homozygotes), the highest in North-West European populations and lower in Southern Europe. The prevalence of H63D mutation is equally high in the regions of North-West and South Europe (1 in 5 heterozygotes and 1 in 50 homozygotes). The aim of this study was to determine the prevalence of C282Y and H63D mutations in the general population in Republic of Macedonia. A total of 100 DNA samples originating from healthy individuals were genotyped for HFE mutations (C282Y, H63D) by PCR-RFLP method. Our results demonstrate low prevalence of C282Y mutation in Republic of Macedonia (1% heterozygotes, no homozygotes) consistent with the low prevalence of this mutation in South Europe and the Mediterranean region. The prevalence of H63D in Republic of Macedonia is in the range published in most European studies (21% heterozygotes, 2% homozygotes). The presence of the HFE mutations in the population in Republic of Macedonia raises questions concerning the screening of this mutation in clinically relevant cases.

Presentation number: C5

RELATIVE RISK FOR VENOUS THROMBOEMBOLIC DISEASE IN FACTOR V LEIDEN CARRIERS

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Factor V Leiden (FVL) is considered to be the most prevalent inherited risk factor for venous thromboembolic disease (VTD). The reported prevalence of this mutation in VTD patients ranges from 20-30%, whereas the estimated prevalence of this mutation in the general population in Western Europe is 3-5%. The estimated relative risk for VTD in the absence of additional acquired risk factor is 7 for heterozygotes and 80 for homozygotes. In order to determine the relative risk for venous thromboembolic disease in Factor V Leiden carriers we genotyped 190 patients and 200 normal healthy individuals for the presence of this mutation. Standard PCR-RFLP methodology was used. VTD patients were divided in the following groups: patients with isolated pulmonary thromboembolism (PTE) (n=45), patients with deep venous thrombosis (DVT) and PTE (n=45), patients with proximal DVT (n=61) and patients with distal DVT (n=84). The prevalence of the mutation as well as the relative risks are presented in the table:

	Number	Factor V Leiden carriers (%)	Relative Risk
Healthy individuals	200	5,5	
Proximal DVT	61	37,7	10,4
Distal DVT	84	13,1	2,6
Secondary PTE	45	20	4,3
Isolated PTE	45	13,3	2,6

The prevalence of the FVL mutation among all patients with VTE was 21 % (relative risk 4.6). The relative risk was highest for proximal DVT (10.4 times), and lowest for isolated PTE (2.6 times). We conclude that Factor V Leiden carriers have the greatest risk of developing deep venous thrombosis of a large venous vessel (ileofemoral or cava vein). This risk is 2-4 times higher than the risk for any other form of venous thromboembolic disease. Patients presenting with ileofemoral or cava vein thrombosis, especially the ones with positive family history, may be the best candidates for routine factor V Leiden screening.

Presentation number: C6

GLUTHATIONE S-TRANSFERASE T1 AND M1 POLYMORPHISMS: SUSCEPTIBILITY TO CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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The GST enzyme family has an important role in the detoxification of endogenous and exogenious chemicals. GST family member's substrates are a group of toxic, mutagenic and envorimental carcinogens. Polymorphic expression of GSTT1 and GSTM1 enzymes are found associated with many cancers such as lung, bladder, stomach and skin cancers. Childhood acute lymphoblastic leukemia (ALL) accounts approximately 25 % of all pediatric cancers. Although aetiology of ALL is still unknown, both genetic and envorimental factors play important roles in the pathogenesis of ALL. Pediatric leukemias give unique investigative models for the study of leukemogenesis. We examined GSTT1 and GSTM1 null genotypes in a Turkish childhood ALL group. Comparison of prevalance of GSTT1 null genotypes between pediatric ALL (23.9%, n=163) and control group (19%, n=100) is not associated with leukemia. But there is an significant difference between GSTM1 null genotype (71.1 %, n=118) and control group (47%, n=100). These data supports that GSTM1 null genotype may be a risk factor for childhood ALL.

Presentation number: C7

MOLECULAR CYTOGENETICS IN BULGARIAN CLINICAL PRACTICE

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Conventional cytogenetic analysis is the first step in the diagnosis of chromosomal abnormalities. However, it has some disadvantages as the need of dividing cell material and perfect metaphase spreads. Introduction of current molecular cytogenetic methods in clinical practice significantly improves the cytogenetic diagnosis. Fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH) and multicolor FISH (MFISH) have been performed in a total of 136 analyses in Bulgaria at the Department of Medical Genetics since 1999. Sixty patients with oncohaematological diseases have been studied by FISH with the following probes – MLL (18 analyses), bcr/abl (21), TEL/AML1 (6), 7 cen (6), c-myc (2), PML/RARA (1), X/Y (6). FISH was also used in prenatal diagnosis (6 cases with 21q22 probe, 1 with X/Y probe) thus enabling us to detect pregnancies at risk – 1 with Down syndrome and 1 with Turner syndrome, confirmed by conventional cytogenetic analysis after abortions. Twenty-nine spontaneous abortions (SA) were examined by CGH. The CGH analysis did not reveal unbalanced chromosomal changes in 11 (38%) cases. In the rest SA investigations were detected: trisomies – 9 (31%); hyperdiploidies – 4 (13.8%); unbalanced chromosomal aberrations, engaging small chromosomal region – 4 (13.8%) and 1 case (3.4%) of combined monosomy 9 with disomy X in male fetus. CGH was also performed in 39 tumors - 36 urinary tract tumors, 1 anaplastic renal cell carcinoma, 1 keratosis vulvae and 1 meningioma. DNA sequence copy number changes in tumor specimens were detected in 29 cases (74.4%). Tumor material from a patient with rare disease - Balkan endemic nephropathy (BEN) was studied with MFISH and complex structural chromosomal aberrations were detected. The introduction of modern molecular cytogenetic methods FISH, CGH and MFISH helped us in clinical practice to reveal chromosomal abnormalities undetected by routine cytogenetics.

Presentation number: C8 (oral presentation)

SURGICAL TREATMENT OF LONG BONES IN CHILDREN WITH OSTEOGENESIS IMPERFECTA - OUR EXPERIENCE

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In the period between 1975 to 1999, 17 patients who were suffering from Osteogenesis imperfecta (O.I.) were treated at our department. All of the patients sustained multiple fractures prior to admission to our unit, while 4 of them had fractures already at birth. Eight patients were suffering from severe form of O.I. We were able to review 14 patients, of whom 8 were treated surgically and 6 conservatively. The follow up period ranged from 3 years to 17 years 4 months - in average 7 years 1 month. The majority of fractures occured in long bones of the extremities (femur 68% and tibia 22%). We treated surgically 44 long bones of the extremities and one severe scoliosis. 21 of the procedures were reoperations. The cause for the reoperations was non union, infection, overgrowth of the bone over intramedullary nail, refracture and inproper placement of the intramedullary rod. For surgical treatment of fractures or deformities of long bones in patients with O.I. we used different intramedullary devises in 35 bones, plate in 5 bones, Ilizarov system in 1 patient and Luque instrumentation for sublaminar fixation of spinal scoliosis in 1 patient. The complication rate was reasonable low. The surgical treatment of deformities and fractures of long bones in children with severe O.I. is beneficial for the patient, improves his mobility and quality of life.

Presentation number: C9

AN UNUSUAL FORM OF OSTEOPETROSIS/ OSTEOSCLEROSIS - EVIDENCE OF INCREASED BONE FORMATION

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Diagnosis was based on radiodense skull x-ray in the second year of life. Other features of this girl, now at the age of 6 years, include: opaque appearance of almost the entire skeleton, dental dysplasia, compression of cranial nerves, seizures, brittle and sparse hair and eyebrows, no eyelashes. So far no fracture occurred and no other organ or nail abnormality was detected. Laboratory findings show no signs of impairment of hematopoiesis. Bone markers indicate continuously increased bone turnover, appropriate for age. Densitometry at 3 and 5 years showed dramatic increment in bone mass Z-score +2.3 and +14.4, respectively. Bone biopsy at he iliac crest revealed increased bone volume and grossly reduced marrow space without hematopoiesis. Bone formation rate was 2-3 times increased. ATP6i gene mutation was not found in this patient. No treatment so far has been introduced. In conclusion, this is an unusual case of progressive osteopetrosis/osteosclerosis with increased bone formation and hair abnormalities.

Presentation number: C10

22q11 MICRODELETION IN POPULATION AT RISK

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Chromosome 22q11 deletion is the most common microdeletion syndrome with the incidence of 1 per 4000. It causes a spectrum of well-described clinical entities which include DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), conotruncal anomaly face syndrome, Opitz G/BBB syndrome and Cayler cardiofacial syndrome. In addition, 22q11 deletion studies are becoming part of a standardized diagnostic procedures for some isolated defects such as conotruncal cardiac anomalies (CHD) or cleft palate CP. In order to assess the cost effectiveness of such practice it is important to get more information on the actual presence of the 22q microdeletion in an unselected group of individuals presenting with symptomes captured in the acronim CATCH 22 (CHD, Abnormal facies, Thymic hypoplasia, CT and Hypocalcemia). Aim was to determine the frequency of 22q11 deletions in an unselected population of patients presenting with CHD, CP, hypocalcaemia or dysmorphic features suggestive of DGS of VCFS. We performed clinical evaluation, high-resolution chromosome analysis, and FISH using double probe (LSI TUPLE1 localised in 22q11 and LSI ARSA) in 90 children divided into 4 groups (1. congenital heart defects, 2. dysmorphic features, 3. cleft palate, 4. hypocalcemia) according to the first presenting symptom. Genetic analysis confirmed hemizygosity for 22q11 in 7.8 (7/90) patients, three patients presenting with CHD, 2 with hypocalcaemia and 2 with dysmorphic features. After clinical assessment 5 patients were diagnosed as having DGS, and two as VCSF, therefore we failed to identify a non-syndromic 22q11 deletion. Detecting 22q11 microdeletion by FISH analysis provides a rapid and definitive diagnosis, ensuring appropriate management, genetic counseling and prenatal diagnosis in inherited cases. As patients with a single CATCH 22 feature are not likely to have 22q11 deletion, quick dysmorphological evaluation by the clinical geneticist prior to cytogenetic evaluation can considerably improve diagnostic rate by preselecting individuals at risk.

Presentation number: C11

SIALIDOSIS TYPE II: CLINICAL, BIOCHEMICAL AND NEURORADIOLOGICAL STUDY OF TWO SIBLINGS

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Sialidosis (neuraminidase deficiency) is a lysosomal storage disease characterized by the accumulation and/or excretion of sialic acid covalently linked to oligosaccharides and/or glycoproteins. Neuraminidase (alpha-NAGA) has an essential role in the removal of terminal sialic acid residues form sialoglycoconugates. This enzyme occurs in a complex with beta-galactosidase and protective protein/cathepsin A (PPCA) and is deficient in 2 genetic diseases: sialidosis (structural deficit in the neuraminidase gene) and galactosialidosis (loss of neuraminidase activity secondary to PPCA deficiency). The disease was classified as sialidosis type 1 - normomorphic (cherry red spot-myoclonus syndrome) and type 2 - dysmorphic, infantile and juvenile form. Her we present clinical, biochemical, and neuroradiological findings in two siblings, 20-year old girl and 15-year old boy affected with type II sialidosis. These two are the first cases of sialidosis in Croatia to be confirmed by enzyme activity. In both patients diagnosis of MPS IV was suspected at the age of 3-5 yrs based on clinical and radiographic findings. They presented with short trunk and relatively long limbs, corase facial features, impaired hearing, dysostosis multiplex and average intellectual development. By the age of 11 years both children developed myoclonic seizures, ataxia and dysarthria. Neither macular cherry-red spot nor hepatosplenomegaly were observed. Brain CT scan showed diffuse cortical atrophy. Both children developed contractures and became immobile between the age of 15 and 20 yrs. Biochemical investigations showed normal or elevated excretion of glycosaminoglicans in urine (chondroitin-6 and chondroitin-4 sulphate, traces of heparan or keratan sulphate, no dermatan sulphate), normal catalytic activities of alpha-iduronidase, GalNAc-6-S sulphatase, beta-galactosidase, arylsulphatase A and B, beta-glucuronidase, alfa-fucosidase, hexosaminidase A and B in skin fibroblasts and leukocytes. Diagnosis was made by finding of elevated levels of sialooligosaccharides in the urine and profound alpha-NAGA deficiency in skin fibroblasts and leukocytes. Presented patients illustrate the clinical variability of neuraminidase deficiency. Clinical presentation presumably correlates with the type of the sialidase molecular defects. Therefore identification of the specific mutations responsible for the enzyme deficiency and the characterisation of the molecular defects are needed to better understand the genotype-phenotype correlation in sialidosis patients.

Presentation number: C12

FURTHER DELINEATION OF THE TORIELLO CAREY SYNDROME

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Toriello-Carey syndrome is a rare multiple malformation/mental retardation syndrome characterised by dysmorphic facial features including telecanthus/hypertelorism, short palpebral fissures, small nose with anteverted nares, malformed ears, and Pierre Robin sequence. Affected patients also show several midline field defects: agenesis of the corpus callosum, laryngeal anomalies, and congenital heart defect. An unbalanced sex ratio was observed and X- linked or sex influenced mode of inheritance was proposed. We summarize 15 so far reported cases and present three new patients with additional findings delineating further the clinical spectrum. Two of our patients were sibs of different sex with a severe phenotype, which argues against the likelihood that this is an X-linked disorder with more severe manifestations in males.

Presentation number: C13

ROUTINE PRENATAL ULTRASOUND DIAGNOSIS OF OMPHALOCELE AND GASTROSCHISIS IN EUROPE

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Evaluation of the prenatal diagnosis of gastroschisis and omphalocele by routine ultrasound examination of the fetus in unselected populations across Europe. Analysis of associated malformations, syndromes and chromosomal abnormalities and of their influence on the pregnancy outcome. We have used data from 19 Congenital Malformation Registries from 11 European countries. Registries collected data on the family history, mother, fetus/infant, results of prenatal scans and outcome of pregnancy. During the study period (01/07/96 to 31/12/98) there were 690,123 monitored pregnancies in the area covered. 243 cases of abdominal wall defects confirmed within one week after birth were included in the study. Prenatal detection rate was 75% (103/137) for omphalocele and 83% (88/106) for gastrochisis. There were significant regional variations in detection rates between European regions. Higher detection rates were observed in Western European countries with screening policy and lower rates in Eastern European countries and Western European countries without screening policy. The detection rate was significantly higher and the mean gestational age at detection lower in multiple affected fetuses. Associated malformations were found in 56.2 % (77/137) of omphalocele and 22.6 % (24/106) of gastroschisis. The overall survival rate was 41% (56) for omphalocele and 73% (62) for gastroschisis. A high number of cases resulted in fetal deaths (30 or 22% of omphalocele and 13 or 12% of gastroschisis). In 51 (37%) cases with omphalocele and in 31 (29%) with gastroschisis parents opted for termination of pregnancy (TOP). Proportion of TOP was higher in multiple affected fetuses compared with isolated cases. There is significant regional variation in the sensitivity of prenatal routine ultrasound in different European regions, reflecting differences in screening policies, equipment and levels of skill. A high proportion of abdominal wall defects is associated with malformations and/or chromosomal abnormalities that clearly influence perinatal outcome. A relatively high pregnancy termination rate is observed also in isolated cases with generally good prognosis after surgical correction.

Presentation number: C14

EPIDEMIOLOGY OF CONGENITAL ANOMALIES IN NORTHWESTERN CROATIA: TEN YEARS EXPERIENCE

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Birth defects are becoming the most important cause of perinatal mortality and morbidity in developed countries. As they include many different and rare disorders in order to arrive to a critical mass of information it is necessary to establish multicentric international network and registries of patients. The EUROCAT (European Registration of Congenital Anomalies and Twins) program is a Concerted Action of the Commission of the European Communities for the epidemiological surveillance of congenital anomalies. Zagreb Registry is included in the network of registries from 1983. Here we want to present the total birth prevalence for major congenital anomalies during the ten-year registration of congenital anomalies in four regional centers (Varaždin, Koprivnica, Pula and Rijeka) of Croatia. To compare the official routine health care statistics data of the Republic of Croatia and the data from the Zagreb EUROCAT Registry with respect to efficiency and uniformity of ascertainment, and diagnostic accuracy. The ascertainment of data, calculation of prevalence rates and statistical methods used are based on EUROCAT methodology. During the monitored period (1990-1999) 1274 children with congenital anomalies per 64, 364 births were registered, with the mean prevalence rate of 19.7/1000 births. Stability in the overall prevalence of malformations was observed, but statistically significant differences ($p<0.01$) in prevalence rates among four regions of Croatia were established. The most common malformations expressed as rates per 10, 000 births were ventricular septal defect (14.4), cleft lip palate (9.0), polydactyly (8.7), atrial septal defect (8.2), and limb reduction defects (4.2). Down syndrome was the most frequent chromosomal aberration with a prevalence rate of 10.7/10 000 births. The prevalence rates of marker anomalies are tenfold higher compared to the routine statistical data of the Republic of Croatia. It is important that national surveillance of congenital malformations should continue. However modifications to the present monitoring system are necessary, including an efficient system for collecting information, greater standardization of data collection, multiple source of information, appropriate population denominator, and the inclusion of data on post-mortem examinations of still-births and therapeutic abortions performed for fetal abnormality.

Presentation number: C15

MOLECULAR DIAGNOSIS OF BETA-THALASSEMIA IN ROMANIAN POPULATION

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Beta-thalassemia is a group of inherited recessive disorder with a defect in synthesis of beta-globin polypeptide chain of hemoglobin is present. From people in all over the world, more than 200 different thalassemia mutations in beta-globin gene have been reported. The aim of our research is to search for distribution of beta-thalassemia mutations in Romanian population. Beta-thalassemia is a frequent genetic disorder in Romania, being estimated at 1,7%. Therefore, the molecular diagnosis is at present a primary goal for heterozygotes screening and diagnostic confirmation. As being the only center in Romania, having such task, we started mutation screening and molecular diagnosis of beta-thalassemia in Romanian patients. Using direct detection by PCR based methods like ARMS-PCR and Restriction Site Analysis we have found out that the most frequent gene mutations in Romania are IVS1-110, IVS2-745 and cd 39. Our data indicated that the mutations identified in Romanian population are of the Mediterranean type. Furthermore, these data will be used in future prenatal diagnosis of beta-thalassemia and for current screening of specific mutations in Romania.

Presentation number: C16

A CASE OF HYPERTELORISM - HYPOSPADIA SYNDROME

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The proband is a newborn boy, born from the second pregnancy which developed on the background of threatened abortion and anemia. His mother is 23; the first pregnancy produced a healthy child. The boy was born in precipitated labor, his mass was 2600g, his length was 49 cm, his head circumference was 32 cm. The fetus was found to be premature, with some inborn anomalies of the uro - genital system, such as multicyst of the left kidney, reno - urethral fistula, perineal hypospadia. It is supposed an inborn cardiac defect. There are also numerous microanomalies: hypertelorism, short nose, high palate, short glossal frenulum, low - placed auricles, cutical navel.

Presentation number: C17

THE PHENOMENON OF GENOME IMPRINTING IN MONOZYGOTIC TWINS AT WAARDENBURG SYNDROME

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The probands are twin sisters of 30 years of age. They were born from the third pregnancy and the third delivery. Their parents are quite healthy. Both sisters demonstrate telecant, partial albinism (a white streak of hair above the forehead), deafness inborn neurosensoric, brachicephalia, wide protruding nose bridge, joined eyebrows, high palate, yellow carious teeth, short neck, skeletial deformities (asymmetry of shoulder blades, scoliosis, valgus deformity of the ankles and ankle joints). One of the sisters has cardiopathia, genital infantilism, ovarian disfunction, and sterility. The other is pregnant and has joint pathology. The twins have two healthy sibs - a brother and a sister. The sisters' caryotypes are normal.

Presentation number: C18

A CASE OF PIERRE ROBIN SYNDROME

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The proband, a newborn girl was born by a mother of 42 in the fourth timely delivery by means of cesarian section. The pregnancy developed on the background of anemia, the state after the thyroid gland resection and obesity of the 4th degree. The proband's brother, 16 years old, has cleft soft and hard palate. The girl was born with the mass of 3640 g, the length of 55 cm, the head circumference of 36 cm. She showed cranium and facial deformities and microanomalies such as microgenia, cleft soft palate, glossoptosis, hypertelorism, flat nose bridge, low-placed auricles, short neck. The cytological examination didn't reveal any chromosome anomalies.

Presentation number: C19

A NEW GENOTYPING METHOD FOR PAI-1 4G/5G POLYMORPHISM

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Plasminogen activator inhibitor-1 (PAI-1) plays important role in fibrinolysis by inhibiting tissue-plasminogen activator (tPA). Although controversial reports are present, 4G/5G single nucleotide I/D polymorphism in the PAI-1 promoter region seems to be associated with myocardial infarction, thrombosis and development of vascular disease in NIDDM patients. Genotyping of this polymorphism is commonly detected with an allele-specific PCR. Thus, our aim was to introduce a new, quick and easy method for genotyping PAI-1 4G/5G polymorphism. The method was based on PCR amplification followed by Single Stranded Conformation Polymorphism (SSCP) analysis and electrophoresis on the precast GMA™ gels in the Elchrom Scientific SEA 2000 apparatus. We analysed 50 whole blood DNA samples of healthy volunteers. DNA was extracted by standard methodology and two PCR fragments (188 and 189 bp respectively) were amplified using previously described primers. SSCP analysis was performed by mixing 6 μl of PCR product with 14 μl of formamide containing 10 mM NaOH, 0.1% bromphenol blue, denatured at 95°C for 5 min and quickly cooled on ice. 10 μl of the mixture were electrophoresed over night (17 hours) with constant buffer temperature (30 mM TAE buffer) at 14°C and 2.5 V/cm. The electrophoresed bands were visualized with SYBR Gold after 1 hour of staining and photographed at 254 nm with Polaroid 667 film. The method allowed us to determine the genotype of all tested samples. Homozygous and heterozygous genotypes were concordant to previously obtained controls. Our PCR-SSCP results showed uniform and reproducible band patterns achieved with constant temperature conditions of the SEA 2000 apparatus and high performance characteristics of GMA™ gels. The method showed to be reliable and cost effective and therefore could be used in laboratories since it is very easy to perform. This analysis could become more important if the PAI-1 genotype is confirmed to be a risk factor for myocardial infarction.

Presentation number: C20

CYP2D6 GENOTYPE AND PSYCHOTROPIC DRUG-INDUCED ADVERSE EFFECTS

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The CYP2D6 gene on human chromosome 22 encodes a cytochrome P450 enzyme responsible for oxidative metabolism of many clinically important drugs. CYP2D6 enzyme activity exhibits a large degree of interindividual variability due to genetic polymorphism of CYP2D6 gene. Genetic polymorphysm has been linked to different phenotypes: EM-extensive metabolizer, PM-poor metabolizer, IM-intermediate metabolizer, and UEM-ultraextensive metabolizer. PM is an autosomal recessive trait, UEM is an autosomal dominant trait. A consequence of polymorphism may be an impaired metabolism of many drugs such as most of the psychotropic drugs with an increased risk of drug side effects or therapeutic failure. The objective of our study was to investigate whether psychiatric patients, on therapy with different psychopharmaca, substrates of CYP2D6 enzyme, exhibiting particular CYP2D6 genotypes, were more likely to develop adverse side effects. One hundred fifty psychiatric patients were included in the study. The CYP2D6 genotype was determined by polymerase chain reaction (PCR)-based amplification, followed by restriction fragment-length analysis or single strand conformation polymorphism. The analysis was performed for most frequent alleles *2, *3, *4, *5, *6, that account for 98% of alleles. Concerning the genotypes, we found that the percentage of functional alleles with extensive metabolic capacity was higher in a group (n = 102) without side effects (88%) whereas the percentage of nonfunctional alleles was significantly higher (p = 0.002) in a group (n = 48) with side effects (42%). The most common deficient allele was CYP2D6*4, followed by 2D6*6, 2D6*5 and 2D6*3. CYP2D6 polymorphism may play a role in drug tolerance and clinical response to treatment with those drugs that are metabolized at least in part, by CYP2D6. By CYP2D6 genotyping we could avoid adverse reactions or therapeutic failure. More prospective studies including plasma levels of drugs and phenotypization are recommended to confirm the results.

Presentation number: C21

POTENTIAL FOR *IN VITRO* AND *IN VIVO* DIFFERENTIATION OF STEM CELLS ORIGINATING FROM THE GASTRULATING RAT EMBRYO

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We have been investigating the developmental potential of gastrulating rat embryo-proper in an unique serum-free *in vitro* model. To discover the remaining developmental potential of cultivated embryos, they were subsequently transplanted to an ectopic, metabolically richer *in vivo* environment. Embryonic parts of 9,5-days-old Fischer rat embryos were micro surgically isolated and cultivated for two weeks at the air-liquid interface in protein-free Eagle's Minimum Essential Medium (MEM), in MEM with addition of transferrin (50μg/ml) or albumin (different concentrations) and in serum-supplemented medium (50%). FGF or 5-azacytidine was used for experiments in different concentrations. Expression of the Proliferating Cell Nuclear Antigen (PCNA) was detected immunohistochemically by mouse monoclonal antibody and labelled streptavidin-biotin kit (DAKO LSAB® 2 Kit, HRP) and stereological analysis was done. Explant diameters were measured by ocular micrometer. For transmission electron microscopy, ultrathin sections were contrasted with lead citrate and uranyl acetate. Some explants were transplanted under the kidney capsule of syngenic rats for additional 14 days. After routine histology, χ^2-test was done to compare incidences of different tissues in explants and in transplants. Rat embryo maintained for two weeks in culture gave rise to a teratoma-like structure containing derivatives of all three germ layers. Transmission electron microscopy confirmed an advanced stage of differentiation of neural tissue, epidermis and cylindrical epithelium. PCNA, the endogenous proliferation marker was detected in cells of the neural tissue, basal layer of epidermis, cartilage cells. FGF enhanced expression of PCNA in neural tissue. Differentiation was optimal in serum-supplemented medium and in the protein-free medium only epidermis could always differentiate with the same incidence. Transferrin or albumin enhanced differentiation of neural tissue. A teratogen 5-azacytidine changed growth and differentiation of cultivated embryos. After subsequent transplantation, skin appendages, ganglionic cells, bone, skeletal and smooth muscle, fat tissue and even some organotypic structures such as tooth differentiated. Differentiation was similar in transplants pre-cultivated with transferrin and with the serum supplement. Protein-free precultivation had no restrictive effect only upon differentiation of epidermis and its derivatives. In this *in vitro* model embryonic tissues could differentiate well in simple chemically defined media but also retained potential for further differentiation *in vivo.*

Presentation number: C22

PREVALENCE OF THROMBOPHILIC RISK FACTORS AMONG CHILDREN WITH CEREBROVASCULAR EVENTS

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Arterial thromboembolic events are rare in childhood. The association of known thrombophilic risk factors and cerebrovascular events in children has not been clarified yet. In order to increase our knowledge about the etiology of cerebrovascular events in childhood we studied the prevalence of thrombophilic risk factors in 21 children (12 males and 9 females; age range 1-15 years) with a history of arterial ischemic stroke (AIS; n=9), transient ischemic attacks (TIA; n=6), migrainous events (n=4) and intracranial hemorrhage (n=2). The diagnosis was based on general clinical and neurological examination, computed tomography and magnetic resonance imaging of the brain. Deficiencies of antithrombin, protein C, protein S or presence of lupus anticoagulant, APC resistance, FV Leiden, and FII20210A were evaluated. Neither antithrombin or protein C deficiency, nor lupus anticoagulant were identified in any of the groups studied. Thrombophilic risk factors were found in 4 out of 9 (44.4%) children with AIS. Protein S deficiency was detected in 2 (22.2%) children. Resistance to activated protein C was demonstrated in 2 (22.2%) other children, one of them (11.1%) showing heterozygosity for the FV Leiden. Among children with TIA, 1 out of 6 (16.6%) children was found to be heterozygous for the FII20210A mutation with associated protein S deficiency. None of the studied thrombophilic risk factors were found in children with migraine or intracranial hemorrhage. The established prevalence of FV Leiden (11.1%) in children with AIS fits into the already reported range of prevalences of FV Leiden in childhood AIS (5.4 –27.2%) and is increased as compared to the prevalence previously determined for the control group (3%). Despite a low number of children in each group it seems that thrombophilic risk factors are more common in children with AIS and TIA.

Presentation number: C23

SINGLE SIGNAL OF THE WILLIAMS SYNDROME CHROMOSOME REGION 1 GENE IN HYPERPLOIDIC BONE MARROW CELLS OF ACUTE LYMPHOBLASTIC LEUKEMIA WILLIAMS SYNDROME PATIENT

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Williams-Beuren syndrome (WS) is a relatively rare autosomal dominant multisystem developmental disease attributed to a microdeletion involving the elastin gene locus on chromosome 7q11. 23. A clinical diagnosis is based on the presence of characteristic facial features and underlying cardiovascular, connective tissue and (or) central nervous system (CNS) involvement. ALL has been reported to occur in patients with congenital chromosome abnormalities. In some of the cases additional karyotypic changes were associated with ALL, but in each case the congenital chromosome anomaly persisted in the leukemic cells. A 14-year-old boy with WS referred at our institution with history of joint pain one-month before the admission. Postnatal growth and development was delayed, congenital supravalvular aortic stenosis with bilateral inguinal hernia was diagnosed in infancy and molecular cytogenetic analysis (FISH) confirmed male karyotype with microdeletion at the long arm of the chromosome #7: 46, XY. ish del (7)(q11.23q11.23). Peripheral blood and bone marrow cytologic analysis revealed acute lymphoblastic leukemia. Immunologic analysis confirmed the diagnosis of ALL. Bone marrow cytogenetic analysis before therapy revealed hyperploidy with 57 chromosomes (57,XY+1B +4C+2D+1E+1F+2G) in 80% of cells (we took to indicate a better prognosis). In a sample of bone marrow hyperploidic cells we performed a FISH analysis with the elastin gene probe to confirm whether the chromosome 7 with microdeletion was duplicated or not. Two signals for centromere of the chromosome 7 (green signals) and one signal of the chromosome 7 with microdeletion (WSCR) (orange signal) was found. We could not find a correlation between the microdeletion disorder and hyperploidy in malignant clones. But we could however confirm that the chromosome microdeletion was stable in the hyperploidic leukemic bone marrow cells of our WS patient with acute lymphoblastic leukemia. This is the first described case of an association between ALL and WS.

Presentation number: C24

GENOTYPE AND PHENOTYPE CORRELATION IN INFERTILE MEN

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Ten to twenty percent of couples encounter difficulty to procreate. In 40-50% of these cases the male partner has qualitative or quantitative abnormalities of sperm production. Spermatogenic failure is associated with microdeletion of the long arm of the Y chromosome. Three regions AZFa, AZFb and AZFc on long arm of Y chromosome are critical for spermatogenesis. These 3 loci act at different stages of germ cell development and deletion of each results in a characteristic phenotype. Deletion of AZFa, AZFb, AZFc regions results in Sertoli Cell Only syndrome (SCO), maturation arrest and hypospermatogenesis respectively. One hundred and two infertile males with oligozoopermia and azoospermia were analysed for cytogenetic and molecular profile. Cytogenetic and semen analysis was done in each case. Testicular Fine Needle aspiration Cytology was collected whenever possible. In 70 cytogenetically normal cases, microdeletion analysis was done using STS-PCR approach using primers sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc). The STS was considered as absent after 3 amplification failures. Eight of the 70 cases showed deletion of at least one of the AZF locus. Four cases had AZFc deletions, three cases had AZFa and AZFb deletions and one case showed AZFb deletion alone. Two cases with AZFa and AZFb had SCO syndrome and 1 case of AZFc deletion showed hypospermatogenesis. In the present study the frequency of microdeletion was 9.9 %. Thus in a significant number of idiopathic cases of male infertility there is an underlying genetic cause. Detection of these microdeletions aids in better management of these infertile couples should they opt for Assisted Reproductive Technology.

Presentation number: C25

SURVIVAL OF PATIENTS WITH CF IN URAL'S REGION

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The world-wide tendency is the prolongation of live expectancy of patients with CF. The results of the work of the Ural's CF center in Ekaterinburg during 30 years are also hopeful. In the last time life expectancy has increased from 8.6 years to in 1988 to 11.1 years in 1993 and to 18.6 years in 1998. In the past 30 years the median survival of patients with CF has increased less 6 years to more 23 years. The improve of the condition is in 34% of patients with CF, the stabilization of the condition is in 39.1%, and the progress of the disease in only 26.9%. Advances in the technologies of drug pharmacology and chest physiotherapy conduced to towards more effective treatment combinations and increase the patient's chance for long-term survival. Patient survival rates have increased because of antibiotic therapy and improved nutrition with pancreatic enzymes replacements. New treatments for the pulmonary disease are under clinical trial and include rhDNase.

Presentation number: C26

THE GENETIC-CARDIAC ASPECT OF TRISOMY 21

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Trisomy 21 is the most common of all autosomal abnormalities which enters deep into the structure and function of more organic individual systems. It represents the most important association between chromosomal defects and congenital heart defects (CHD). The aim od this study is to evaluate the genetic-cardiac aspect of trisomy 21 in the post-war period in B&H (FB&H). During 1996-2000 at Paediatric Clinic of Sarajevo retrospectivelly were evaluated 28 pts (56% boys), age 1-12 years with citogenetically (G-band) proved Down syndrome and diagnosed CHD (clinical examination, ECG, X ray and ultrasound). Mental retardation and hypotony were present in 99.9% cases of trisomy 21, and CHD in 67.8%; ASD 31.6%, AVSD 26.1%, DAP 26.3%, VSD 15.8%, Pentallogy Fallot 5.26% and FoA 5.26%. All pregnancies were single; nullipara in 35.7% cases. In 12/28 mothers it was 2^{nd}, 3^{rd}, 4^{th} or 5^{th} pregnancy. In 55.5% cases (15/28) mothers were younger than 35 years of age; 8/15 mothers (29.6%) from 21-15 years and from 38 till 40 years of age (25%) 7/28. The mean fathers age was 32.5 years (from 25-54 years). The prenatal diagnosis reffered to only routine ultrasound examination, and screening maternal serum (AFP, hCG, U3) was not available. Prenatal diagnosis has been based on routine ultrasound examination, and screening of maternal serum was not available, which point out necessity for its development in the future period. High percent of CHD compared to world statistics could be explained by possible environmental influence, insufficient food intake during the organogenesis, exaggerated intake of multivitamin tablets during war time. A changing pattern of risk in relation to maternal age was identified compared to those in world statistics. Genetic consulting (informing) is important factor for decreasing the risk of having children with chromosomal defects.

Presentation number: C27

ANALYSIS OF 35DELG MUTATION OF THE CX26 GENE IN PATIENTS WITH NON-SYNDROMIC RECESSIVE DEAFNESS IN VOLGA-URAL REGION

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Autosomal recessive non-syndromic deafness is the most common cause of hearing loss and is highly heterogeneous genetically. Mutations in connexin 26 (GJB2) gene have been recently shown to be one of the most often hereditary defect in human with the approximate frequency 20% in various populations. This point mutation is responsible for most of this autosomal recessive hearing loss, DFNB1. However, mutation 35delG in GJB2 is sufficiently frequent in some population, for example in Caucasoid. Approximately, 3% of people in Western Europe are 35delG heterozygotes. We studied patients with hereditary deafness, homozygous alleles were detected in 46,1% cases, compaund heterozygous alleles were detected in 15% cases. This results served as the base for analising frequency mutation 35delG carriers in Volga-Ural region populations, belonging to Turkic brunch of Altai language family according to linguistic classification (Bashkirs (n=208), tatars (n=85), Chuvashs (n=42), and Finno-Ugric brunch the Uralic language family (Mari (n=49), Mordovians (n=70), Udmurts (n=61), Komi (n=76)). The most important fact is 35delG heterozygous allele is definitely present in Mordovian population (2,85%), Udmurts (1,6%), Mari (1,02%), Tatars (0,5%). From our point of view 35delG mutation in Finno-Ugric populations of Volga-Ural region was spreading from West to East. This fact is supported by the lack of mutation in Komi population, and also by high rate of this mutation in Estonia, which population belongs to Finno-Ugric group. The data received as a result of this investigation provide the tool for molecular diagnosis, mutation carriers detection and prenatal diagnosis of autosomal recessive non-syndromic deafness.

Presentation number: C28

OSTEOBLASTIC DIFFERENTIATION IN TRANSGENIC MOUSE MEDIATED BY RCAS (AVIAN RETROVIRUS)

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We have used Replication Competent Avian Splice-acceptor-containing (RCAS) retroviral vectors to study the effect of ectopic expression of proteins and antisense RNA on chick osteoblast differentiation. These vectors allow relatively easy preparation of high titer virus stocks, which can efficiently infect primary cell cultures. Previously the use of these vectors has been restricted to avian cells. Although the well established chick calvarial osteoblast cell culture has been useful for our studies, it is advantageous to be able to use the RCAS system with mouse cells, because of the availability of knockout mice, transgenic lines containing bone directed promoter-reporter constructs and the mouse marrow stromal cell culture system. A transgenic mouse line (β – AKE) has been generated which expresses the receptor for the RCASBP (A) vector, so cells from this mouse are infectable with this normally avian specific vector. In previous studies we showed that RCASBP (A) can infect primary calvarial osteoblasts and bone marrow stromal cells derived from β – AKE mice. Our laboratory has previously demonstrated that a homeodomain protein-binding site plays an important role in osteoblast specific transcription of the Col1a1 gene (Dodig et al, J. Biol. Chem. 271, 16422-16429), and that Dlx5, a homeodomain-containing protein, binds to this site. In addition, we have reported that Dlx5 overexpression stimulates differentiation of primary chick calvarial osteoblasts. In the current study we have used the RCASBP (A) vector to study the effect of Dlx5 on mouse osteoblast differentiation. Primary calvarial osteoblast cultures were established from fractions 2-4 of cells released by staged collagenase digestion of calvaria from 7-day-old β – AKE mice. Bone marrow stromal cells were derived from long bones of 2-month-old β – AKE mice. Cells were initially plated in DMEM containing 10% FCS, and after confluence (day7) were maintained in αMEM with 10% FCS. Calvarial osteoblast cultures were infected with RCASBP (A) Dlx5 or RCASBP (A) on days 1, 2, and 3 of culture, while marrow stromal cells were infected on days 3, 4 and 5. RNA was extracted at different time points for analysis. Northern blot hybridization in multiple experiments consistently detected increased Col1a1, osteocalcin and bone sialoprotein mRNA in cells infected with RCAS (A) Dlx5 compared with control cells. In conclusion, in both mouse calvarial osteoblasts and bone marrow stromal cells, Dlx5 overexpression mediated by an RCAS vector stimulates osteoblastic differentiation. RCAS vectors used with cells from β – AKE mice should be a useful system for studying the role of proteins in primary osteoblast differentiation.

Presentation number: C29

IDENTIFICATION OF THE FAMILIAR DEFECTIVE APOLIPOPROTEIN B-100 IN CROATIA

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Familiar defective apolipoprotein B-100 (FDB) is an autosomal dominant disorder characterized by the presence of LDL that are defective in their ability to bind to the LDL-receptor. The defect is caused by the mutation in the codon for amino acid 3500 leading to Arg>Gln substitution in the receptor binding region. The incidence of FDB in Caucasian hypercholesterolemic patients is approximately 1 in 500. Apo B-100 gene haplotype analysis suggests that the alleles of affected Caucasian individuals originate from a common ancestral chromosome. The aim of the study was to determine the incidence of apo B 3500 mutation in Croatian hypercholesterolemic patients and to analyze whether it originated from the same ancestral allele as in other Caucasian populations screened so far. A total of 960 unrelated hypercholesterolemic patients with LDL-cholesterol above 3.9 mmol/L were selected. Nine family members of the affected heterozygous patients were also tested. Secondary hypercholesterolemia was excluded in all of them. Part of exon 26 of the apo B-100 gene containing the codon 3500 was screened for apo B 3500 mutation using restriction isotyping with Sca I. Eight diallelic markers were used for haplotyping of the mutant alleles of subjects and their kindreds: SP ins/del, ApaLI, HincII, PvuII, AluI, XbaI, MspI and EcoRI, Apo B 3500 mutation was found in two patients and in four family members of one of them. Haplotype analysis confirmed the common origin of the mutation as in other Caucasian populations studied. Apo B 3500 mutation prevalence in Croatian hypercholesterolemic patients is estimated to be 0.21%, similar to those published for other Caucasian hypercholesterolemic groups screened.

Presentation number: C30

EFFECTS OF ISCHAEMIA AND REPERFUSION ON MOUSE KIDNEY POLYRIBOSOME STRUCTURE

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Polyribosome sedimentation pattern was analyzed by sucrose density gradient centrifugation of postmitochondrial supernatant prepared from mouse kidney that had been exposed to short ischaemia without or with reperfusion. Ischaemia was provoked by clamping of renal artery for 3 or 5 min. When reperfusion was required, the clamp was removed and the kidney taken after 10 min, or 1, 2, 6 and 24 hours, respectively. Ischaemia provoked a polyribosome disaggregation after 5 min, and this effect was significantly augmented during 6 hours of reperfusion. Pretreatment with elongation inhibitor cycloheximide prevented the effect of ischaemia, but had no significant influence on polyribosome disaggregation during reperfusion. That finding indicates a decrease in the rate of initiation relative to the rate of elongation of the polypeptide chain synthesis, presumably as a mechanism of polyribosome breakdown in ischaemia, likely because of energy depletion. Lack of cycloheximide effect in reperfusion implicates other mechanisms, probably in the first place that polyribosome disaggregation is due to damaging effects of free oxygen radicals. Nevertheless, in our model we could not prevent reperfusion injury with allopurinol, xanthine oxidase inhibitor. The results show that analysis of polyribosome sedimentation pattern could be effectively used for studies of early ischaemic and reperfusion cell injury.

Presentation number: C31

POLYMORPHISM OF THE SEROTONIN TRANSPORTER GENE (HSERT) IN PATIENTS WITH DRUG ADDICTION

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Aim was to study hSERT polymorphism in patients with opiate drug addiction, to study the role of the neurotransmitters (serotonin) of the central and peripheral nervous system in the drug addiction development and 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. Analysis of polymorphism in intron 2 of the hSERT was analysed for allelic association with in 177 patients in the age between 13-40 years with opiate drug addiction of the 2nd stage. The most frequent were homozygotic genotypes 12/12 and 10/10 in patients and 10/12 in controls. Genotypes and alleles frequencies distribution between patients and controls statistically were not significant. The patient sample was divided into 2 age subgroups. The first subgroup included patients with the opiate narcotization onset in the age of 16 years old and younger (“early onset”), the second subgroup-the rest patients (“late onset”). The genotypes frequency distribution analysis shown the veracious difference between “early onset” patients and controls. (X^2 = 9,77; P = 0,025). There is an authentically more higher homozygotic fraction in “early onset” patients with controls (X^2 = 7,95; P = 0,001) and with “late onset” patients (X^2 = 7,11; P = 0,012). These differences with the controls are due to 10/12 genotype authentic decreasing (X^2 = 6,16; P = 0,014) and 12/12 and 10/10 homozygotic fraction increasing compare to the controls and “late onset” patients. Thus, heterozygotic genotype 10/12 could be a factor of resistance to early narcotization. Analysis polymorphism depending on narcotization experience duration was performed. In the more than 4 years narcotization experience duration patients the considerable increasing of 12/12 and 10/10 homozygotic genotypes fraction compare to the control due to heterozygotic genotype 10/12 fraction decreasing compare to the controls.In the group of patients belonging to a Slavic ethnicity (Russians, Belorussians, Ukrainians) there is a considerable 10/12 fraction decreasing in “early onset” patients and in more than 4 years narcotization experience duration patients compare to the controls and all other groups of patients. In the group of patients belonging to a Turkic group (Bashkir, Tatars) there is a considerable 10/12 heterozygotic genotype fraction decreasing “early onset” patients and in 4 years and less narcotization experience duration patients due to a considerable homozygotic genotypes growth in these groups. Thus, the results certificate that the serotonin transporter gene is involved in the development of drug addiction formation and points at a possible interconnection of the homozygotic genotypes 12/12 and 10/10, with the early drug addiction onset and more extended drug addiction experience duration. (RR= 2,02). The heterozygotic genotype 10/12 could be a factor of resistance to early narcotization.

Presentation number: C32

USING MULTIPLEX PCR TO DETECT BETA-THALASSEMIA MUTATIONS IN THE VIETNAMESE PATIENTS

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Beta-thalassemia is known as a prevalent genetic disease. The aim of our research is getting distribution of beta-thalassemia mutations in a Vietnamese population. Further more, those data will be served for prenatal diagnosis of thalassemia mutations in the future. Multiplex PCR was applied with control and specific primers for 6 mutations: frameshift codons 41-42(-CTTT), codons 71-72(+A), nonsense codon 17(A-T), codon 28(A-G), codon 29(A-G) and IVS II-654 (C-T) to screening 150 Vietnamese thalassemia patients. The result of our study shows that all kinds of these mutations were found from those samples. The mutation at codons 41-42 is the most common in Vietnam (42,6%). Whereas mutations 28, 71-72 and IVS II-654 are less frequent (3,6 - 3,1 and 2,0%). Forty-nine beta-thalassemia families were performed for those mutations, as well:
In 18 cases: a child and mother/father had mutation at codons 41-42
7 cases had mutation at codon 17
3 cases had mutation at codon 29
1 case had mutation at codon 28
3 cases had mutation at codons 71-72
2 cases had mutation at IVS II-654.
One patient had 2 mutations (71-72 from his mother and IVS II-654 from his father).

Presentation number: C33

CYSTIC FIBROSIS MUTATIONS IN CROATIAN PATIENTS

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The frequency of cystic fibrosis (CF) causing mutations varies among different ethnic groups and geographical regions around the world. The mutations present within a particular population need to be defined in order to provide meaningful carrier screening and testing for rare mutations in affected individuals. To determine the frequency of common CFTR mutations in Croatian cystic fibrosis patients in order to develop a practical CF mutation panel that can provide maximal information for our population. We also want to analyze the correlation between the genotypes which have been identified and the main clinical features in order to add some new information to the classification of CF mutations. We have screened 44 CF patients attending Children's University Hospital Zagreb for 16 relatively common mutations: ΔF508, I507, G542X, G551D, W1282X, 3905insT, N1303K, 3849+10kbC-T, R553X, 621+1G-T, R1162X, 1717-1G-A, 2789+5G-A, 3849+4A-G, 1898+1G-A, R117H. Genotype was compared with clinical and laboratory data. Eight mutations: ΔF508 (61.4 %), R117H (4.5%), G542X (3.4%), 1717-1G-A (3.4%), N1303K (2.3%), G85E (2.3%), R1162X (1.1%) and 621+1G>T (1.1%) - were found to account for 78,5% of the CF alleles in Croatian patients. Sixteen (36%) were homozygous for ΔF508, with severe phenotype and pancreatic insufficiency (PI). Three compound heterozygotes for G542X and one for 1717-1G-A had severe form of the disease, while one patient heterozygous for 621+1G>T was pancreatic sufficient (PS). All heterozygotes for R1162X, N1303K and G85E presented with PI. Two ΔF508/ R117H patients had serious clinical course, but one patient had mild phenotype. From nine ΔF508/ N patients, six were PS. Four N/N patients had severe pulmonary disease without pancreatic involvement. The CFTR gene analysis demonstrated the notable heterogeneity of our CF population, which could be explained by the strong influence of the genetic pool from other southern European countries. A carrier-screening program should be postponed until reaching a proportion of at least 90% of known CF alleles. Further studies are necessary to identify other common and uncommon mutations in our population and to better delineate genotype/phenotype correlations. This will enable more accurate genetic counseling of Croatian CF families.

Presentation number: C34 (oral presentation)

CELLULAR AND MOLECULAR INTERACTIONS BETWEEN THE IMMUNE SYSTEM AND BONE

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Functional interdependence between the immune and bone systems is reflected in a number of regulatory molecules acting on the cells of both systems and common precursors for bone and immune cells. Consequently, the disturbances of the immune system may affect bone metabolism, and *vice versa*. We will here address the roles of two major immune cell populations, T and B lymphocytes, in the regulation of bone metabolism. Experimental models and human diseases demonstrated that T lymphocytes may produce a number of bone cell regulatory cytokines, including two essential stimulators of osteoclastogenesis, RANKL and M-CSF. The effect of T lymphocytes on osteoclastogenesis may be both stimulatory and inhibitory, and depends on the activation stage and pattern of cytokine production. We showed that acute removal of T lymphocytes stimulated osteoclast differentiation *in vitro* and enhanced new cartilage and bone formation at non-osseous sites *in vivo*. B lymphocytes may be even more closely related to bone cells, as B-lymphopoiesis requires an intimate contact with osteoblastic/stromal cells, and estrogens, powerful regulators of bone mass, are also involved in the differentiation of the B-lymphocyte lineage. Also, B-lymphocyte progenitors may give rise to functional osteoclasts. Both B and T lymphocytes may act through the RANKL/RANK/OPG cytokine system, which has been independently discovered within immune and bone systems. These cytokines have crucial roles in the development and function of osteoclasts, dendritic cells, and T and B lymphocytes, as well as in the thymus and lymph node organogenesis. The cytokines produced by immune cells may affect bone cell function and *vice versa*, but the full complexity of these interactions awaits further investigation.

Presentation number: C35

MOLECULAR DIAGNOSIS OF CHARCOT-MARIE-TOOTH 1A DISEASE AND HEREDITARY LIABILITY TO PRESSURE PALSIES IN CROATIAN PATIENTS

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The most frequent form of hereditary sensomotor polyneuropathy Charcot-Marie-Tooth (CMT) is type 1A which is result of duplication on chromosome 17 in band p11.2-p12. This region contains PMP22, a gene expressed in peripheral myelin. The cause of mutation is unequal crossing-over involving repeat sequences, CMT1A-REP located on both sides on duplicated chomosomes. Deletion in the same region is reciprocal product of this recombination, which is associated with hereditary neuropathy with liability to pressure palsies (HNPP). The aim of this study was to estimate the duplication and deletion frequencies in Croatian CMT patients and HNPP. We analyzed 39 patients with CMT for the presence of duplication and 39 HNPP patients for the presence of deletion using polymerase chain reaction/restriction fragment length polymorphism analyses. Nerve conduction velocities were lower than 38 m/s in all patients with CMT1A. CMT1A-REP sequences can be distinguished by EcoRI digestion. Presence of duplication produces an EcoRI site and generates fragments of 3600, 3300 and 300 bp. If no duplication is detected only 3600 bp fragment is visualized. In the case of deletion EcoRI digestion generates fragments of 3600, 3300 and 300 bp. If no deletion is detected only 3300 and 300 bp fragments are visualized. In 39.2% of 23 unrelated and 56.3% of 16 related CMT patients, the 17p11.2 duplication was present. In 13.3% of 15 unrelated and 16.7% of 24 related HNPP patients, the 17p11.2 deletion was present. Duplication frequencies in unrelated Croatian patients are within the range of frequencies for Greek (30%), Italian (50%) and German patients (50%). Deletion frequencies in unrelated Croatian patients were lower than found for other European countries by Nelis et al. 1996.

Presentation number: C36

MOLECULAR ANALYSIS OF SHORT TANDEM REPEAT LOCI TO FOLLOW CHIMERISM AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Relapse after bone marrow transplantation (BMT) remains a significant problem. Previous studies have used standard cytogenetic techniques, changes in red cell phenotype or appearance of both Y chromosomes for following chimerism after allogeneic BMT. In the case when any of these markers is not useful, short tandem repeat (STR) loci can be a potential tool for the diagnostic. In order to establish the strategy for the follow up of chimerism, in this study 12 patients (before and after BMT) and their HLA identical donors were analyzed for five STR loci (HUMTH01, HUMVWA31, HUMFES/FPS, HUMF13A1, and SE33), as well as for one VNTR locus (D1S80 locus). DNA was isolated by standard salting out method. After amplification samples were run on 6% polyacrilamid gel and detected automated by a Pharmacia ALFexpres. Assignment of alleles was performed using a Fragment Menager program. In all 12 HLA identical pairs we found differences between recipient and donor for at least two loci. Namely, these tested loci have six and more known alleles. A complete chimerism was present in all 12 patients. The results of this study showed the application of polymorphism of these loci for monitoring post-transplant chimerism. Further prospective studies will examine the clinical implications of potential mixed chimerism and the detection of residual haematpoiesis as a sign of early relapse.

Presentation number: C37

CHARACTERISATION OF AN X;AUTOSOME TRANSLOCATION AND IDENTIFICATION OF SUPERNUMERARY MARKER CHROMOSOME IN A STERILE FEMALE BY CONVENTIONAL CYTOGENETICS AND FISH

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High resolution cytogenetic analysis of 28 year old woman affected by premature ovarian failure (POF) revealed a unique combination of a balanced X; autosome translocation t(X;17)(q22;q11) and supernumerary bisatelited marker chromosome. An X-replicating study by cytogenetic BrdU banding found the normal X chromosome constantly inactive, and the POF phenotype was probably caused by disruption of the specific gene(s). Cytogenetic investigation by fluorescence *in situ* hybridization (FISH) was focused on chromosomal origin and composition of marker chromosome in attempt to define the karyotype/phenothype correlation. The results, using alpha satellite 14/22, wcp 22 and specific region probe 22q11.2 allowed the identification of supernumerary marker chromosome as a dicentric marker, derived from chromosome 22, idic(22)(q11). Although the patient carried tetrasomy for the centromere and proximal euchromatic region of chromosome 22 usually associated with cat-eye-syndromes (CES), the clinical examination didn't reveal any of adverse phenotype effects.

Presentation number: C38

PRENATAL DETECTION OF COMMON ANEUPLOIDIES AND FETAL SEX BY QUANTITATIVE FLUORESCENCE POLYMERASE CHAIN REACTION

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The existence of fetal DNA in maternal blood is well known phenomenon. The possibility of maternal peripheral blood use for noninvasive prenatal diagnosis is very attractive. Molecular diagnosis of chromosomal aneuploides from amniotic fluid, chorionic villus and fetal blood samples was the first purpose of our research. Consecutively we focused on the detection of fetus-derived Y-sequences from plasma of women bearing male fetuses. DNA was extracted from fetal blood, amniotic and chorionic cells or from plasma of pregnant women blood using the QIAamp Mini Kit (Qiagen) Highly polymorphic fluorescent STR markers were used for the detection of common chromosomal aneuploides of fetal DNA. D21S11, D21S1411, D21S1412, D21S1414 were used for the evidence of trisomy 21 (Down sy) and D18S51, D18S535 for trisomy 18 (Edwards sy). Gonosomal aneuploidies (Klinefelter sy) were detected using primers for HPRT gene and homologous region of the amelogenin gene (AMXY). Male and female DNA in maternal plasma samples was detected by the fluorescent STR marker for amelogenin gene (AMXY). Electrophoretic analysis was performed on a 310 ABI PRISM DNA sequencer (Applied Bisystems, USA). 38 fetal samples were tested for major chromosomal abnormalites. Trisomies of chromosome 21 were proved in 24 cases, trisomy of chromosome 18 in 4 cases, aneuploidy of X-chromosome (Klinefelter sy) in 9 cases. Chromosomal polyploidy was detected in 1 case. Among the 68 women bearing male fetuses, Y-positive signals were detected in 54 plasma samples (specifity 79%). None of the 6 women bearing female fetuses had Y-positive result. Our noninvasive molecular diagnosis results were confirmed by the cytogenetical ones. Our results show convenience of QF-PCR in molecular detection of common chromosomal aneuploidies. It was confirmed that the presence of fetal DNA in maternal plasma is in sufficient quantity for noninvasive prenatal diagnosis. The success rate in different gestational age was variable.

Presentation number: C39

OUR EXPERIENCE IN BISPHOSPHONATE THERAPY FOR OSTEOGENESIS IMPERFECTA

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Severe osteogenesis imperfecta (OI) is a hereditary disorder characterized by increased bone fragility and progressive bone deformity. Secondary osteoporosis is an important feature of OI. So far, no effective medical treatment is available. Antiresorptive activity of the aminobisphosphonates may improve clinical outcome in children. Aim is to asses the clinical impact of the administration of bisphosphonates in Croatian OI patients. We introduced therapy in 1998 encouraged by parents from Croatian society of OI (HUOI). Here we report results of 1-3 years treatment with intravenous pamidronate (APD) in six children (four girls) of age 3 months – 11 years at entry, with severe OI. Pamidronate was administered in cycles as monthly infusions at a daily dose of 1-1,5 mg/kg during 6 months following pause for three months, or the same dose for three days every four months. All took 500-1000 mg calcium from food or supplements and 1000 IU of vitamin D daily. During treatment DEXA measurements showed a gradual increase in bone density in all patients. Biochemically assessed bone turnover rate fell. Number of confirmed fractures decreased in all. The reduction in pain and improvement in well being and ability were impressive in two boys who had been confined to a wheelchair and now they walk using crutches. No adverse side-effects were noted, apart from the well known acute phase reaction during the first infusion cycle in two children. Although the bisphosphonates do not correct basic abnormalities in OI, they significantly alter the natural course of the disease and improve patient's quality of life. For the time being they seem not only effective but also avoid of any adverse effects on bone growth and remodeling.

Presentation number: C40

EFFECTS OF PARATHYROID HORMONE, 1,25-DIHYDROXYVITAMIN D3 AND INTERLEUKIN-1A ON COL 1A1 PROMOTER ACTIVITY IN TRANSGENIC MOUSE CALVARIAE: 5' AND INTERNAL DELETION ANALYSIS

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Chronic high concentrations of parathyroid hormone (PTH), 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and interleukin-1α (IL-1) can inhibit bone formation via repression of COL1A1 transcription in osteoblasts. To map promoter elements mediating this repression, we have examined the effects of PTH, $1,25(OH)_2D_3$ and IL-1 on chloramphenicol acetyltransferase (CAT) activity in cultured calvariae derived from Col1a1 promoter-CAT reporter (ColCAT) transgenic mice. Previous studies have shown that all of these agents repress Col1a1 promoter activity in osteoblasts via cis-acting elements located downstream of –1719 bp. Moreover, the COL1A1 promoter contains a homeodomain protein motif immediately downstream from –1683 bp that is required for high levels of promoter expression in osteoblasts in vivo. The goal of this study was to elucidate DNA sequences downstream of –1719 bp that are required for the inhibitory effect of these agents on Col1a1 promoter activity in osteoblasts. CAT activity was measured in calvariae derived from 6 to 8-day-old ColCAT transgenic mice and cultured for 48 h. Transgenes tested were ColCAT1719 and ColCAT1683, with 1719 or 1683 bp of COL1A1 promoter sequence, respectively, and ColCAT1719(-1284/-318) with an internal deletion extending from –1284 to –318 bp. We found that the ability of PTH (10^{-8}M) and $1,25(OH)_2D_3$ (10^{-8}M) to repress promoter activity up to 60% was maintained in all constructs tested. On the other hand, IL-1 (1 ng/ml) inhibited ColCAT1719 promoter activity by 40%, whereas ColCAT1683 and ColCAT1719(-1284/-318) showed only a 10 to 20% repression. These observations suggest that down regulation of the COL1A1 promoter by PTH and $1,25(OH)_2D_3$ most likely involves cis-elements located between –1683/–1284 bp or in the proximal promoter downstream from –318 bp. In contrast, repression by IL-1 appears to be complex, requiring multiple cis-elements located between –1719/–1683 bp and between –1284/–318 bp. These studies should provide a better understanding of the molecular mechanisms involved in repression of COL1A1 transcription in osteoblasts leading to reduced rates of bone formation.

Presentation number: C41

MENKES DISEASE: TWO CASES PRESENTING WITH EARLY WHITE MATTER LESIONS

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Menkes disease is an X-linked recessive disorder of the copper metabolism due to malabsorption and defective distribution of dietary copper. It is caused by a defect in the Menkes (ATP7A) gene, which encodes a transmembrane copper-transporting P-type ATPase. The classic form of the disease is characterized by hypothermia, impairment of growth, characteristic hair abnormalities (thin, breakable, kinky and/or gray-hair) and facial appearance, seizures and neurodevelopmental delay. Neuroimaging usually shows cortical atrophy, chronic subdural effusion or hygroma, vascular abnormalities, progressive and extensive degeneration of gray matter with secondary demyelination. White matter lesions are rarely present before other features of the disease and may evolve into atrophy. Clinical case: We report two cases of Menkes disease with predominant early diffuse white matter involvement on neuroimaging which have lead to diagnostic difficulties suggesting Krabbe disease. Clinical progression suggested the diagnosis of Menkes disease, confirmed consequently by low levels of serum copper and ceruloplasmin, high 64CU uptake in fibroblasts and DNA analysis. In Menkes disease white matter lesions may be present before other features of the disease leading to the diagnostic difficulties in the early stage of the disease. In the differential diagnosis of leukoencephalopathies Menkes disease should be also considered.

Presentation number:C42

ARYLSULFATASE A PSEUDODEFICIENCY IN A CROATIAN POPULATION

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Deficiency of lysosomal enzyme arylsulfatase A (ASA) causes metachromatic leukodystrophy, rare autosomal recessive disorder characterized by the storage of cerebroside sulfate mainly in the nervous tissue. Low ASA activities have been also reported in healthy individuals and several neurologic and pshychiatric disorders, due to condition termed ASA pseudodeficiency. Two mutations in the ASA gene responsible for the majority of pseudodeficiency alleles are designated as N350S and 1524+95 A>G mutation. It has been suggested that suboptimal enzyme activity and sulfate accumulation resulting from pseudodeficiency mutations could contribute to pathogenesis of certain neuropsychiatric disorders in later life. The frequency of arylsulfatase pseudodeficiency has been estimated within the range of 7-15% in clinically healthy individuals from different populations. The aim of this preliminary study was to establish the frequency of both previously described mutations associated with ASA pseudodeficiency in 150 healthy individuals from Croatian population. For this purpose, genomic DNA was extracted from leukocytes and two fragments of ASA gene were amplified using specific primers. After digestion with adequate restriction enzymes, the reaction products were analyzed by electrophoresis on 8% polyacrylamide gel. The results are expressed as frequencies of mutations responsible for ASA pseudodeficiency in analyzed group. This preliminary study presents the first data on frequencies of two mutations associated with arylsulfatase A pseudodeficiency in healthy individuals in a Croatian population.

Presentation number: C43

STEROL REGULATORY ELEMENT BINDING PROTEINS IN MOUSE TESTIS

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Sterol regulatory element binding proteins (SREBPs) are membrane-bound transcription factors which control the metabolism of cholesterol and fatty acids in animal cells. Cleavage of SREBPs and release of the bHLH-zip domain, which enters the nucleus and binds to sterol regulatory elements (SRE), is enhanced by sterol depletion and inhibited by sterol supplementation. Previous studies suggested that SREBP-dependent pathway is responsible for regulation of cholesterol and fatty acids biosynthesis in liver and other somatic tissues, while cAMP-dependent transcriptional activator CREMτ may predominate in activation of cholesterogenic gene CYP51 in male germ cells. The aim of this study was (1) to determine the expression of SREBP mRNAs in mouse germ cells and (2) to localize SREBPs proteins in mouse testis at the ultrastructural level. The expression of SREBP mRNAs was analyzed by RT-PCR method using specific fluorescent-labeled primers for SREBP-1a, -1c and SREBP-2. Amplification of the β-actin gene was used as an internal standard. RT-PCR products were analyzed by capillary electrophoresis on Abi Prism Genetic Analyzer. The cellular localization of SREBPs in mouse tissue was analyzed by immunogold electron microscopy using primary antibodies that recognize SREBP-1 and SREBP-2, and secondary antibodies bearing 10 nm gold particles, which were applied to ultrathin sections of mouse testis. The analysis of SREBP mRNA expression showed the presence of SREBP-1c and SREBP-2 transcripts in mouse testis and interstitial cells, as well as in isolated germ cells from both prepubertal and adult animals. Preliminary immuno-electron microscopy data showed the presence of SREBPs in nuclei of primary spermatocytes, in nuclei of round and elongated spermatides, and in nuclei of interstitial cells. Detection of SREBP mRNAs shows that SREBP genes are transcribed in mouse germ cells. Preliminary data indicate that the SREBP mRNA is translated and properly cleaved in spermatocytes as well as in spermatides and interstitial cells, since gold-labeled SREBP particles have been detected in nuclei of those cell types. A more detailed analysis is needed to evaluate the quantity of mature SREBP protein in different types of germ cells and its potential role in transcription of cholesterogenic genes in male germ cells.

Presentation number: C44

DIGEORGE SYNDROME – CLINICAL AND GENETICAL DIAGNOSIS

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DiGeorge syndrome is reported in association with hemizygosity for a region of chromosome 22q11 (the CATCH22 phenotypes, with cardiac defect, abnormal face, thymic hyperplasic, cleft palate and hypocalcaemia). Cytogenesis analysis was performed in 42 patients with suspected DiGeorge syndrome. Indication for FISH was phenotype – facial abnormalities, cleft soft and/or hard palate, congenital heart disease and hypocalcaemia. In 10 patient micro deletion was detected with FISH in DiGeorge region. Among those 10 patients 7 (70%) had significant congenital heart defects – 4 with tetralogy Fallot, 2 with persistent truncus arteriosus and 1 child with pulmonary atresia. All of the CHD's are surgically successfully corrected. Clinical findings of immunodeficiency were present in 4 and hypocalcaemia in 3 children, respectively. In other 3 patients no CHD was detected but child had typical face and in 2 cases cleft of soft palate. In 32 cases genetical diagnosis of DiGeorge syndrome was not established with commercially available kit (D22S75 or N25). In that group 14 (43%) patients had CHD (3 – persistent truncus arteriosus, 2 – interruption of aortic arch, 5 – pulmonary atresia, 2 - single ventricle with pulmonary atresia, 2 - aortopulmonary window), while other 18 patient had only phenotype that suggested possible DiGeorge syndrome (12 – hypertelorism, 9 – low set small ear lobes, 7 – micrognatia, 4 – cleft palate). Clinical findings of immunodeficiency were present in 2 and hypocalcaemia in 3 children, respectively.

Presentation number: C45

RENAL OSTEODISTROPHY IN CROATIA BASED ON BONE HISTOMORPHOMETRY-FIFTEEN YEARS OF EXPERIENCE

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The metabolic bone disease and mineral metabolism disturbances are frequent complication in renal failure, and still a great challenge of the modern medicine. Renal osteodystrophy is the generic term generally used to describe the skeletal complications of renal failure and it encompasses a wide spectrum of bone disorders. Based on the predominant histopathologic patterns, it is often classified as: predominant hyperparathyroid bone disease, mixed uremic osteodystrophy, low turnover, osteomalacio, adynamic uremic bone disease, mild uremic bone disease. But because of the great variability of histopathologic patterns no current classification is completely satisfactory, so we could consider the classification of renal osteodystrophy as not final. Since the current therapeutic approach to the renal osteodystrohy is to normalize the defect in bone remodeling, histopathologic pattern is the best basis for the accurate diagnosis and planning the appropriate treatment. Thus, bone biopsy and histomorphometry are the crucial diagnostic procedures in the evaluation of renal osteodystrophy. This article provides review of renal osteodystrophy in Croatia describing the hystomorphometric characteristics of bone in the group of 1000 uremic patients on regularly hemodialysis randomized among 2600 bone biopsies performed in last fifteen years.

Presentation number: C46

FISH INVESTIGATION OF CHROMOSOME ABNORMALITIES IN CLINICAL CYTOGENETICS

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From November 20, 1996 to June 1, 2001 we have performed 906 molecular cytogenetic studies in 288 samples of peripheral blood, 49 amniotic fluids, 4 CVS, 4 chordocentesis and 10 tissues, 548 bone marrows and only 3 solid tumors. A variety of probes were used with numerous applications such as wcp, alpha satellite and specific region probes. Hybridised signals were scored under a fluorescence microscope using appropriate absorption and excitation filters. Microdeletion syndromes, rearrangements and marker chromosomes have been detected or characterised by those techniques. Examples of chromosome markers include 29 small markers derived from chromosome 15, 22, 21, X and Y, a small marker 22 in case that also had a translocation (X;17). Several cases with "*de novo"* rearrangements were recognised. We found that an add(9)(p14;24) was a der(9)t(4;9) with monosomy 9p and partial trisomy 4p, der(13) was inversion duplication of 13(q 22), cx translocation t(7;4;14) with insertion hence a partial trisomy of der 4, derivate chromosome 1 with centromire 15 on the terminal part of 1(p36) and that Elastin locus was disrupted by translocation t(7;14)(q11.23;p12). Molecular cytogenetic analyses of 12 workers exposed to the constant level of vinyl-chloride monomer were performed for detection of stabile genome damage in order to estimate the genome risk. FISH studies in bone marrows and solid tumors characterised the origin of multiple chromosome rearrangements and helped in the interpretation of the diagnoses and/or prognoses. FISH was used for both, initial detection of specific abnormality with major prognostic and biologic impact and to the follow-up of treated patients by detection of MRD and identification of the origin of bone marrows cells following stem cell transplantation. I-FISH permits the evaluation of a large number of cells in detection of numerical changes and specific translocations. Hybridised signals were scored in about 100-700 interphase nuclei for each patient, with increased precision, since hundreds of cells (including nondividing cells) can be examined in a short time. Metaphase FISH was useful in detection of cryptic rearrangements and identification of marker chromosomes. The subtelomeres has been investigated with PNA probes in children leukemia. We conclude that the availability of new probes and the use of specialised techniques, such as FISH, M-FISH have improved significantly the services offered by clinical cytogenetic laboratory in our hospital.

Presentation number: C47

TRANSGENIC MICE EXPRESSING DUAL REPORTER TRANSGENE DRIVEN BY 3.6 COL1A1 AND 2.3 COL1A1 PROMOTERS

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To overcome the problem of quantitative and spatial analyses of promoter constructs in intact bone we designed a construct for a dual reporter transgene containing CAT and GFP under the control of 3.6kb and 2.3kb fragments of the rat COL1A1 promoter. Previously we have demonstrated that 3.6 or 2.3 COL1A1 promoter fragment that includes 1.6kb of the collagen first intron driving GFP produces fluorescent osteoblasts in intact bones of transgenic mice. We used the pIRESeGFP vector (Clontech) in which the CMV promoter drives a transcript containing viral internal ribosome entry site (IRES) that is fused with eGFP. Multicloning site is placed upstream of the IRES into which a second gene can be placed. Resulting bicistronic mRNA is translated into two separate proteins. We modified the pIRESeGFP vector by inserting the CAT gene upstream of IRES and by replacing the CMV promoter with a 3.6COL1A1 or 2.3 COL1A1 promoter. eGFP was replaced by GFPsaphire(Packard) or GFPcyan(Clontech). Resulting constructs, COL3.6CATiresGFPsaph and COL2.3CATires GFPcyan were shown to be functional transgenes in transient transfection experiments prior to pronuclear microinjection. Two founders for each construct were produced. Tail biopsies were GFP positive by microscopic examination and tail protein extracts showed CAT activity. Marrowstromal cell cultures from heterozygous COL3.6CATiresGFPsaph and COL2.3CATires GFPcyan mice were established under differentiating conditions. COL3.6CATiresGFPsaph culture had CAT activity at very low level on day7, which increased with time, reaching the peak on day16. The GFP positive cells started to appear on day11 and their number increased throughout the culture period. GFP positive cells were predominantly localized within the mineralized nodules. Cells in COL2.3CATiresGFPcyan culture were becoming GFP positive on day13 and they were restricted only to mineralized nodules. CAT data are being analyzed. Tissue sections of paraffin embedded femurs and calvariae are in the process of analysis. This work shows that the dual reporter concept appears to be a useful tool for the simultaneous visual and quantitative analysis of transgene activity in bone.

Presentation number: C48 (oral presentation)

EXPRESSION OF BONE MORPHOGENETIC PROTEINS IN STROMAL CELLS FROM LONG-TERM CULTURE OF HUMAN BONE MARROW

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Interaction between hemopoietic and stromal cells of the bone marrow is well known. It has been recently demonstrated that highly purified primitive human hemopoietic cells express mRNA for BMP type I receptors as well as their signaling transducer molecules, and respond to exogenously added BMPs. To explore the possibility that bone morphogenetic proteins (BMPs) might be involved in hemopoietic differentiation, we have tested stromal cells from long-term culture of normal human bone marrow (LTC), known for their supportive role in hemopoiesis, for the expression and presence of these factors. The mRNA expression for BMP-3, BMP-4 and BMP-7 was detected in bone marrow stromal cell population from the first to the eight week of culture and the protein presence was confirmed by using specific BMP antibodies. The cells did not produce BMP-2, BMP-5 and BMP-6. Short-term in vitro colony assay revealed the presence of clonogenic progenitors throughout the entire investigation period. Furthermore, bone marrow stromal cell population was found to express BMP type I receptors, activin-like kinase (ALK)-3 and ALK-6, as well as their down-stream transducers SMAD-1, -4 and -5. We conclude that normal human bone marrow stromal cells synthesize and produce several BMPs, which may target hemopoiesis in a paracrine manner and osteogenesis in an autocrine manner.

Presentation number: C49

BONE MORPHOGENETIC PROTEIN EXPRESSION IN DIFFERENTIATING OSTEOBLASTS IS REGULATED BY PARATHYROID HORMONE

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We have recently shown that MC3T3-E1 cells during normal differentiation process in vitro synthesize only BMP-4 which is necessary and sufficient for osteoblastic differentiation and extracellular matrix deposition. In the same system the function of BMP-4 could be replaced by BMP-7, another BMP family member. In this study we examined the effect of parathyroid hormone (PTH) addition on regulation of BMP gene expression by osteoblastic MC3T3-E1 cells. Cells were routinely cultured and after reaching confluency, beta-glycerophosphate and ascorbic acid were added. At designated time points (days 0, 3, 7, 12 and 17), parallel cultures were treated with PTH (10-9 M) for 12 and 72 hours. Total RNA was isolated using TRIzol reagent and cDNA was synthesized from 4 μg of total RNA with Superscript II RNase H-Reverse Transcriptase (Gibco BRL), and the expression of BMP-2 to BMP-7, alkaline phosphatase, collagen type I, osteocalcin, osteopontin, BMP receptors type I (ALK-3 and 6) and G3PDH was analyzed by RT-PCR. Compared to control samples, the addition of PTH slightly delayed the maximum expression level of BMP-4 mRNA, reached on day 7. After that point, the BMP-4 gene expression gradually decreased in time. Interestingly, during early differentiation, PTH transiently stimulated the expression of BMP-2 and BMP-3. The expression of genes associated with osteoblast differentiation, osteopontin, collagen type I, alkaline phosphatase and osteocalcin were markedly stimulated by PTH, as well as bone nodule formation at day 21. These results suggest that BMP signal is necessary for initiation of the osteoblastic differentiation, and that the effect of parathyroid hormone on this process can be in part achieved via regulation of BMP gene expression.

Presentation number: C50

COMPARISON OF IN SITU HYBRIDIZATION CATALYZED SIGNAL AMPLIFICATION SYSTEM AND POLYMERASE CHAIN REACTION FOR THE DETECTION OF HPV

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Human papillomavirus (HPV) is declared as a human carcinogen. Its role as oncogenic factor in the development of cervical carcinoma and its precursor lesions is well established. Paraffin embedded biopsy specimens are a very important resource for molecular and epidemiological studies of HPV infection. Modern molecular biology techniques are sensitive and the quality of routinely processed tissue in clinical laboratories does not always satisfy. Aim of our study was to introduce and optimize two molecular techniques - polymerase chain reaction (PCR) and in situ hybridization (ISH) for the detection and genotyping of HPV on our paraffin embeded material. We compared a sensitivity of three types of consensus and type specific primers in PCR diagnosis of HPV. For ISH we used a new catalyzed signal amplification (CSA) system which supposed to be significantly more sensitive then conventional ISH method. Finally, we compared results of HPV testing obtained with PCR and ISH method. We determined the prevalence of HPV types on our material. PCR HPV L1 consensus region was first amplified with My09/11 primers and Gp5/6+ primers in nested reaction. After reaction with CpI/IIG primers from the E1 region, all specimens were typed with type specific primers for HPV 6/11,16,18,33. Non-radioactive ISH signal amplification system-CSA (Genpoint, DAKO, Denmark) with biotynilated probes for HPV 6/11, 16/18, 31/33/51 (ENZO, NY, USA) was used according to supplier protocols. Both techniques are optimized on our formalin-fixed, paraffin embedded material. Results of PCR independently showed that testing with My09/11 primers followed with Gp5/6+ primers in nested reaction gave the highest number of positive results. ISH and PCR results showed high concordance although there were some exceptions. HPV16 was the predominant type. Only a few comparisons of ISH and PCR on paraffin material have been presented and they showed various degree of concordance between these two assays. We did not find any comparison of the new CSA system with PCR. Our conclusion is that both techniques are valuable, and the best way is to use both assays because that increases the probability for precise virus detection and typing.

Presentation number: C51

INCIDENCE OF NEOPLASMS IN FAMILIES

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Family physician could contribute to the early detection of carcinoma by creating genograms, an important part of family medical records. By using that principle it is possible to follow up families and individuals at risk for the disease. Being acquainted with the heredity of carcinoma we could perform targeted diagnostic procedures which would be efficient and cheaper than very expensive mass screening procedure. Genograms of the patients recently affected by carcinoma from the city area of Požega (approximately 30 000 citizens) were analyzed during 1995. All data analyzed in this study were obtained from the County cancer registry. During this year cancer was diagnosed in 21 man, aged 35 to 74 and in 22 women aged 29 to 70. Family history was taken in personal contact with the patients, or from its family members or from the family physician in charge. Localization of carcinoma in proband and in its sick relatives was analyzed as well. Out of 19 patients with positive family history 8 of them had only one member of the family with the same disease while 11 had more than one cousin with the cancer during three generations. Further, from the all analyzed cousins with the cancer several of them had the same organs involved (2 with respiratory system involved, 3 with the gastrointestinal system involved, 1 with breast and uterus cancer). Relatives of the patients suffering from carcinoma are in the 9 cases sisters, brothers or a first cousins, while in the 4 cases are brothers and ancestors .

Presentation number: C52

HERITABILITY OF DIABETES MELLITUS

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In early dignosis of diabetes mellitus, family physician has to separate individuals at the risk from the other population, control them more often and include them in program of education and prevention. In this work genograms of the patiens with diabetes mellitus type I and II, gestational diabetes and obese patients with positive family history were analysed and heritability index was calculated. The total number of analysed persons was 2147. Nine of them had IDDM (4 females and 5 males) who had 5 relatives with one type of diabetes mellitus, while 107 had NIDDM (81 females and 26 males) who had 81 relatives with diabetes mellitus. Twenty women had gestational diabetes mellitus and 15 of them had relatives with diabetes mellitus (7 where their mothers). Ninety eight obese patients (42 females and 56 males) had relatives with diabetes mellitus (in total number of healthy probands with sick relatives was 142). Heritability index for this population is 69,88%.

Presentation number: C53 (oral presentation)

FLOW CYTOMETRY TECHNOLOGY - APPLICATIONS IN IMMUNOLOGY AND HEMATOLOGY

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Flow Cytometry technology finds its first commercial instruments in the mid-seventies. The early attractiveness of the technology stems from its unique capability of sorting single cells and providing a convenient technique to prepare cell lines and monoclonal antibodies. With time, also its analytical power and capabilities have come to be appreciated and today most laboratories use flow cytometry for its powerful multiparameter capabilities. Indeed, flow cytometry allows the analysis of many thousands of cells to be handled within a few seconds only, including detailed quantitative analysis of multiple subpopulations from complex samples. While originally limited to select leading research institutes, flow cytometry has found its way to the vast majority of research and indeed clinical laboratories over the last ten years. Compactness and simplicity have been provided through the continued development in the electronics and the computer industry. A strong reduction in size as well as cost was supported with the advent of air-cooled lasers. The key to the general utilization of flow cytometers in many hundreds of laboratories was made possible thanks to a major redesign towards ease-of-use. Fixed laser alignments, integrated fluidics and automated instrument setup routines now provide ready access to this powerful technology. The increased intensity of the research work of so many laboratories has resulted in a tremendous progress in the fields of hematology, immunology, oncology and genetics. The general public finding of research areas in cancer, leukemia and AIDS have further fueled new scientific discoveries, leading in turn to more sophisticated and refined analytical subroutines in these research disciplines. While originally used to detect cellular - single - fluorescence, more expensive models, based on dual laser designs, could deliver combined dual fluorescence measurements. With the development of new dyes and better staining methods, today's single laser instruments are capable of measuring at least three different fluorochromes in addition to two light scatter parameters and a time marker. Multiple laser instruments today can handle more than eight simultaneously acquired fluorescence parameters. With the help of these compact flow cytometers many questions can be resolved in the clinical and clinical research laboratories. The applications routinely used in a vast number of these labs range from the detection and the enumeration of stem cells to the screening of residual leukocytes in blood bags. Patients with AIDS can be monitored with absolute CD4 counting. More complex analysis of blood samples is being performed for leukemia diagnosis and clinical follow-up. Based on lineage development schemes, aberrations in the cells of the patients can now be precisely evaluated. Also, with the help of a four color antibody labeling, all major leukocyte subpopulations can be enumerated and compared against target values. Recent studies with this reagent combination indicate that this test might allow for fast and quantitative screening of abnormal cytology samples in the hematology laboratory.

Presentation number: C54

EVALUATION OF ENVIRONMENTAL AND INBORN RISKS FOR CHROMOSOMAL DISORDERS IN CHILDREN

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Prevalence of livebirth Down syndrome (DS) may be affected by the maternal age distribution of the population, and as yet unidentified genetic and environmental factors. Associations between environmental hazards and the prevalence of chromosomal anomalies was not significant. Many studies on seasonality in DS have been performed and have come to different conclusions. It is suggested that seasonal variation in hormone production by the hypothalamus-pituitary-ovarian axis just before ovulation leads to seasonality in conception rates of DS. This study aimed to determine whether there is seasonal variation in the prevalence of DS at birth as a proxy for seasonality in DS at conception. Seasonality or time/space clusters were not observed. In this study we showed that there are zones of geological abnormalities (22%) on the map of Ekaterinburg City. In this zones were born 40,2% patients with chromosomal diseases. All of cases of birth sick child with translocations “de novo” were in places of geological fault earthly surface. The detectable have been carried out by seeking evidence of non-random occurrence of cases (clusters). There have been a some few number of occurrences of clusters of DS in zone of geological fault. In these clusters 39,7% sick child were born. Thus, our study discover a drift of prophylaxis of there were many several heritable diseases.

Presentation number: C55

HEMOPHILIA SITUATION IN NORTHWEST OF IRAN

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Despite molecular advances in carrier detection and prenatal diagnosis, in many rural and border area of Iran and Turkey, hemophilia is still a major problem. RFLP/PCR method for factor VIII mutations screening (intron 18 BCL1 and intron19 HindIII RFLP) were carried out. Questionnaires for all patients and their families who are receiving clothing factor were obtained. The necessary DNA was gathered using peripheral blood and Proteinase K method. PCR and RFLP analysis were carried out according to the literature. In 60 percent of the cases this method is informative and it is possible to give clear answer to the family. Interestingly in many of the villages still it is possible to see affected female with hemophilia A. Even in some families more than 5 members are affected. This is happening mostly because of the consanguineous marriage. In more than 70% of the cases the carrier females are marrying with theirs affected first cousin. Therefore their entire child is affected. Despite prenatal facilities in the region, still they are not seeking any of them but they have access to free colting factors in the hospitals. In more than 60 percent of the cases family has noted to the disease by accident such as dental operation or general surgery. This makes the duty of the cytogenesis and counseling centers much heavier. Even it gives an idea to WHO organizers that if they going to offer any help in establishing any center in developing countries check many factors like cultural, educational and traditional background of needed people. In this study we are going to present the results of our study in carrier detection as well as sharing experience with other colleagues for working in rural area.

Presentation number: C56 (oral presentation)

INSULIN-LIKE GROWTH FACTOR II IN HUMAN CANCER

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Insulin-like growth factor II (IGF II) appears to be involved in the progression of many tumors by a variety of mechanisms including: loss of imprinting (LOI), loss of heterozygosity (LOH) with parental duplication, excessive transcriptional activation, loss of transcriptional suppression or alteration in IGF-binding proteins. IGF II binds at least on two different types of receptors: IGF type 1 (IGF IR) and IGF type 2/mannose-6-phosphate (IGFII/M-6-P) receptors. Overexpression of IGF IR, independent of exogenous peptides, transforms cells to a phenotype of anchorage-independent growth. Physiological levels of IGF IR are an obligatory requirement for the establishment and maintenance of the transformed phenotype at least for several cell types both *in vitro* and in the intact animal. IGF II/M-6-P has tumor suppressor function; it has been identified as a coding region target of microsatellite instability in human gastrointestinal tumors. IGF II/M-6-P normally has two growth-suppressive functions: it binds and stimulates the plasma-mediated cleavage and activation of the latent transforming growth factor β_1 (LTGF β_1) complex, and it mediates the internalisation and degradation of mitogen - IGF II ligand. In genetically unstable gastrointestinal tumors, mutation of a microsatellite within the coding region of IGF II/M-6-P functionally inactivates this gene, causing both diminished growth suppression and augmented growth stimulation. Our recent results suggest that expression of IGF II gene was much higher in gastric cancer than in tissue adjacent to the tumor. There was significant difference between IGF II m RNA expression in tumor tissue from more aggressive diffuse type of gastric cancer than in tissues from intestinal type of gastric cancer. Two IGF II immunoreactive peptides of Mr 7500 and Mr 15 000 were secreted at higher levels in the medium of cultivated gastric cancer cells. IGF II peptide levels in the media collected from culture were significantly higher in diffuse than in intestinal type of cancer cells. IGF I receptor number was significantly higher in cells from diffuse type of tumor compared with cells from intestinal type. The levels of IGF II peptide in conditioned media strongly correlated with ^{3}H-thymidine incorporation and cell proliferation. The overproduction of IGF II resulted in an alteration in routing of newly synthesized cathepsin D. Consequently, IGF-positive gastric cancers have greater invasive potential than IGF II negative ones. On the other hand IGF II/M-6-P expression was decreased in aggressive type of gastric cancer. Some tumors showed mutation in microsatellite within the coding region of IGF II/M-6-P gene that functionally inactivate this gene causing diminished growth suppression of gastric tumors. Alpha IR3 monoclonal antibody diminished strongly cell count, inhibited ^{3}H-thymidine incorporation and inhibited a number of colonies in soft agar of IGF II positive cells in which IGF II gene is overexpressed. Tumors with extensive involvement of the IGF II pathway would be excellent candidates for the therapeutics strategies aimed an interference with this pathway.

Presentation number: C57

THE LONGITUDINAL FOLLOW UP THE DYNAMICS OF TUMOR MARKER CYFRA 21-1 ESTABLISH A FACT OF LOCAL RELAPSE IN PRIMARY NSCLC (36 MONTHS FOLLOW UP STUDY ON 500 PATIENTS)

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The clinical application of cytokeratin marker CYFRA 21-1 was standardized on 3000 patients. In all patients the start value of CYFRA 21-1 was determined before therapy (Electrochemiluminescence method, Roche Diagnostics). Tumor marker CYFRA 21-1 was assessed longitudinally 1 to 36 months after the therapy, or before the next therapy. One to three months after the surgical treatment in 97% of the patients the marker values decreased to the healthy population level, depending on the therapy and TNM stage ($p<0,0001$). The decrease appeared earlier in SQC then in AD and LCLC (Large Cell Lung Carcinoma) patients. Comparing clinical follow up data and CYFRA 21-1 marker as a prognostic factors for local relapse, it was found that tumor marker predicted the local relapse 1 to 12 months earlier in 235 out of 500 patients. Among those the majority had the start value above cut off level, which pointed to the earlier onset of local relapse and shorter survival period. The CYFRA 21-1 values depend on the tumor type, the location of the local relapse and the measurement interval. The assessment of chemotherapy efficiency using CYFRA 21-1 marker will be shown in 141 patients. The elevated cytokeratin marker CYFRA 21-1 value is a biological signal of early events in lung tumor, which is why it becomes unavoidable diagnostic and prognostic marker within clinical procedure.

Presentation number: C58

APPLICATION OF FLUORESCENCE IN SITU HYBRIDIZATION (FISH) IN THE DIAGNOSIS OF CONGENITAL ABNORMALITIES

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Constitutional chromosome abnormalities occur with an incidence of 1.8/1000 live births, and they are often associated with malformations, developmental delay, poor physical growth and mental retardation. Genomic imbalance may involve whole chromosome, chromosomal segment or subtle submicroscopic region. Precise identification of chromosomal rearrangement is very important in determining diagnosis, prognosis and treatment of the patient. In past ten years we are aware of rapid cytogenetic development and introduction of powerful methods for precise chromosome analysis. We report the results of the study performed to evaluate the advantages and limitations of conventional cytogenetics and FISH method in a selected group of 20 patients referred for suspected chromosomal aberrations. Routine chromosome analysis was performed on slides obtained by peripheral blood cultures. FISH analysis of chromosomal rearrangement require sometimes multiple hybridizations and use of multiple probes. In present study commercial whole-chromosome painting, centromeric, subtelomeric or locus specific probes were used. This study demonstrates a number of advantages of FISH method over conventional cytogenetic: 1. The major advantage of FISH over traditional karyotyping is the possibility to investigate genomic imbalance in dividing and nondividing cells. 2. FISH is very useful in the detection of cytogenetically invisible microdeletions such as 22q11 in DiGeorge syndrome. FISH using metaphase and interphase cells may unequivocally diagnose this and other microdeletion syndromes. 3. Combination of metaphase analysis and interphase FISH may be an important tool in the detection of chromosomal mosaics. 4. FISH is powerful method in evaluating the chromosomes involved in translocations, and sometimes may give useful information regarding chromosome break points. FISH method, however, presents some limitations: 1. In FISH experiments the information from single hybridisation is limited to 2-3 chromosome targets, while traditional cytogenetics is able to identify every chromosome in a metaphase spread. 2. FISH application usually requires some prior knowledge of chromosomal aberrations or phenotype that strongly suggests specific microdeletion syndrome in order to select the appropriate probe. 3. FISH cannot detect intrachromosomal rearrangement like inversions or chromosomal segment involved in duplications. It is necessary to point out that FISH cannot replace the traditional karyotyping, but serve as a very useful adjunct to routine chromosome analysis.

Presentation number: C59

CHROMOSOME ABNORMALITIES IN NEUROBLASTOMA: CYTOGENETIC AND FISH ANALYSIS

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Neuroblastoma is one of the most common solid tumors in children. Cytogenetic analysis revealed recurrent chromosome aberrations. The chromosome rearrangements important in tumor evolution are near-diploid or tetraploid chromosome number, terminal deletions of the short arm of chromosome No. 1, double minutes, homogeneously staining regions and amplification of the N-myc protooncogene. These genomic imbalances contribute to the tumor phenotype and carry useful prognostic information. In this report the results of cytogenetic analysis in 10 children with neuroblastoma are reported. The aim of this study is to identify numerical and structural aberrations and determine the frequency and types of acquired genomic abnormalities in our group of children with neuroblastoma. Cytogenetic investigation was performed on slides obtained by direct method of tumor tissue treatment. GTG and CBG-banding method were used for chromosome identification. FISH analysis was carried out using locus specific and chromosome specific centromeric probes (Vysis). The analysis of malignant cells revealed chromosome abnormalities in 9 cases, while in one tumor normal diploid karyotype was identified. Most tumor presented near-diploid or tetraploid chromosome number while one was near-triploid. Aberrations of chromosome No. 1 were the most frequent clonal rarrangemetns including translocations, terminal deletions, duplication and isochromosome. N-myc amplification was detected by cytogenetic and FISH analysis in one patient. This study confirms cytogenetic heterogeneity of neuroblastoma, and advantages of FISH, especially interphase FISH in the detection of genomic changes in neuroblastoma.

Presentation number: C60

PARENTAL ORIGIN OF THE STABLE DICENTRIC X CHROMOSOME

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Dicentrics are chromosomes with two centromers. This structurally aberrant chromosomes are highly unstable, and rare constant member of human karyotype. On the other hand, stable dicentrics usually present one active and one inactivate centromere and behave like monocentrics. The investigation conducted so far disclosed several types and different mechanisms of origin of dicentric chromosomes. In this report we present the results of cytogenetic and molecular study in a 5-years old girl with mild dismorphism, grow retardation, behavioral problems and structural rearrangement of X chromosome. Cytogenetic analysis was performed on short-term lymphocytes cultures of the proband and her parents. Chromosomes were GTG and CBG banded, while X chromosome inactivation was studied using RBG method. Fluorescence in situ hybridisation (FISH) was carried out on patient's metaphase chromosomes and interphase cells with X chromosome painting and centromeric probes. The parental origin of aberrant chromosome was investigated by DNA polymorphisms analysis. Barr body analysis was performed on buccal mucosa smears stained by routin acetic orcein. Both parents presented normal karyotype. Leukocyte chromosome analysis of the proband revealed one normal and one aberrant X chromosome. The rearranged X is a large submetacentric with one primary constriction and two blocks of C-stainig. FISH analysis revealed that aberrant chromosome is composed entirely of the X chromosme material. Interphase FISH with alpha satellite X centromere probe revealed two mosaic cell lines. Three signals were observed in 84.5% and one signal in 15.5% of interphase cells. Replication analysis showed that the normal X always-early replicates while the dicentric was late replicating in all investigated cells. The buccal smear revealed X-chromosome positive cells, and in some cells the Barr body was bipartite. Molecular analysis of DNA polymorphisms indicates that the dicentric is of paternal origin. Based on this study the karyotype of the patient is 45,X/46,X, psu idic(X)(q22.3), with the trisomy Xpter -> q22.3 and monosomy Xqter -> q22.3. We suggest that dicentric X is the result of isolocal break in the two chromatids of the paternal X chromosome, subsequent rejoining of broken ends, followed by inactivation of one centromere.

Presentation number: C61

CYTOGENETIC, FISH AND MOLECULAR ANALYSIS IN THE GIRL WITH UNBALANCED t(X;6) AND HER MOTHER PRESENTING t(6;10)

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It is well known that unbalanced translocations are mostly familiar, and cytogenetic analysis of parents usually reveal the reciprocal exchange of chromosome segments identified in a given unbalanced rearrangement. Here we report on the girl with unbalanced t(X;6) and unusual mosaicism involving abnormalities of chromosomes 6 and 10 in the mother. Our patient is 6-year-old girl with short stature, failure to thrive, moderate mental retardation, deficiency of IgG, mild facial dysmorphism, and no malformation or abnormalities of visceral organs. Cytogenetic analysis was performed on slides obtained by peripheral blood cultures of the proband and her parents. The slides were stained by GTG and RBG banding methods. FISH was carried out using whole chromosome painting, and centromeric probes specific for chromosomes 6 and X (Vysis). In this study we used polymorphic DNA markers to investigate the parental origin of aberrant chromosome. The chromosome analysis of the proband showed a 46, X, der(X)t(X;6)(q22;p11) karyotype. The derived X was late replicating in all investigated cells with variable spreading of X chromosome inactivation onto translocated 6p. The molecular analysis revealed that the chromosome X involved in the rearrangement is of the paternal origin. The normal karyotype was observed in the father, while the mother presented 46,XX/ 46,XX, der(10)t(6;10)(p11;p11). The mother is a mosaic with unbalanced t(6;10) in 4% of cells. To best of our knowledge, this unusual mosaicism has not been reported yet. We suggest that chromosome constitution in the mother is due to postzygotic recombination involving chromosome 6 and 10 at S/G2 phase of the cell cycle. Cytogenetic analysis of the proband revealed unbalanced karyotype resulting in a trisomy for the segment 6pter->p11, and loss of the segment Xqter->q22. She had only discrete facial features characteristic of partial trisomy 6p. This mild phenotypic expression is due to X chromosome inactivation spreading onto translocated 6p.

Presentation number: C62

PHYSICAL MAPPING AND CHARACTERIZATION OF A CONSTITUTIONAL TRANSLOCATION T(2;3)(Q33;Q21) ASSOCIATED WITH FAMILIAR RENAL CELL CARCINOMA

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We have identified a Polish family in which multifocal clear cell renal carcinoma segregates with a balanced constitutional chromosome translocation, t(2;3)(q33;q21), possibly with translocation positions similar to those of the renal cell cancer-associated t(2;3)(q35;q21) reported in a Dutch family. YAC and BAC contigs encompassing the 2q and 3q breakpoints were constructed and BACs crossing the breakpoints were partially sequenced. All known regional markers, genes and ESTs were mapped relative to the contigs, as well as to our breakpoint sequences. Two single ESTs map within the 2q breakpoint BAC, while the repeat rich 3q breakpoint region is gene poor. Physical mapping suggests that the 3q break is in 3q13, possibly near the border with 3q21 and is 10-20 megabases centromeric of the Dutch 3q break. Physical mapping illustrated that the 2q break is closely telomeric to the 2q31 *FRA2G* site, suggesting the band position 2q33 is correct. Molecular mapping data available for the Dutch 2q break was insufficient to determine similarity of the Polish and Dutch 2q breaks, although the cytogenetic data suggests different band positions. Characterization of full-length cDNAs for the ESTs near the 2q break will determine if a gene(s) is altered by this familiar RCC-associated chromosome translocation.

Presentation number: C63

A NOVEL GENE (DIRC1) ON CHROMOSOME 2 DISRUPTED BY A CONSTITUTIONAL TRANSLOCATION T(2;3)(q33;q21) COSEGREGATING WITH FAMILIAR RENAL CELL CARCINOMA

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We have described a Polish family with multifocal clear cell renal carcinoma that segregates with a reciprocal, balanced chromosome translocation, t(2;3)(q33;q21); we have characterized the genomic regions surrounding the 2q and 3q breakpoints by building YAC and BAC contigs, partial sequencing and localizing genes, STSs and ESTs. Here we describe the full-length gene for EST AI468595. We have designated this 57 kb gene at chromosome region 2q33, the *DIRC1* gene (Disrupted in Renal Cancer) and show that in this family the gene is disrupted by the translocation. The 1.5 kb mRNA encodes a predicted protein of 104 amino acids with a molecular weight of ~11.4 kDa. Low level expression of *DIRC1* can be detected by RT-PCR amplification in adult placenta, testis, ovary, and prostate and fetal kidney, spleen and skeletal muscle tissues. A GFP-Dirc*1* fusion protein has been expressed *in vitro* and polyclonal anti-Dirc*1* peptide serum prepared. A panel of tumors and cancer cell lines have been examined for *DIRC1* mutations but only a rare polymorphism has been observed. The two familiar tumors examined showed loss of the derivative 3 chromosome, as has been observed in Dutch kindreds with t(2q;3q) and Japanese family with t(1q;3q) associated renal cancers. Mutations in the second *DIRC1* allele were not observed in the two tumors. Additional studies will be required to determine if disruption of the *DIRC1* gene contributed to development of clear renal cancer in this family. But so far, our results further support a three step model for tumorigenesis in this family. A constitutional translocation t(2;3) increased the susceptibility to loss of derivative 3 chromosome which might be then followed by somatic mutations of the RCC-related *VHL* gene located in the remaining copy of chromosome 3.

Presentation number: C64 (oral presentation)

OSTEOGENESIS IMPERFECTA: CURRENT CONCEPTS

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Osteogenesis imperfecta (OI), or brittle bone disease is a heritable disorder characterized by increased bone fragility. Four different types are commonly distinguished that range from a mild condition (type I) to a lethal one (type II). Types III and IV are the severe forms surviving the neonatal period. In most cases, there is a reduction in the production of normal type I collagen or the synthesis of abnormal collagen as a result of mutations in the type I collagen genes. These "classic" forms of OI will be described in this paper in great detail. There are instances, however, where alterations in bone matrix components, other than type I collagen, are the basic abnormalities. Recently, three such discrete types have been identified by histomorphometric evaluation (types V and VI) and linkage analysis (Rhizomelic OI). They provide evidence for the as yet poorly understood complexity of the phenotype-genotype correlation in OI. Here will also be discussed a bisphosphonates treatment as well as a fracture management and surgical correction of deformities observed in the patients with OI. However, ultimately, strengthening bone in OI will involve steps to correct the underlying genetic mutations that are responsible for this disorder. In this review we will also give different genetic therapeutic approaches that have been tested either on OI cells or on available OI murine models.

Presentation number: C65 (oral presentation)

SNP GENOTYPING USING MEGAPLEX PCR AMPLIFICATION AND LINEAR PROBE ARRAYS

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There has been some interest expressed in having a highly discriminating single nucleotide polymorphism (SNP)-based assay for forensic applications. Because SNPs have only 2 alleles compared to 5 or more alleles in STR loci, many more SNPs (~50) are required to provide a significant level of discrimination between individuals. Therefore, to be of most value to the forensics community, 50 SNPs should be co-amplified from a few nanograms of DNA and the genotyping results should be accurate and quantifiable. Most of the available SNP genotyping assays are designed to amplify 1-10 SNPs per PCR amplification using over 10 ng DNA for each reaction. Consequently, 5-50 amplification reactions per sample would need to be run to achieve a level of discrimination appropriate for human identification applications. As the number of required amplification reactions increases, so do the chances of sample mix-up and contamination. The cost and set-up time of the assay are also greatly increased when more PCR amplifications have to be performed per sample. Perhaps of greatest importance to the forensics community is the need to consume a total of 50 or more nanograms of DNA per sample to obtain genotypes at all of the SNPs. We have been developing SNP-based genotyping assays for the cardiovascular and inflammatory diseases research communities. At present, we routinely co-amplify 50-55 SNPs in a single reaction and perform two hybridizations to obtain genotypes. Under current assay conditions, accurate results can be obtained from as little as 5 ng and from as much as 200 ng DNA. Quantitation of DNA is not essential for the SNP assay, a feature that makes it very attractive to the research and diagnostic communities. In our assay, SNP detection is performed using an array of SSO probes immobilized in a line format on a strip of nylon membrane. Each strip can accommodate 58 probes and, using new instruments, up to 48 strips can be processed at a time. The hybridization instrument is being modified so that an image of the strips can be generated and transferred for analysis without removing the strips from the trays. By choosing a different panel of SNPs and further optimizing the megaplex PCR conditions, this assay could be valuable for human identification applications.

Presentation number: C66

INCIDENCE OF HIV, HBV AND HCV INFECTIONS DETECTED BY PCR METHOD IN BIH

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Direct monitoring by sensitive nucleic-acid tests would provide data accurately to measure the risk and to assess risk-reduction procedures. We adapted PCR method for investigation the incidence of HIV, hepatitis C virus and hepatitis B virus infections during last two (HBV, HCV) or three years (HIV) in Bosnia and Herzegovina. For PCR testing individual plasma samples were analyzed. Viruses were concentrated by centrifugation and nucleic acids were extracted by QIAamp nucleic acids extraction method or by guanidinum isothiocyanate (GTC)/silica gel extraction of nucleic acids. In the case of HCV i HIV an additional reverse transcription step before PCR amplifications was performed. Amplified virus-specific sequences by specific complementary primers were detected by agarose-gel electrophoresis. Using this validated methodology routine we checked 184 persons on HIV infection from July 1998 up to the end of 2000. 8 (4.4%) of them were HIV-RNA positive. One of positive persons was a patient from Clinic of Infectious Diseases, other 7 HIV-RNA positive (6 injection drug users and 1 from promiscuity-risk group) were tested on their personal request. During 1999 and 2000 we performed 54 tests for identification HBV infected individuals and found 11 (20.4%) HBV positive persons. 7 of them were patients from Clinic of Gastroenterohaepathology, 1 from Pediatric Clinic and 3 from external medical institution. The HCV tests for the same period included 148 tested persons. 9 (6.1%) of them were HCV-RNA positive. It was 1 patient from Clinic of Infectious Diseases, 1 patient from Institute of Nephrology, 5 patients from Clinic of Gastroenterohaepathology and other 2 patients were from external medical institution. None of PCR-tested persons had HBV/HCV or other dual positivity. HIV, HBV and HCV have been the viruses most intensively subjected to PCR analysis. It was necessary to have an overview of incidence these types of infectious diseases for a transition-state country such as BiH. In consideration of number of our population there is relatively high percentage of HIV, HCV and especially HBV positively. It would be necessary to realize better transmission control preventing infections by these viruses in BiH. Those are the results of PCR diagnostics without serological investigations.

Presentation number: C67

METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) C677T MUTATION ASSOCIATION WITH SCHIZOPHRENIA AND DEPRESSION

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Schizophrenia clinical phenotype is heterogeneous entity with respect to response to medication and clinical outcome. It is also possibly influenced by genetic heterogeneity. T677 allele encodes for a thermolabile enzyme associated with hyperhomocysteinemia if present in homozygous genotype, and is a common missense mutation (C677T) of the methylenetetrahydrofolate reductase (MTHFR) gene. It has been reported to be associated with schizophrenia, depression and bipolar disorder. Aim of our study was to estimate frequencies of the C677T mutant alleles and genotypes and to determine possible association of mutated allele with schizophrenia and depression. We compared a group of 69 patients with schizophrenia (diagnosed as F20 and F25 according to International Classification of the Disease - ICD 10), 46 depression patients (F33) and a group of 111 healthy volunteers, with regard to MTHFR allelic and genotype frequency. Among 111 healthy controls, C677T genotype frequencies were as follows: 51.4% C/C, 47.7% C/T and 0.9% T/T, while allelic frequencies of C and T alleles were 75.2% and 24.8%, respectively. In schizophrenia group of patients, genotype frequencies for the C/C, C/T and T/T were 53.6%, 36.2%, and 10.1%, respectively. In depression group there were 43.5% C/C, 47.8% C/T and 8.7% T/T. Allelic frequencies of C and T alleles in schizophrenia was 71.7% and 28.3% while the C and T frequency in depression was 67.4% and 32.6% respectively. Genotype frequencies between schizophrenia and healthy subjects differed significantly (P=0.009 - Chi square test), what emerges from the difference for the T/T genotypes (P=0.011; Z-test), and value of the T/T odds ratio (OR= 12.42; 95%CI 2.28-67.46). Somewhat lower level of significance was observed among depression patients (P=0.037 - Chi square test), P=0.042 - T/T genotype Z-test, and T/T odds ratio (OR= 10.48; 95%CI 1.69-64.95), compared to controls. Our preliminary results strongly suggest that MTHFR gene homozygosity could be involved in the pathogenesis of schizophrenia and probably in somewhat smaller extent is linked to pathogenesis of depression.

Presentation number: C68

ATTENUATED ADENOMATOUS POLYPOSIS OF THE COLON DUE TO A MOSAICISM FOR A DELETION REMOVING EXONS 2-14 OF THE APC GENE

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Attenuated Adenomatous Polyposis of the Colon (AAPC) is a variant of the familial adenomatous polyposis characterized by the presence of <100 polyps in the large intestine, which eventually progresses to malignancy in the 4th decade of life. The molecular defect in most patients with AAPC is the presence of point mutations or small deletions/insertions in the very 5' or 3' parts of the APC gene. In this paper we describe a patient with AAPC from Macedonia in whom we detected a deletion spanning exons 2-14 of the APC gene. The patient was diagnosed with an advanced adenocarcinoma of the caecum at the age of 39; at the same time 3 additional polyps, 1-2 cm in size and located in colon ascendens, were also detected. The patient was treated with a total colectomy but she died 2 years after the primary surgery due to disseminated metastatic disease. Detailed DNA analysis did not reveal the presence of any mutation in the APC gene. However, nested RT/PCR analysis of lymphocytic RNA revealed the presence of an abnormal fragment which was shown to be an APC transcript containing complete exon 1 linked to exon 15. Although these data were consistent with the presence of a deletion removing exons 2-14, detailed mapping of the APC gene region did not reveal any abnormal fragments. A plausible explanation of our results is that the patient is a mosaic for an exon 2-14 deletion that is present only in a small subset of cells which precludes its detection by standard Southern blot analysis. The AAPC phenotype in our patient is consistent with this hypothesis, i.e. a deletion present in only a subset of cells resulting in a truncated product containing only the very N-terminal domain of the APC protein. Our data strengthens the notions that deletional types of defects in the APC gene should be considered when performing molecular analysis of FAP patients and that mosaicism can be present in cases in which the mutation could not be detected by standard methodologies.

Presentation number: C69

GENETIC POLYMORPHISMS OF THE RENIN-ANGIOTENSIN SYSTEM AND PROGRESSIVE RENAL FAILURE IN CHILDREN

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Progressive renal failure is a complex phenotype that results from an underlying kidney disease and superimposing environmental and genetic factors. The renin-angiotensin system (RAS) is likely to be important in the progression of renal diseases because of its effect on tissue hemodynamics and glomerular cell function. Therefore, the genes of the RAS and their relation to renal diseases are of great interest. The aim of our study was to evaluate the role of polymorphisms in the genes encoding for components of the RAS in the development and /or progression of renal failure. We studied the effect of the insertion/deletion gene polymorphism of the angiotensin-converting enzyme gene (ACE I/D) and the A1166C gene polymorphism of the angiotensin type 1 receptor gene (AT1R A1166C) on the rate of renal function deterioration in 107 children patients (Caucasians - Eastern Slovakia) with a variety of renal diseases. In patients (12,2 ± 6,6 years) was study the relationship among the ACE, AT1R genotypes and adverse renal progression, serum creatinine (S-cr) and 24-h proteinuria. Individual genotypes were determined by polymerase chain reaction amplification using allele-specific primers. We were unable to find any correlation with the presence of ACE-DD genotype and adverse renal progression. Only AT1R polymorphism significantly associated with progressive deterioration of renal function. The C-allele of the AT1R polymorphism was associated with an increased risk of elevated S-cr (odds ratio (OR) 6,0; 95% confidence interval (CI) 1,7-24,5) and with an increased risk of proteinuria (OR 3,4; 95% CI 1,3-8,9). Morever, AT1R gene polymorphism shows synergy with the deleterious effects of the ACE DD genotype distribution on elevated S-cr. Among the DD patients with the serum creatinine >170 mmol/l were 100 % CC/CA, compared to 23,5% among the DD patients with the serum creatinine <170 mmol/l (p=0,004, Fisher exact test). This study provides evidence that progression of renal disease is more rapid in CC/CA genotypes of the AT1R gene polymorphism. Our results indicate a synergistic contribution of ACE and AT1R polymorphisms to the risk of increased serum creatinine level. The data provide further evidence of the possible role of the RAS system genes in the rate of progression of renal failure, although further studies are required to evaluate the role of this and other proposed candidate genes in renal diseases.

Presentation number: C70

GLYCOSYLATION OF PLACENTAL PROTEINS IN MISSED ABORTION

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Contemporary understanding of missed abortion, as a case of spontaneous abortion where embryo after death is retained in the uterus of the mother for four weeks or more, is very poor. Therefore we have undertaken comparison of glycosylation patterns of placental proteins between samples from normal pregnancy and samples from missed abortion. Olygosaccharide branches were detected by Western-blot method using lectins: SNA, DBA, PHA-E and UEA-I, after preliminary separation of proteins by discontinuous SDS-PAG electrophoresis. Comparison of the recognized sugars from both samples at the same gestational age, identifies changes in protein glycosylation between normal and pathological placentas. Lectin UEA-I detects in normal placenta during the ninth week of gestation the glycoprotein GP 74, which is absent from sample of missed abortion, indicating reduced fucosylation of this glycoprotein. Lectin DBA identifies during eleventh week presence of GP 105 in placental glycoprotein pattern of normal pregnancies, which is missing in pattern from missed abortion. Lectin PHA-E identifies GP 71 during thirteenth week only in the control samples of normal placenta, while it is absent from the pathological placenta of the same gestational age. There are other glycoproteins identified with lectins used, displaying similar relative quantitative proportions in normal and pathological placentas, as well as those displaying minor variations in microheterogeneity between normal pregnancy and missed abortion. It was interesting to note the appearance of glycoprotein GP 25 with lectin SNA in the sample of missed abortion only during tenth week of gestation. These preliminary results encourage the hypothesis that olygosaccharide structures of placental glycoproteins play important role during placental development in normal gestation and their potential malfunction in missed abortion.

Presentation number: C71

FLUORESCENCE IN SITU HYBRIDIZATION IN DETECTION OF CHROMOSOME ABNORMALITIES IN PATIENTS WITH ACUTE LEUKAEMIA

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Fluorescence in situ hybridization (FISH) is molecular biology techniques, which enhanced visualization and identification of specific DNA and m-RNA sequences (part of genes, genes, specific chromosome regions and whole chromosomes). FISH is using metaphase chromosomes and interphase nucleus. Method is based on the specific hybridization of two complementary sequences. This is also one of basic principles of molecular biology. Signals are depending on the size of the probe that means that different probes have different signal size. Signal is concentrated in the specific chromosome region e.g. Centromere. To obtain relevant result more than 200 interphase nuclei should be analyzed separately. For metaphase analysis more than 50 good metaphases should be analyzed. Signals are analyzed by using fluorescent microscope and different kind of filters (DAPI, FITC). FISH resolution size is 1 Kb, which allows us to analyzed specific changes, which are to small for classical cytogenetic analysis. To evaluate efficacy of FISH in detection of chromosome changes in patients with acute leukemia 40 bone marrow aspiration samples were analyzed. In 50% of above mentioned patients chromosome changes have been obtained using classical cytogenetic analysis (G-banding). In 15% of those patients whom had normal karyotype FISH analysis showed specific chromosome findings. In conclusion we could stated that FISH method is reliable method for detection of specific chromosome findings which are invisible by classical chromosome techniques. But the main disadvantage of FISH techniques is that FISH is discovering only specific chromosome changes and is unable to detect other chromosome changes. In those cases classical cytogenetic methods are superior to any FISH techniques.

Presentation number: C72

A NEW FACTOR V LEIDEN PCR-SSCP DETECTION METHOD

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Factor V Leiden mutation is the most common cause of thrombophilia. The overall prevalence of Factor V Leiden in healthy population is rather high, and it is therefore necessary to develop and introduce a simple and cost-effective method for screening this genetic abnormality in population at increased risk. We herein present a new, simple, reproducible and cheap PCR-SSCP method that allows us to identify the carriers of Factor V mutation. Our PCR-SSCP method for detection of Factor V Leiden mutation was optimized using the precast GMA™ gels in the Elchrom Scientific SEA 2000 apparatus. We performed PCR-SSCP analysis on 109 whole blood DNA samples previously genotyped by PCR-RFLP method using the Mnl I restriction endonuclease. Exon X of Factor V was amplified using previously described primers and denatured for 5 minutes at 95°C. Denatured PCR products were loaded and run overnight on precast GMA™ gels in SEA 2000 electrophoresis apparatus. Gels were stained for 30 min with SYBR Gold nucleic stain and photographed at 254 nm with Polaroid 667 film. We observed reproducible and uniform band patterns for mutant and wild type variants of Factor V. Genotype frequencies were 92.7, 7.3 and 0 for wild type, heterozygotes and homozygotes respectively. Allele frequencies were 96.3 for wild type and 3.7 for mutated allele. The reported frequencies were consistent with those reported in the literature for population of whites. Our PCR-SSCP results were verified using the restriction pattern after Mnl I digestion of the same PCR product. The concordance between both methods was 100%. Technique is highly reproducible because of the high performance characteristics of GMA™ gels and constant temperature maintenance by Elchrom Scientific SEA 2000 apparatus. In conclusion, we recommend herein presented PCR-SSCP procedure as a highly reliable, time saving and cost effective Factor V Leiden detection method.

Presentation number: C73

UGT1A1 GENETIC VARIABILITY AND GILBERT SYNDROME IN CROATIAN PEDIATRIC POPULATION

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Homozygous mutation in UGT1A1 gene - $(TA)_7$ (allele 7) is associated to Gilbert syndrome (GS), compared to wild type $(TA)_6$ (allele 6). Aim of our study was to estimate allelic and genotype frequencies in healthy children and children diagnosed as Gilbert syndrome. Association of mutant 7/7 genotype with elevated total serum bilirubin (TSB) concentration was also determined. 48 control subjects (mean age 15 years; 63% females) and 48 patients (mean age 15 years; 52% females) who were clinically diagnosed or suspected as Gilbert syndrome were included in the study. Associated diagnose was anorexia nervosa (AN) in 8 patients. For every subject TSB concentration was measured (μmol/l) and UGT1A1 genotype was determined by PCR. Genotype distribution among controls was: 42% 6/6 (TSB 9.83; SD 5.60), 40% 6/7 (TSB 10.79; SD 6.05) and 19% 7/7 (TSB 20.39; SD 13.5). The allelic frequencies in this group for 6 and 7 alleles were 61% and 39% respectively. In GS group 6/6 genotype was not found, and 6/7 and 7/7 genotype distribution was 6% (TSB 25.73; SD 8.66) and 94% (TSB 41.88; SD 21.93) respectively. Allelic frequencies of 6 and 7 alleles were 3% and 97% respectively. There was no significant difference for genotype, allelic frequencies and TSB between males and females within groups. Genotype and allelic frequencies between GS and controls differed significantly: CHI(p)=0.001. TSB values among controls with 7/7 compared to 6/6 or 6/7 genotypes revealed significant difference: ANOVA(p)=0.001-0.011. TSB difference between 6/7 and 7/7 genotypes in GS group was not significant, what could be explained with small number of 6/7 genotypes. Among AN patients two were of 6/7 genotype while others were 7/7. Our preliminary results confirmed association of UGT1A1 7/7 genotype with Gilbert syndrome and elevated serum TSB values. Furthermore, it seems that there is some association between AN and clinical expression of GS. The future objective is to continue this investigation in order to estimate UGT1A1 frequencies in Croatian population on a larger number of subjects.

Presentation number: 74 (oral presentation)

APPLICATIONS OF ABI PRISM TECHNOLOGIES FOR HUMAN DISEASE RESEARCH AND MOLECULAR DIAGNOSTIC

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DNA is a powerful source of useful information for diagnostic purposes. Interrogating DNA we can have answers that can help us understand, predict, cure several diseases. The information we can get through DNA goes from presence or absence, to quantitation, to identification and genotyping. Applied Biosystems automated multicolor fluorescent detection technologies enable to get precise answers from nucleic acids. Real Time PCR is the method of choice for getting yes or no and quantitative detection of pathogens and viruses as well as accurate gene expression profiles. Capillary Electrophoresis based Sequencing and Fragment Analysis offers several solutions dedicated to Mutation Detection, SNP screening, Genotyping and Identification. Some of the most common applications of this technology in Molecular Diagnostic are: HIV Genotyping, HLA Typing, mutation analysis of hereditary diseases like Cystic Fibrosis and Fragile-X.

Presentation number: C75

PRENATAL DIAGNOSTICS OF TUBEROUS SCLEROSIS, USE OF LOSS OF HETEROZYGOSITY EXAMINATION BY QUANTITATIVE FLUORESCENT PCR

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Tuberous sclerosis (TSC) is a frequent hereditary autosomal-dominant disease characterised by hamartomas developing in many organs. Disorder is caused by mutations affecting either of the presumed tumor-supressor genes, TSC1 and TSC2. About two-thirds of the TSC cases are sporadic, representing new mutations. Loss of heterozygosity (LOH) was proven in 20 – 70 % of hamartomas. Prenatal diagnosis is often hampered by lack of detection of causing mutation. More diagnostic methods are needed to provide genetic counselling for more TSC patients and their families. Mutation analysis of TSC patients involves Southern hybridization, SSCP analysis of 21 TSC1 and 41 TSC2 exons followed by sequencing and ASO hybridization. Analysis of flanking polymorphic markers for linkage purposes and LOH test in DNA from biopsies and paraffin embedded hamartomas is performed on capillary electrophoresis with fluorescent PCR products. In 45 unrelated Czech TSC families 4 prenatal diagnosis were performed since 1998. One diagnosis excluded large deletion by Southern hybridization, ASO probes were prepared for two families with known mutation and prenatal linkage analysis was done in the family with multiple TSC patients. Search for causing mutations is very laborious, time consuming and the efficiency varies from 30 – 80% depending on precision of clinical diagnosis, screening method and involved gene. A case is demonstrated here, where linkage analysis in family with multiple affected persons could be replaced by test of LOH in proband`s hamartoma. Use of LOH test for prenatal diagnosis is also shown in two sporadic cases. We suggest, that examination of LOH is useful for prenatal diagnostics in special cases. First situation is a demand for prenatal diagnostics in couples where one partner is a sporadic case and the female is already pregnant, so that there is not enough time for mutation screening. Other case arises, when mutation screening yielded no causing mutation and the sporadic patient is planning own baby.

Presentation number: C76

MOLECULAR BIOLOGICAL TECHNOLOGIES FOR THE IDENTIFICATION AND DETECTION OF ANTIBIOTIC RESISTANT BACTERIA IN RAPID DIAGNOSTICS OF NOSOCOMIAL INFECTIONS

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The aim of this work is to carry out the comparative analysis on the basis of the author's own investigation and the data from original papers in molecular biological technologies of identification and testing for antibiotic susceptibility of bacteria. The trends in development and application of molecular biology techniques for the rapid diagnostics of hospital infections and the monitoring antibiotic resistance of microorganisms is analyzed. The traditional microbiological methods are antimicrobial susceptibility test discs, gas chromatography, immunogaschromatographic method, polimerase chain rection (PCR). The prevalence of pathogens from clinical isolates were identified. The carried study showed that the most frequent of dangerous microorganisms were methicilin resistant Staphylococcus aureus strains (MRSA) and vancomycin resistant Enterococcus spp., Streptococcus pneumoniae with resistance to penicillin and Chlamydia spp. Currently existing traditional microbiological methods for determination of pathogens are laborious and time consuming. Cellular fatty acids and ester linked fatty acids of the membrane phospholipids are now firmly established as the means of rapid identification of monocultures or as tool to assess the microbial community structure in clinical specimens. We elaborated an effective express immunogaschromatographic method that involves the use of antibody labeled to detect specific cell surface marker of Staphylococcus aureus strains. This immunogaschromatographic technique was developed to test the detection of patogens in urine and blood. These studies have demonstrated that the polymerase chain rection (PCR) and sequence of the 16S rRNA genes as powerful alternative in clinical diagnosis of nosocomial infection. The possibilities of applying PCR amplification with sequencing the 16S rRNA genes have been revealed to identify biochemically inert and closely related bacterial strains. It has been shown to have the discriminatory power for the strain typing. Also, oligonucletide PCR microchips array has emerged as a new molecular technique to identify pathogenic microorganisms and testing of antimicrobial resistance. The trends in the development and application of biochip technology for epidemiological monitoring of nosocomial infections has been reviewed. The advance in the application of microchips in clinical research are represented. Recent developments in coupled gas chromatographic techniques makes fully automated the determination of bacteria in clinical microbiology. Combined complex use molecular biological technology (PCR amplification on the microchip array) to perform bacterial identification and detection of drug-resistance genes allow to monitor pathogenic microorganisms. Reliable methods of infection control are needed for elaborating effective biochips for resolving issues of therapeutic efficacy. All these technology posses a clear perspective for future application in rapid diagnostics of intrahospital infections.

Presentation number: C77 (oral presentation)

STEM CELL TRANSPLANTATION FROM FULL - HAPLOTYPE MISMATCHED DONORS IN LEUKEMIA

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Hematopoietic stem cell transplants are being performed more and more often at our Center since we showed that an immuno-myeloablative conditioning regimen followed by the infusion of large numbers of extensively T-cell-depleted stem cells prevents both graft rejection and GvHD (Aversa et al. Blood 1994,84:3948; N Engl J Med 1998,339:1186). To date, a total of 112 acute leukemia (53 AML, 59 ALL) patients have been treated. Ages ranged from 2 to 53 years, median 24. Almost all were at high-risk for relapse and TRM because of the advanced stages of disease at transplant (11 bad-risk CR I, 49 in CR II, 52 in relapse). The first 36 patients received an inoculum made up of lectin-separated bone marrow and PBPCs. The 43 patients in the second pilot study received PBPCs depleted of T-cells by one-step E-rosetting followed by a positive selection of the CD34+ cells with the CellPro device. Since January 1999 we have been using the CliniMacs instrument to select CD34+ cells for 33 patients to date. All grafts contained a median of 10x106/kg CD34+ cells. When we started using positive selection of the CD34+ cells we reduced the number of T-cells in the final inoculum by one log (from 22 x 104/kg of the lectin-separated grafts to the current 1 x 104/kg). Furthermore, in October 1995 we substituted fludarabine for CY and in January 1999 we decided to stop post transplant G-CSF administration to the recipients because of its immunosuppressive effects (Volpi et al. Blood 2001;97:2514). Overall, full donor-type engraftment was achieved in 108/112 (96.5%), 9 after second transplants. Acute GvHD grade II-IV occurred in 9 patients (6/36 lectin-separated group and 3/76 CD34-selected group). EFS at 7 years is 35% for AML (n=53) and 13% for ALL (n=59) patients. The patients who were in either first CR (n= 7) or stable CR II (n=20) have 0.50 probability of EFS at 7 years. TRM and EFS are even better if we analyze the 11 patients in stable CR II who benefited from the changes we introduced in the last study (0.10 and 0.70, respectively). Today high risk acute leukemia patients are treated at less advanced stages of disease, receive a reasonably well-tolerated conditioning regimen, and benefit from advances in post-transplant immunological reconstitution. T-cell depleted mega dose stem cell transplant from a mismatched family member, who is immediately available, can be offered as a viable option to candidates with high risk acute leukemia.

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ACKNOWLEDGMENTS

The Second European-American Intensive Course in Clinical and Forensic Genetics gratefully acknowledges the support of the following sponsors as per date of printing:

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We would also like to thank the following institutions and organizations for supporting The Second European-American Intensive Course in Clinical and Forensic Genetics:

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