

Rapid detection of GYPA, LDLR, HBGG, D7S8 and GC alleles by real-time fluorescence PCR

M. Nata and M. Hashiyada

Division of Forensic Medicine, Department of Public health and Forensic Medicine, Tohoku University Graduate School of Medicine, Japan

We have developed an allele-specific TaqMan Polymerase Chain Reaction (PCR) assay in the glycoprotein A (GYPA), low density lipoprotein receptor (LDLR), hemoglobin G (HBGG), D7S8 and group specific component (GC). This assay combines allele-specific primers and hybridization of the TaqMan probe to the target DNA. The TaqMan probe (Applied Biosystems, USA) contains a reporter dye at the 5' end and quencher dye at 3' end of the probe. During PCR, AmpliTaq Gold DNA polymerase (Applied Biosystems, USA) cleaves the TaqMan probe by its 5' nuclease activity. As a result of separation of the reporter dye and the quencher dye by cleavage of probe, amount of fluorescence of the reporter increases, and accumulation of PCR products is detected. Genotypes are detected according to the different threshold cycles of the each allele-specific primer, respectively. The results of the allele-specific TaqMan PCR assay were consistent with the results obtained from PCR-reverse dot hybridization method by AmpliType PM PCR Amplification and Typing Kits (Applied Biosystems, USA).

We have also applied the real-time fluorescence PCR based on SYBR Green, which is double-stranded DNA-selective fluorescent dye, to genotyping of 5 loci. Genotypes are separated according to the different threshold cycles of the each allele-specific primer, without the TaqMan probe.

These two assays could be applied to forensic investigation because of their simplicity and rapidness.

Dr. Masayuki Nata, Division of Forensic Medicine, Department of Public health and Forensic Medicine, Tohoku University Graduate School of Medicine, Seiryomachi 2-1, Aoba-ku, Sendai 980-8575, Japan, Tel. 00 81 (0)22 717 8110; Fax: 00 81 (0)22 717 8112, e-mail: nata@forensic.med.tohoku.ac.jp

Validation of SNPs as molecular markers for individual identification

E. Petkovski^{1,2}, C. Keyser², B. Ludes² and R. Hienne¹

¹*CODGENE, Strasbourg, France*

²*Institute of Legal Medicine, University Louis Pasteur, Strasbourg, France*

Nowadays, the DNA markers used for individual identification in forensic science are on one hand based on repeat sequences (STR and VNTR) on genomic DNA and on the other hand the chondrioms' (mtDNA) hypervariable regions 1 and 2. An alternative to those classical markers could be single nucleotide polymorphisms (SNPs).

SNPs, alterations in DNA sequence at a single nucleotide position, are and will be even more used in medical and agricultural research. Presenting advantages over other markers (STR, VNTR, mtDNA and Y chromosome markers) SNPs should be carried over for forensic purposes.

Even if most of SNPs are bi-allelic and therefore of limited discriminatory value, a vast number of those loci exist and are being catalogued at a rapid rate. Furthermore those markers have a sex unspecific transmission, are less subjects to mutations as they can be found in coding regions of DNA and their detection and analysis are relatively easy to achieve. Finally, the analysed DNA sequence being much shorter than the one used for an STR research, SNP profiles could have an application in paternity testing especially in anthropology where samples are old and often degraded.

The aim of this study is the selection of about 20 informative SNPs i.e. conserved, phenotypically not expressed and represented at high frequencies in population, their analysis by microsequencing and the confirmation of their mendelian transmission.

This method could allow the achievement of a genetic profile specific of an individual in extremely short time (ex. within custody delays or even within 24 hours) and at low costs.

The number of SNPs present on human genome being vast, the list of the selected ones could be even longer.

Correspondence to:

Elizabet Petkovski, laboratoire CODGENE, 11 rue Humann, 67000 Strasbourg, France, Tel. 00 33 (0)3 90 24 31 67; Fax: 00 33 (0)3 88 24 00 85, e-mail: elizabet@esbs.u-strasbg.fr

Mass spectrometric analysis of human microsatellite markers

S. Hahner¹, U. Schmidt², A. Kiehne¹, D. Wunderlich¹, A. Ingendoh¹ and T. Fröhlich³

¹Bruker Daltonik GmbH, Bremen, Germany

²Institut für Rechtsmedizin, Universitätsklinikum Freiburg, Germany

³Bruker Saxonia GmbH, Leipzig, Germany

Introduction

Genotyping based on the analysis of multiple short tandem repeat (STR) loci has been validated for routine use in human identification and parentage testing. Currently, the analysis of the polymorphic variations in allele length of these genetic markers depend on electrophoretic separation of fluorescently labeled PCR products. Another technique accepted for sizing and typing of microsatellite markers is capillary electrophoresis which, compared to slab gel electrophoresis, is capable for a more automated procedure with increased resolution of the separation and reduced time for an electrophoretic run. Despite the improvement toward automation and throughput difficulties remain associated with electrophoretic methods. Mainly the size determination which is correlated to the mobility of an internal standard in the gel matrix is error prone due to sequence specific effects and unresolved secondary structures.

With the introduction of gentle ionization techniques for large biomolecules such as electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) has become an alternative method for the size determination of DNA fragments. The generation of intact gas phase ions for separation and detection offers the advantage of short analysis time and the ability to obtain molecular weight information without the need for labeling and size standards. However, mass analysis of PCR products is complicated by the presence of various salts as well as of surplus nucleotide triphosphates and primers used in the amplification reaction of relevant regions in the genome. Thus, purification of the PCR reaction is a severe demand for the use of both, MALDI and ESI MS.

Results

For the analysis of human microsatellite markers using ESI ion trap MS a new purification method based on reversible binding of PCR products onto magnetic particles is applied. This approach has the capability for fully automated handling and is directly compatible with ESI ion trap MS analysis.

The purified PCR products derived from several polymorphic STR loci dissociates during ionization resulting in detection of the sense and the anti-sense strand with a mass accuracy of less than 0.5%, sufficient to determine single base substitution in a mass range of 30 kDa. The sensitivity of this approach is below 500 fmol/μl.

Dr. Stephanie Hahner, Bruker Daltonik GmbH,
Fahrenheitstr. 4, D-28359 Bremen, Germany

Tel.: ++49 (0)421 2205 327; Fax: ++49 (0)421 2205 103;

e-mail: sha@bdal.de

Single nucleotide polymorphisms detected by Temperature-modulated high-performance liquid chromatography

Y.P. Hou, H.Y. Meng, H. Tang, L.C. Liao, J. Zhang, Y.B. Li and J. Wu

Institute of Forensic Medicine, Sichuan University, Chengdu, PR China

Single nucleotide polymorphisms (SNPs) are new generation markers. An open question in research on SNPs is how to find novel SNPs in a nature population? To this end, we developed an approach of high-throughput genotyping by applying the temperature modulated heteroduplex analysis (TMHA) based upon ion-pair reversed-phase high-performance liquid chromatography. This approach allowed a very efficient resolution of identically sized PCR products with a single nucleotide change. This approach was also known as denaturing high performance liquid chromatography (DHPLC). However, the terms of both TMHA and DHPLC should be considered carefully. Firstly, it is DNA molecular that is denatured in high performance liquid chromatography but not the method or the instrument. Secondly, the temperature in the liquid chromatograph is modulated according to T_m (melting temperature) of the DNA fragments. As it is known, T_m is the temperature at which a population of double-stranded nucleic acid molecules becomes half-dissociated into single strands, and under this temperature, half of the DNA molecules is still not denaturing. Thirdly, the targets for detection are both the homoduplexes and the mismatched heteroduplexes, which are double-stranded nucleic acid molecules and are annealing. The term of DHPLC may lead to confusion in annealing and denaturing. In addition, since TMHA can be carried out using gel electrophoresis and HPLC, there is a need to distinguish HPLC from gel electrophoresis. Therefore, we suggest a new term for this technology, which is TmHPLC (temperature-modulated high-performance liquid chromatography). This term not only provides a clear definition for this new method, but also includes both TMHA and HPLC. More importantly, TmHPLC implies that the temperature in HPLC is modulated according to T_m of the DNA fragments. Our results show that this approach leads to significant reduction of sequencing efforts for searching novel SNPs. This implies that TmHPLC is a sensitive, accurate, and cost effective approach to screening sequence variation in human genome.

Prof. Yi Ping Hou, Institute of Forensic Medicine,
Sichuan University (West China University of Medical Sciences),
610041 Chengdu, PR China,

Tel. 0086-28-5501549; Fax: 0086-28-5501549,

e-mail: rechtsme@wcums.edu.cn

Application of the DNA IQ™ and AluQuant™ Human DNA Quantitation Systems

B. Krenke, M. Mandrekar, P. Mandrekar, J. Shultz and A. Tereba

Promega Corporation, Madison, Wisconsin, USA

The streamlining of amplification and analysis components of human identification has increased the demand for simpler DNA isolation and quantitation technologies. The DNA IQ™ System was designed to provide an easy and flexible DNA isolation method using magnetic particles. This system has been demonstrated to extract and purify DNA from a large variety of sample types while giving the option to automatically quantitate total DNA. For applications requiring human-specific quantitation, the AluQuant™ Human DNA Quantitation System has been developed. This system uses probes specific to repetitive genetic elements allowing quantitation without target amplification. The AluQuant™ Human DNA Quantitation System was designed to use a series of solution-based enzymatic reactions to produce a luminescent signal proportional to the quantity of human DNA present. This approach eliminates many of the drawbacks of membrane-based hybridization and allows quantitation of degraded DNA. This system has demonstrated a range of quantitation from 0.1–50 ng of human DNA. Signal from non-human DNAs tested was insignificant. Furthermore, addition of non-human DNAs into a human sample did not alter quantitation. The combination of these technologies provides an efficient, automatable platform for sample preparation that is compatible with current STR-based genotyping systems.

*Benjamin E. Krenke
Research Scientist, Genetic Identity, Promega Corporation
2800 Woods Hollow Road, Madison, WI 53711, USA
e-mail: bkrenke@promega.com
Telephone: 608-274-1181 x1713*

Y Chromosome SNP markers discriminate between Basque, Georgian and Berber populations

**M. Alvarez-Alvarez¹, J.L. Rodríguez-Filgueira¹,
I. Fernández-Fernández^{2,3}, A. Castro^{2,3} and M. M. de Pancorbo^{1,3}**

¹*Dpto. de Z. Dinámica Celular A., F. Farmacia, U. País Vasco, Vitoria-Gasteiz, Spain*

²*DataGene, Sondika (Bizkaia) Spain*

³*Instituto Vasco de Criminología, U. País Vasco, San Sebastián-Donostia, Spain*

Introduction

The Y Chromosome is an important tool for discriminating related males and populations. It is characterized by absence of recombination during meiosis, except in the pseudoautosomal fragment, meaning that the different alleles of multiple polymorphisms of the Y chromosome can be combined to obtain compound haplotypes. The short tandem repeat (STR) markers of the Y chromosome are highly variable and can therefore discriminate between chromosomes of different paternal lineages, which is of great usefulness in forensic casework.

The Power of Discrimination of each single nucleotide polymorphism

(SNP) marker is lower than that of STR markers, although a broad combination of them also provides high discrimination capacity.

SNP markers provide an additional advantage over STRs in that they are better suited for distinguishing human population groups. These polymorphisms, which are better preserved due to their lower variability, make it possible to distinguish groups of related Y chromosomes within specific populations. Thus they are a useful tool for evaluating similarities between Basque, Georgian and Berber populations. The relationships found to date between these populations are based on linguistic and genetic data. Certain linguists, such as Laffon (1951), Ruhlen (1992) and Gamkrelidze & Ivanov (1990) posit a common – albeit remote – ancestor for Basque and certain languages of the Caucasus. Similarities have also been found between the Basque and Berber languages (Allières 1979, Alonso-García 1996a, 1996b). Regarding the genetic data obtained to date, few markers (proteins, blood type and mtDNA_{hV1}) have corroborated the linguistic hypotheses relating Basques to Georgians (Bertorelle 1995) or to the Berbers.

Objective

Given the evidence reported so far, a more detailed study appeared necessary to evaluate the genetic relationships between the Basque, Georgian and Berber populations. Accordingly, we decided to use SNP markers of the Y chromosome as the best tool for discriminating among populations.

Methodology

This work presents a study of 8 biallelic markers, YAP (DYS287), Sy81 (DYS271), Tat, SRY-1532 (SRY10831), SRY-8299 (SRY-4064), SRY-2627 (SRY-2628), M9 and DYS257. Using the phenol-chloroform method, peripheral blood DNA was analyzed from 166 unrelated males from 3 different regions: 59 autochthonous Basques (34 from Arratia, 25 from Gohierri), 40 males residing in the Basque Country, 49 from Georgia (12 residents and 32 autochthonous Georgians from Swanetia) and 18 Berbers from Morocco. 30 ng of each sample was amplified by PCR and the genotypes analyzed following digestion with the corresponding restriction enzymes, except in the case of Alu insertion. Genetic and haplotype frequencies were calculated and molecular variation analysis was made. Population differentiation was estimated by F_{st}. Hierarchical cluster analysis was also performed, followed by factorial analysis of principal components and, finally, by discrimination to determine the haplogroups influencing the formation of the clusters found.

Results

The Berbers exhibit a unique haplotype that does not appear in the populations of Georgia and is rarely found in the Basque Country. On the other hand, the Basque populations have the highest frequencies of haplotypes not detected in the populations of Georgia. Such dissimilar results are indicative of the high degree of differentiation among these populations. The statistical analysis made supports these results.

Conclusions

This study of the haplotypes of SNPs does not show the existence of similarities between the Basque and Berber populations reported in earlier works, and fails to confirm the possible relationships between these populations suggested by linguistic studies published to date.

Address for correspondence:

*M. M. de Pancorbo, Dpto. de Z. y Dinámica Celular,
Facultad de Farmacia, Universidad del País Vasco.
Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain.
Tel. 00 34 945 013045; Fax: 00 34 945 013756;
e-mail: gcpmagom@lg.ehu.es*

Analysis of Mitochondrial 12S rRNA Gene Sequence Variation in four Ethnically defined Populations

C. Albarrán¹, P. Martín¹, O. García², P. García¹, L. Fernández de Simón¹, R. Deka³, S. Guangyun³, M. Sancho¹ and A. Alonso¹

¹Instituto de Toxicología. Sección de Biología. Madrid. Spain

²Area de laboratorio Ertzaintza. Sección de Biología. Bilbao. Spain

³Department of Environmental Health. University of Cincinnati. Cincinnati. USA.

MtDNA control region sequencing has become a powerful tool to investigate the identity of biological evidence that cannot be analysed by the use of single-copy nuclear markers. However, the power of discrimination of mtDNA testing is very limited. Targeting additional variation outside of the hypervariable regions will enhance the power and efficiency of mtDNA profiling. In this context, we have screened for the 12S rRNA gene sequence variation in four ethnically defined population groups. In this study, we have analysed the nucleotide sequence variation of the 12S rRNA mitochondrial gene (648 bp to 1601 bp) from five different populations (Spanish Caucasian, Autochthonous from the Basque Country, Chinese, New Guinea Highlander and Africans of Benin) by full sequencing of two overlapping PCR fragments using d-rodhamine cycle sequencing coupled with an ABI377 sequencer. Preliminary data indicate different patterns of sequence variation between Africans and non-Africans. Africans are much more polymorphic than non-African populations, which have only a very restricted subset of haplotypes. Furthermore, the greater part of Africans analysed showed two specific nucleotide substitutions (769G → A and 1018G → A) that were not observed in non-African individuals. In conclusion, the mtDNA 12SRNA gene in combination with other systems could be an interesting ethnic marker that could help to differentiate between African and non-Africans maternal lineages.

Address for correspondence:

Prof. Cristina Albarrán. Instituto Nacional de Toxicología. Sección de Biología. C/Luis Cabrera, 9. 28002 Madrid. Spain. Tel. 00-34-(1) 5629190. Fax: 00-34-(1) 5636924. e-mail: biologia@mad.inaltox.es

Significant differences between the Leeward and Windward groups of the Cabo Verde archipelago (West African Coast)

A.T. Fernandes¹, A. Rosa¹, A. Brehm¹ and A. Carracedo²

¹Centro de Ciências Biológicas e Geológicas da Universidade da Madeira

²Instituto de Medicina Legal, Santiago de Compostela, Galicia, Spain

Introduction

The Cabo Verde archipelago is composed of nine islands sub divided into the Barlavento (Windward) and Sotavento (Leeward) groups.

The archipelago was discovered in 1419 by the Portuguese and was part of the slave trade route, which flourished in the 15th and 16th centuries. Slaves originated in the West African coast (especially the coast of Guinea) were sold in Cabo Verde and then proceeded to Lisbon. They were also used to colonise the islands of Santiago, Fogo and Brava. The Leeward islands were essentially populated with slaves brought from Guinea (West African Coast), the Europeans colonisers never been more than 10% of the total population.

The Windward islands were colonised later in the 18th century by some slaves from the Leeward and together with some deported portuguese men.

In this study we used five autosomal STRs (CD4, TPO, FES/FPS, TH01 and VWA31/) and a three Y-biallelic markers YAP, SRY₈₂₉₉ and sY81 to trace the composition of y-related markers in the present day population.

The YAP polymorphism is due to an Alu insertion, while SRY₈₂₉₉ and sY81 occur because of a base substitution; these markers together define three haplotypes known as 4, 21 and 8. The haplotype 8 being characteristic of sub-saharian population and the 21, although occurring in Europe has its highest frequency in North African populations

Materials and methods

We collected DNA from unrelated individuals whose ascendancy goes back to the same island for at least three generations. Sample sizes and origins were the following: Cabo Verde Leeward (N=120) and Cabo Verde Windward (N=110)

DNA was extracted using the chelex method and PCR amplification was done using specific primers for each STR and Y marker.

PCR products were separated in polyacrilamide gels and visualised by silver staining.

In case of sY81 and SRY₈₂₉₉ markers, we used restriction enzymes NlaIII and BsrBI to digest the PCR products.

Discussion

The two populations were found to be in Hardy-Weinberg equilibrium. Haplotype and allele frequency values of populations were compared. Using Arlequin software we found significant differences between the two groups of islands on the basis of both STRs and Y-markers.

Address for correspondence:

Prof António Brehm, Centro de Ciências Biológicas e Geológicas, Universidade da Madeira, Campus da Penteadá, 9000 Funchal, Portugal. Tel: 00 351 291 705383; Fax: 00 351 291 705399, e-mail: brehm@uma.pt

Dynamics of Molecular Genetic Diversity in the East Midlands, UK: Forensic and Paternity implications

S.S. Mastana and D.R. Lee

Human Genetics Lab., Department of Human Sciences, Loughborough University, Loughborough, UK.

The objectives of this investigation were to establish the database of Minisatellite (VNTRs) Microsatellite (STRs), and ALU Insertion allele frequencies for the regionally sub-divided populations of the East Midlands, which is suitable for population genetic and forensic investigations. The secondary objective was to determine if Caucasian sub-population heterogeneity exists within the United Kingdom, within Europe and World Caucasian and racial populations at these loci. To evaluate the efficiency of these markers for forensic and paternity purposes in the East Midlands populations

Blood samples (600) were taken at random from the Caucasian East Midlands populations (5 sub-populations). Using standard molecular genetic techniques, we analysed MS1, MS31, YNH24, MS43a VNTRs and HUMTHO1, F13A, F13B, FES,LPL, VWA31 and CSF1PO STRs. Alu insertion polymorphisms studied included, ACE, TPA, PV92, D1, APO and FXIII B. Overall efficiency of these loci for forensic and paternity work in the East Midlands populations is at par with other Caucasian populations. Average value of PE is more than 0.999 and cumulative PM was 5.3×10^{-13} for UK populations with some regional and local variation. Significant heterogeneity chi-square values for MS43a, MS1 and YNH24 were found in a large number of FBI bins in UK Caucasian, European, world Caucasian and UK racial population comparisons. Overall comparisons provided interesting results suggesting caution should be exercised in usage of pooled or general population databases for forensic and paternity investigations.

Address for correspondence:

*Dr. Sarabjit Mastana
Human Genetics Lab.,
Department of Human Sciences
Loughborough University
Loughborough LE11 3TU,
UK
Tel: 01509223041
Fax: 01509223941
e-mail: S.S.Mastana@lboro.ac.uk*

A study of the Genetic Polymorphism of GPT in the Beijing population

Peili, Zhaoxingchun, Denghua, Jiangchengtao, Changcaiqin, Wangjian

Institute of the forensic science, Ministry of Public Security, PRC

Genetic Polymorphism of human red cell glutamate pyruvate transaminase (GPT) was investigated in blood sample from Beijing in north China, 156 unrelated individuals were studied using AMP-RFLP in polyacrylamide gels. The allele frequencies were as follows: $GPT^*1 = 0.4294$, $GTP^*2 = 0.5705$.

The population data fitted the Hardy-Weinberg law.

Genetic Polymorphism of the A Subunit of Coagulation Factor 13 in the Beijing Population

Denghua, Peili, Jiangchengtao, Zhaoxingchun, Changcaiqin, Wangjian

Institute of the forensic science, Ministry of Public Security, PRC

Coagulation factor 13 A polymorphism in a random population sample from Beijing in north China ($n = 287$) was studied using isoelectric focusing in polyacrylamide gels followed by immunoblotting with enzyme immunoassay. The allele frequencies were as follows:

$F_{13A}^*1 = 0.8833$, $F_{13A}^*2 = 0.1167$.

The distribution of phenotypes of F13A agrees with the Hardy-Weinberg equilibrium. Comparing these allele frequencies with those reported in other populations, it was found that the allele frequencies of F_{13A}^*1 in the Beijing population were higher.

A multicentric study of SE33 allele frequencies in the Italian population

L. Buscemi, C. Turchi, M. Pesaresi, A. Tagliabracci¹
 L. Caenazzo, E. Ponzano, P. Cortivo²
 C. Previderè, G. Peloso, P. Grignani, G. Pierucci³
 C. Toni, I. Spinetti, S. Presciuttini, R. Domenici⁴

Universities of Ancona (1), Padova (2), Pavia (3), Pisa (4)

The HumACTBP2-human beta-actin-related pseudogene H-beat-Ac-psi-2 locus-SE33- is a complex tetranucleotide short tandem repeat locus and it is one of the most informative and polymorphic genetic markers (Rolf B. et al, Int J Legal Med 1997, 110:69–72)

A collaborative research on the polymorphism of this system was carried out by the Institutes of Legal Medicine in four Italian regions (Marches, Veneto, Tuscany and Lombardy). The aim was to expand the database in view of HumACTBP2 application in forensic identification and paternity testing. The goal for each participating laboratory was to study at least 100 genotypes of unrelated, locally residing individuals.

After DNA extraction, PCR was carried out using the amplification conditions proposed by Wiegand et al. (Int J Legal Med 1993, 105:315–20), with minor modifications; PCR fragments were detected by capillary electrophoresis (ABI Prism 310 Genetic Analyzer, PE Biosystems). Allele assignment was performed by comparison with allelic ladder kindly provided by the Institute of Legal Medicine in Lousanne. The nomenclature used is in accordance with the recommendations of GEDNAP (Schneider HR et al, Int J Legal Med 1998, 111:97–100).

The allele variants were sequenced by Taq-cycle-sequencing using BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI Prism 310 Genetic Analyzer.

Prof. Dr. Adriano Tagliabracci, Istituto di Medicina Legale, Policlinico, Torrette, I-60020 Ancona (Italy), tagliabr@popcsi.unian.it

Ecuadorian Quichua Population Data on 3 Tetrameric Short Tandem Repeat Loci-HUMTH01, TPOX and CSF1PO-Derived using STR Multiplex System

D. Sánchez de González¹, F. González-Andrade^{2,3},
 B. Martínez-Jarreta², R. Chriboga³, D. Colina³, V. Aguirre³

¹Chief of Molecular Genetics Laboratory, Cruz Roja Ecuatoriana, Quito (Ecuador)

²Department of Legal Medicine, Faculty of Medicine, University of Zaragoza, (Spain)

³Molecular Genetics Laboratory, Cruz Roja Ecuatoriana, Quito (Ecuador)

Abstract

Ecuador is a small South American country with almost 12 million inhabitants comprised by three main ethnic groups: Caucasian mestizos, Amerindian natives (more than 100 multiethnic and pluricultural groups), and a small group of negroids descendants of African slaves. In 1990, the National Institute of Statistics (INEC) report that exists 910,416 indigenous in our country (9.4% of the total population). The most important ethnic group is Quichua with 170,000 persons approximately (66,964 in the mountains, 72,528 in Amazonia and 27,648 on the coast). They are descendants of Incas and local tribes and can be found in other South American countries as Peru, Bolivia and Colombia.

There is a lack of information on Quichua population from a genetic point of view and therefore no previous publications on the distribution of STRs is available.

In this paper, we report the allele frequency distribution of 3 STR loci that have proven to be extremely useful for forensic casework and human identification: HUMCSF1PO, HUMTPOX, HUMTH01 in a population sample of Amerindian Quichuas from Ecuador (n = 179).

Allele and genotype frequencies for the 3 loci were determined using STR Multiplex System, electrophoresis of the PCR products in denaturing polyacrylamide gels and subsequent detection of allelic fragments by silver staining.

All loci met Hardy-Weinberg expectations and there was no evidence for association of alleles among the three loci.

There was obtained some statistical parameters of medico-legal interest as allelic diversity value and the chance of exclusion in paternity cases. These parameters reveal the high forensic efficiency of the three STR loci analysed.

The Quichua population data for the three STRs loci were compared with those from other populations. Significant differences were observed with other Ecuadorian and Spanish populations.

The data presented in this work will allow the calculation of matching probabilities in forensic casework if Ecuadorian Quichua individuals are considered as source of DNA evidence.

The tetranucleotide repeat polymorphism C 2_4_4: Sequence and population data

S. Stadlbacher, E.M. Dauber, B. Glock and W.R. Mayr

University of Vienna, Medical School, Clinical Department for Blood Group Serology, Austria

The STR locus C 2_4_4 situated in the HLA class I region (6p21.3) near the HLA-B and HLA-C locus was investigated in an Austrian Causasoid population sample of 247 unrelated individuals. PCR amplification and subsequent denaturing polyacrylamide gel electrophoresis was carried out with automated detection by laser induced fluorescence on the A.L.F. DNA Sequencer using a Fluorescein labelled primer. 13 different alleles were observed ranging in size from 227 to 275 bp. Sequencing revealed a (RAAR)₉₋₂₁ repeat pattern. Some alleles showed a sequence variation in addition to length polymorphism. An allelic ladder out of 12 of these alleles (all except 15.1) was composed and used for typing. The resulting allele frequencies and further statistic data are shown below:

Allele name	Allele frequency
9	0.146
10	0.421
11	0.024
13	0.014
14	0.002
15	0.014
15.1	0.002
16	0.142
17	0.077
18	0.081
19	0.043
20	0.014
21	0.020

Rate of heterozygosity: 0.781

Power of exclusion: 0.565

Polymorphism information content: 0.74

Matching probability: 0.078

Power of discrimination: 0.922

Typical paternity index: 2.29

References

Tamiya G, Ota M, Katsuyama Y, Shiina T, Oka A, Makino S, Kimura M, Inoko H (1998) Twenty-six new polymorphic microsatellite markers around the HLA-B, -C and -E loci in the human MHC class I region, Tissue Antigens 51:337-346

Address for correspondence:

Simone Stadlbacher, Universität Wien-Universitätsklinik, Klinische Abteilung für Blutgruppenserologie, A-1090 Wien, Währinger Gürtel 18-20, Austria, Tel: +43-1-40400-5320, Fax: +43-1-40400-5321, e-mail: simone.stadlbacher@univie.ac.at

Allele Frequencies of the Profiler Plus STR loci in Canary Islands (Spain)

A. Hernandez, I. Frias, J.J. Sanchez, J.M. Perez, J.A. Cuellas

Instituto Nacional de Toxicología, Delegación de Canarias, Spain

Canarian archipelago is a crossroad for populations from Europe, Africa and South America, what makes it a particular zone for crime activities and investigations. Profiler Plus (Applied Biosystems) is a widely used STR system in forensic casework, and it is included in the Combined DNA Index System (CODIS).

In this study we present the results of a survey aimed at investigate the allele and genotype frequency distribution of these loci in Canary Islands. DNA was isolated from blood or saliva samples, randomly selected from routine forensic casework, paternity testing or volunteers. More than 200 unrelated individuals were included in the Profiler Plus STR data bank. Amplification products were analyzed by capillary electrophoresis using the ABI 310[®] Genetic Analyzer. Statistical analysis was carried out to determine the compliance of allele frequencies with the Hardy-Weinberg equilibrium, and other population parameters of interest were also obtained (loci association, combined chance of exclusion, power of discrimination, heterocigosity, population substructure, etc).

Some of the samples were previously analyzed by silver-stained electrophoresis gels using Triplex GenePrint[®] Systems (Promega), so the concordance in some loci between both data pools was checked as a validation of our capillary STR analysis system.

Allele Frequency distribution of Cofiler STRs in Canary Islands Population (Spain)

I. Frias, A. Hernandez, J.M. Perez, J.J. Sanchez and J.A. Cuellas

Instituto Nacional de Toxicología. Delegación de Canarias (Spain)

Allele and genotype frequencies for seven STRs included in the AmpliFSTR Cofiler kit (D3S1358, D16S539, TH01, TPOX, CSF1PO y D7S820) were obtained for a sample of more than 200 unrelated individuals from routine casework and paternity testing and from volunteers.

Samples analyzed were blood or saliva and PCR and typing by Capillary electrophoresis were according to manufacturer's instructions (Applied Biosystem).

All systems and loci were analyzed for Hardy-Weinberg equilibrium and the, others statistical parameters of forensic interest are also reported (homo and heterocigosity, combined chance of exclusion and combined discrimination power.

Allele frequency distribution of four STR loci vWA, TH01, TPOX and F13A01 in three Asian populations (Japanese, Indonesians and Bangladeshis)

A. Kido¹, R. Susukida¹, M. Oya¹, N. Fujitani², H. Kimura³ and M. Hara⁴

¹Department of Legal Medicine, Yamanashi Medical University, Japan

²Department of Biochemistry, Okayama University of Science, Japan

³Department of Forensic Medicine and Human Genetics, Kurume University School of Medicine, Japan

⁴Department of Forensic Medicine, Saitama Medical School, Japan

Population genetic studies of four STR loci vWA, TH01, TPOX and F13A01 were carried out on 200 unrelated Japanese, 104 unrelated Indonesians and 139 unrelated Bangladeshis. The genotype frequency distributions of each locus for the three populations did not deviate from the Hardy-Weinberg equilibrium. There were no significant differences of allele frequency distributions of vWA, TH01 and TPOX among the three populations. As for the locus F13A01, larger alleles (13–17) were observed at a considerable frequency in Bangladeshis, while they were rare in Japanese and not at all in Indonesians. Moreover, in Bangladeshis the commonest allele at the locus F13A01 was the allele 5, while in Japanese and Indonesians the allele 6 was the commonest. Some statistical parameters of forensic importance such as observed/expected heterozygosity, mean exclusion chance, polymorphism information content and power of discrimination were calculated. The combined power of discrimination of the four loci was 0.9995 for Japanese, 0.9998 for Indonesians and 0.99991 for Bangladeshis.

Dr. Akira Kido, Department of Legal Medicine, Yamanashi Medical University, Tamaho, Yamanashi-ken 409-3898, Japan
Tel: +81-55-2739548, Fax: +81-55-2736753,
e-mail: akido@res.yamanashi-med.ac.jp

STR data for thirteen loci from Jewish populations

A. Picornell, C. Tomàs, J.A. Castro and M.M. Ramon

Laboratory of Genetics, Department of Biology, University of Balearic Islands, Spain

Different methodologies have been used in studies on genetic variability in human populations to assess their genetic composition, their relationships and the evolutionary factors to which they are subject, as well as for forensic purposes. Among these, short tandem repeats or STRs are a well-known group of highly polymorphic markers. Since their description in the late eighties they have been increasingly used as populational, forensic and clinical markers, especially those based on a 4 bp motif because of their high level of informativeness. The technique of multiplexing is well established and some multiplex systems are now commercially available, which provide a fast tool for forensic DNA testing. The "AmpFISTR Profiler Plus PCR Amplification Kit (PE Applied Biosystem)" co-amplifies nine tetranucleotide STR loci: D3S1358 (Chr. 3p), vWA (Chr.12p12-pter), FGA (Chr. 4q28), D8S1179 (Chr. 8), D21S11 (Chr. 21), D18S51 (Chr. 18q21.3), D5S818 (Chr. 5q21–31), D13S317 (Chr. 13q22–31) and D7S829 (Chr. 7q) and a segment of the gene amelogenin (Chr. Xp22.1–22.3 and Yp11.2). This set of markers has been approved in the combined DNA Index System (CODIS) database in the USA. Additionally, the STRs D4S243 (Chr.4), HUMF13A1 (Chr. 6p24–25), D18S535 (Chr. 18) and D12S391 (Chr.12) have been studied in this work.

Jewish populations have been studied by geneticists since the turn of the twentieth century in an attempt to unravel what must be a complex system of interrelations among Jewish communities and their non-Jewish neighbours, with whom they lived during the Diaspora (the numerous migrations of Jewish populations and their subsequent residence in various countries in Europe, North Africa and West Asia). Several works have attempted to study the evolutionary factors that have come into play during the Diaspora and controversial results can be found in to what extent the non-Jewish admixture in the modern Jews is. Most of the studies used "classical" genetic markers, but also DNA markers have been used (mtDNA RFLPs, chromosome Y and other nuclear markers). But a few data on Short tandem repeats have been published to date in Jewish populations.

The purpose of this work was to study the genetic variability of thirteen STR loci in Jewish populations. We analyzed 124 Jewish individuals. Following the classical criteria, they were categorized in four groups: Ashkenazi (25 individuals), Sephardic (36 individuals from Turkey), North African (13 Moroccan, 13 Tunisian and 13 Lybian individuals) and Oriental (12 Iranian and 13 Iraki individuals). All these samples belong to the collection of The National Laboratory for the Genetics of Israeli Populations at Tel-Aviv University.

No significant differences were found between the four Jewish groups, except in the FGA system. No system was statistically different from HW equilibrium. The heterozygosity ranged from 0.744 to 0.888 and the number of alleles observed was from 6 to 15. The usefulness of the thirteen systems studied can be judged by their combined PD, which corresponded to 1 in 2.392 E+15 individuals and their combined CE, 0.999996. No significant pairwise correlation was observed between the thirteen markers, with the exception of the D18S5-D7S820 pair. Therefore, the a priori statistical power of this set of STRs in Jewish populations indicated that they are very informative for the application of these results in paternity and forensic casework.

Dr. M.M. Ramon; Laboratory of Genetics, Department of Biology, University of Balearic Islands, Crta. Valldemossa Km 7,5,
07071-Palma de Mallorca, Balears, Spain, Tel. 00 34 971 173152;
Fax 00 34 971 173184; e-mail: dbsmrjo@clust.uib.es.

Tunisian population genotype and alleles frequencies on 15 PCR-based loci

C. Brandt-Casadevall¹, M. Ben Dhiab², C. Gehrige¹,
N. Dimo-Simonin¹, F. Taroni¹, M. Zemni², P. Mangin¹

¹Institut universitaire de Médecine légale, Lausanne, Switzerland

²Service de Médecine légale, Hôpital Farhat Hached, Sousse, Tunisie

Genotype and allele frequencies distribution for fifteen PCR-based loci (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX and FGA) were determined for a Tunisian population sample of 200 unrelated individuals. The examined population is constituted by a mixture of Arabic and Berber individuals coming from the three different regions of the country: North, Centre and South.

Blood samples were deposited on FTA-paper were directly amplified without quantification using the multiplex PowerPlex16 (Promega). Separation and detection of the alleles were carried out using capillary electrophoresis on an ABI310 instrument.

Possible divergence from the Hardy-Weinberg expectations was tested. Our data was compared with those of European, North-African and Arabic populations.

Dr. C. Brandt-Casadevall
Institut universitaire de Médecine légale
Rue du Bugnon 21
1005 Lausanne
Switzerland
Tél.: 41 21 3147070
Fax: 41 21 3147090
e-mail: Conxita.Brandt@inst.hospvd.ch

Allele distributions and genetic relationship with at 13 CODIS core STR loci in various Asian populations in / near Japan

T. Yamamoto¹, M. Mizutani¹, R. Uchihi¹, M. Tanaka¹, T. Yoshimoto¹,
S. Misawa², N. Saitou³ and Y. Katsumata¹

¹Department of Legal Medicine & Bioethics, Graduate School of Medicine, Nagoya University, Japan

²Department of Legal Medicine, Institute of Community Medicine, University of Tsukuba, Japan

³Laboratory of Evolutionary Genetics, National Institute of Genetics, Japan

Short tandem repeat (STR) polymorphisms are mainly used in forensic fields for paternity tests and personal identification at present. Multiplex PCR amplification and typing system with multi-colored fluorescent labeled primers have made it convenient to genotype STRs using commercially available kits. The FBI in USA adopted 13 STR (D3S1358, vWA, FGA, D16S539, TH01, TPOX, CSF1PO, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820) to construct a huge database for criminal investigation (CODIS: the Combined DNA Index System). As internationalized crimes have been increased, each allele frequency at these 13 STR loci is having been calculated all over the world. We investigated allele frequency distributions at these 13 STR loci using each more than 100 DNA samples in Japanese (Hondo and Ryukyu), Korean (Soul), Chinese (Beijing, Shanxi, Hunan, Guangdong, Fujian and Jiangsu), Thais (Bangkok) and Burmese (Yangon). We analyzed these databases by a genetic distance DA to construct a tree based on the Neighbor-Joining method, and obtained one being well coincident with their geographical distributions. We also presented a genetic relationship including the published ethnic data in the world.

Dr. Toshimichi Yamamoto, Department of Legal Medicine & Bioethics, Graduate School of Medicine, Nagoya University, 65 Tsurumai-cho, Showa-ku, Nagoya, 466-8550 Japan, Tel: +81-(0)52-744-2119, Fax: +81-(0)52-744-2121, e-mail: yamachan@med.nagoya-u.ac.jp

Population genetic analysis in Hungarian populations using the Powerplex™ 16 system

B. Egyed¹, S. Füredi¹, M. Angyal², I. Balogh³, L. Kalmár⁴,
A. Tordai⁴, J. Woller¹ and Z. Pádár¹

¹Department of Haemogenetics, Institute for Forensic Sciences,
Budapest, Hungary

²Department of Forensic Medicine, University Medical School Pécs,
Hungary

³Department of Clinical Biochemistry and Molecular Pathology,
University Medical School Debrecen, Hungary

⁴Molecular Biology Laboratory, National Institute for Haematology
and Immunology, Budapest, Hungary

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 analyses 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) were used for genotyping.

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 in-between 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 FST 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.

Correspondence:

Balázs Egyed

Institute for Forensic Sciences

POB 314/4

H-1903 Budapest

Hungary

Tel. 00 36 1441 1474

Fax. 00 36 1441 1473

e-mail: egyed.b@free-mail.c3.hu

Allele Frequencies of Eight STR Loci in a Japanese Population Detected by the Fluorescent Image Analyzer

A. Nagai¹, Y. Nozaki², I. Nakamura¹, Y. Bunai¹, R. Nakashige²
and I. Ohya¹

¹Department of Legal Medicine, Gifu University School of Medicine,
Gifu, Japan

²Research & Development Department, Hitachi Software Engineering
Co., Ltd., Yokohama, Japan

Population studies on eight short tandem repeat (STR) loci D16S539, D7S820, D13S317, D5S818, CSF1PO, TPOX, TH01 and vWA were carried out in a sample of 460 unrelated Japanese individuals living in Gifu Prefecture (central region of Japan).

The eight loci were co-amplified using the *GenePrint*™ PowerPlex™ Fluorescent STR System (Promega). The amplified products were electrophoresed using 4% denaturing polyacrylamide. After the electrophoresis, the gels were scanned on a FMBIO II Multi-View fluorescent image analyzer (Hitachi Software Engineering) and the fluorescent DNA bands of the eight loci were detected simultaneously. The fragment sizes of the bands were determined using FMBIO Analysis Software (Hitachi Software Engineering), and typed using Excel STaR-Call Genotyping Software (Hitachi Software Engineering).

The following allele frequency ranges were found: D16S539: 7 alleles with frequencies ranging from 0.002 (allele 8) to 0.329 (allele 9); D7S820: 8 alleles with frequencies ranging from 0.003 (allele 14) to 0.316 (allele 11); D13S317: 8 alleles with frequencies ranging from 0.001 (allele 7) to 0.265 (allele 8); D5S818: 10 alleles with frequencies ranging from 0.003 (alleles 6, 7 and 15) to 0.263 (allele 11); CSF1PO: 8 alleles with frequencies ranging from 0.003 (allele 15) to 0.396 (allele 12); TPOX: 6 alleles with frequencies ranging from 0.003 (allele 14) to 0.463 (allele 8); TH01: 6 alleles with frequencies ranging from 0.005 (allele 10) to 0.399 (allele 9); vWA: 9 alleles with frequencies ranging from 0.002 (allele 21) to 0.279 (allele 17). The combined power of discrimination (PD) for the eight loci was 0.99999992 and the mean exclusion chance (MEC) 0.998. For all loci, no deviations from Hardy-Weinberg expectations were detected using the homozygosity, the likelihood ratio, and the exact tests. The results indicate that these eight loci are useful genetic markers for forensic personal identification and paternity testing in the Japanese population.

Address for correspondence:

Atsushi Nagai, Department of Legal Medicine,

Gifu University School of Medicine, Tsukasa-machi,

Gifu 500-8705, Japan, Tel: +81 58 267 2250; Fax: +81 58 267 2957,

e-mail: anagai@cc.gifu-u.ac.jp

PowerPlex™ 16 analysis in Japanese population

M. Hashiyada¹, Y. Itakura² and M. Nata¹

¹Division of Forensic Medicine, Department of Public Health and Forensic Medicine, Tohoku University Graduate School of Medicine, Japan

²NTT Data Technology Corporation, Japan

Short tandem repeat (STR) analysis is a useful tool in forensic sciences to get information of individual identification. Recently, several STR loci can be amplified in one tube using multiplex PCR STR kit, which are commercially available.

We investigated 16 STR loci in 350 Japanese by GenePrint® PowerPlex™ 16 System (Promega, USA). Genomic DNA was extracted from EDTA whole blood and bloodstain using SDS-proteinase K or Chelex 100 treatment followed by the phenol/chloroform extraction. PCR was performed in accordance with manufacture's protocols. Electrophoresis was carried out on an ABI 377 sequencer and the alleles were determined by GeneScan™ 2.0.2 software (Applied biosystems, USA).

The statistical data of analyzed 15 STRs, excluding Amelogenin locus, are relatively high rate, and no significant deviation from Hardy-Weinberg Equilibrium was detected. In 15 STRs, the Penta E locus found to be a most highly polymorphic locus. The matching probability of this system showed 1 in 1.94×10^{17} and the power of exclusion was 0.999998982 in Japanese population. Our results suggest that this system provide powerful discrimination.

Mr. Masaki Hashiyada, Division of Forensic Medicine, Department of Public Health and Forensic Medicine, Tohoku University Graduate School of Medicine, Seiryomachi 2-1, Aoba-ku, Sendai 980-8575, Japan. Tel. 00 81 (0)22 717 8110; Fax: 00 81 (0)22 717 8112, e-mail: hashiyad@forensic.med.tohoku.ac.jp

Multiplex STR genotyping: comparison study, population data and new sequence information

C. Alves¹, L. Gusmão¹, L. Pereira^{1,2} and A. Amorim^{1,2}

¹Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal

²Faculty of Sciences, University of Porto, Portugal

A simultaneous study was performed using Perkin Elmer Profiler Plus/GenePrint CTTv and Promega Powerplex 16 kits which share the same STR loci, in two population samples from North Portugal (N = 110) and Mozambique (N = 110).

Population data for the three STRs included only in the Powerplex 16 kit (D16S539, Penta D and Penta E) are reported for both populations. No deviations from Hardy-Weinberg equilibrium were observed for all markers in both population samples. A comparative analysis between our data and Promega's (Caucasian-Americans and African-Americans) is also presented.

As the primers chosen by both manufacturers are distinct, inconsistencies between genotypes obtained by the different kits may be detected and are due to polymorphisms in the primer annealing regions. Out of 110 unrelated individuals from North Portugal we found 3 inconsistencies between the genotypes obtained from the different manufacturers for D5S818. In these cases, the inconsistencies are due to a Promega primer annealing failure, resulting in false homozygotes. Sequencing analysis using a new set of primers revealed a T to C substitution at the 36th position downstream of the last AGAT repeat, inside Promega's Powerplex reverse primer annealing region, to be the cause for this failure. For this locus in both populations, other point mutations outside the repeat region and, in some alleles, structural differences from the consensus repeat motif were also detected.

Out of 110 unrelated individuals from Mozambique we found 1 inconsistency between the genotypes obtained by the different kits for D8S1179. In this case, the inconsistency is due to a Perkin-Elmer primer annealing failure. Sequencing analysis for this locus is currently under study.

Corresponding author:

Cíntia Alves

IPATIMUP

Rua Dr. Roberto Frias, s/n

4200 Porto

Portugal

Tel: +351 22 5570700

Fax: +351 22 5570799

e-mail: calves@ipatimup.pt

Allele frequency Distribution of 13 STRs in an Italian and Immigrant Population Sample

N. Cerri, M. Franchi, S. Mascadri and F. De Ferrari

Institute of Forensic Medicine University of Brescia, Italy

Recently foreigners, especially Asians, North Africans and East Europeans, have been immigrating to Italy and they are sometimes involved in criminal action or in paternity test to rejoin their's family.

Consequently it is necessary for a forensic lab to create an adequate database to calculate the probabilities in personal identification, bloodstain analysis and paternity testing.

The aim of this study is to analyze allelic and genotypic distribution of 13 STRs loci (D3S1358, vWa, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, D2S1338, D19S433, TH01) in a champion of natives and immigrant population living in Brescia area (Northern Italy) to establish a DNA database wich allow us to solve some of the cases requested by the Court.

Blood samples were collected from a champion of about 120 unrelated individuals; DNA was extracted by the standard procedure with pCIA. PCR amplification was performed in a Gene Amp PCR System 2400 by Perkin Elmer using the AmpFISTR Profiler Plus and SGM Amplification Kit, according to the manufacture's recommendation.

The amplified products were sized by capillary electrophoresis using the Genetic Analyzer 310 by ABI and the sizing results were determined with Genotyper 2.5 by a comparison with supplied allelic ladders and an internal size standard.

Evaluation of Hardy Weinberg equilibrium and a comparison between the different population has been estimated.

Prof. Dr. Med Francesco De Ferrari, Institute of Forensic Medicine, University of Brescia, c/o Spedali Civili, Piazzale Ospedale, 1 25100 Brescia, Italy. Tel. ++39/030/3995838; Fax. ++39/030/3995839, e-mail: deferrari@master.cci.unibs.it

Population Genetics of 9 STR Loci in Turkish Population

M. Kurtuluş Ülküer¹, Ü. Ülküer², C. Elma² and T. Kesici³

¹*Department of Biology, Faculty of Kırşehir Education, Gazi University, Turkey*

²*General Directory of Security, Criminal Police Laboratories, Turkey*

³*Biometry-Genetics Unit, Faculty of Agriculture, Ankara University, Turkey*

The use of polymorphic short tandem repeat is becoming important in genetics applications, such as gene mapping, identification and paternity. In forensic application it is possible to reach result even though the material is highly degraded and mixed. Because of the advantages of STR analyses, it is increasingly being used in forensic applications.

The purpose of this study is to investigated the population genetics of STR polymorphism. In the research, the allele frequency distribution of 9 STR loci which were D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820 was established using the blood samples of 100 randomly selected individuals. The 9 STR loci were amplified and detected by using multiplex PCR with fluorescent-labelled primers.

The frequency distributions of alleles were evaluated by using various statistical methods, such as HWE, polymorphisms information content (PC), power discrimination (PD) and probability of exclusion (PE). The allele frequency distributions found in this study were compared with the other allele frequency distributions reported in literature.

Melihat Kurtuluş Ülküer PhD

Emniyet Genel Müdürlüğü

Kriminoloji Polis Laboratuvarı

Anıttepe-Ankara-Turkey

Tel: 00 90 312 4124537

e-Mail: melahatkurtulus@hotmail.com

Genetic Structure of the Contemporary Cuban Population for Nine STR Loci

R. Leonart¹, Y. Carpio¹, M.V. Sainz de la Peña², M. Santiesteban², G. Rodríguez⁵, R. Ferreira⁵, F. Amaro³, E. Riego¹, K. Bacallao³, M. Blanco⁴

¹Center for Genetic Engineering and Biotechnology, Havana, Cuba.

²Laboratorio Central de Criminalística, Havana, Cuba.

³Legal Medicine Institute, Havana, Cuba.

⁴AIDS Research Laboratory, Havana, Cuba.

⁵Hermanos Ameijeiras Hospital, Havana, Cuba.

Short tandem repeats are at the moment the most convenient and powerful way to infer personal identification out of genome data obtained from scarce and highly degraded DNA. In the forensic context, personal identification from bones and other human remains are one of the most challenging tasks to solve in cases where traditional anthropology is insufficient to achieve that goal. In order to assess the usefulness of a set of microsatellites containing the loci HUMTH01, HUMTPOX, HUMCSF1PO, HUMvWA, HUMFESFPS, HUMF13A01, HUMF13B, HUMHPRTB and HUMLPL, reference databases were constructed for the three main racial groups of the contemporary Cuban population (Caucasoids, Mestizos and Negroids). Minimal allele frequencies were estimated following the expression described by Budowle (Int J Legal Med (1996) 108: 173–176) based on the predictions of the Infinite Alleles Model to avoid negative bias when rare alleles are considered. The distributions of allele frequencies were found to be similar to the ones reported by other authors at close related population groups. A rare allele 5 was found, sequenced and characterized in Mestizo group at the HUMCSF1PO locus. Private alleles were found for the three groups. The three Cubans databases were validated for the presence of Hardy-Weinberg equilibrium and Linkage Disequilibrium using Markov Chain Monte Carlo methods. A few significant *P* values were eliminated when Bonferroni type corrections were done for the control of type I error, indicating both the validity of the sampling scheme done and the possibility to use the standard formulae to estimate the frequency of a multilocus profile in the Cuban population. The way genetic variation is distributed at the contemporary Cuban population was tested by estimating *F* and *Rho* statistics and testing their significance by permutation analysis. Genetic Distance for microsatellites ($\delta\mu^2$) was also calculated between each racial group as well as rate of Migration (*Nm*) to further characterize the dynamics of genotypes at the population. Paired comparisons indicate that there is very high level of genetic information exchange between groups as judged from the estimated values of *Nm*. Inbreeding coefficients (*f*) were estimated using the method of moments for each racial group being in close agreement with the values reported by other authors. Results are also shown which confirm that among all three groups there is significant genic differentiation for all the loci except for HUMLPL, and significant genotypic differentiation for all loci except HUMHPRTB and HUMLPL.

The forensic usefulness of this group of nine STR loci was demonstrated by the very high values obtained for combined Discrimination Power which ranged from 1:1.5 x 10⁸ (Caucasic group) to 1:2.6 x 10⁹ (Blacks group).

Taken all together, these results confirm the validity of the use of both the Cuban databases and this STR set for Personal Identification purposes in this population.

Dr. Ricardo Leonart Cruz, Center for Genetic Engineering and Biotechnology, P.O. Box 6162, La Habana, CP 10600, CUBA.
Tel. (537) 218008. Fax. (537) 218070.
e-mail: rleonart@cigb.edu.cu or rleonart@yahoo.com

Population genetics of three STR markers (CYP19, D8S1132 and FGA) in north-east Italy

R. Perossa¹, F. Cossutta¹, B. Gregoret², A. Peretti¹, B.M. Altamura¹ and P. Fattorini¹

¹Department of Sciences of Public Health, University of Trieste, Trieste, Italy

²Departmente of Biomedical Sciences, University of Trieste, Trieste, Italy

More than one hundred unrelated subjects living in north-east Italy were analysed at loci CYP19, D8S1132 and FGA. PCR was performed following standard procedures with minimal modifications. The PCR products were analysed by polyacrylamide gel electrophoresis and silver staining. Sequenced allelic ladders were employed as internal standards.

Allele frequency distributions and other forensic parameters (OH, EH, PM and DP) useful for personal identification and paternity testing are provided.

Correspondence to:

Paolo Fattorini, PhD, Department of Sciences of Public Health, UCO of Legal Medicine, University of Trieste, via Molino a Vento 123, 34147, Trieste, Italy; Tel. 0039 (0)40 946153; Fax: 0039 (0)40 392399; e-mail: fattorin@univ.trieste.it

Allele frequencies of the STR-loci F13A01, F13B, TPOX in population sample from the Ukraine

Yu.M. Sivolap, S.V. Chebotar, G.F. Krivda

Molecular-Genetics Expertise Center of Odessa Region Forensic Service Odessa, Ukraine

Before installation of PCR-technique to expert practice of such region forensic service for which genetic-population research is absent, preliminary analysis of alleles and genotypes distribution in concrete population is necessary. This analysis includes the evaluation of individual genotypes distribution correspondence to the equilibrium law Hardy-Wainberg, the estimation of expected loci heterozygote (H_{exp}) and following parameters of selfdescriptiveness selected loci: probability of casual concurrence of two unrelated persons genotypes (probability of random match, pM), probability of distinguishing of two unrelated persons genotypes (power of discrimination, PD), average probability of typing sample exception (mean exclusion chance, W), selfdescriptiveness of given locus polymorphism (polymorphism information content, PIC).

Till now analysis data on alleles frequencies matching and polymorphism information contents of researched loci in Ukraine populations are absent in general, that hinders to use DNA-typing technique for person identification.

We estimated the alleles frequencies of F13A01, F13B, TPOX loci in Ukrainian population among the unrelated representatives.

Distribution of alleles and genotypes frequencies is showed in the tables 1 and 2. In the investigated population the availability 14 alleles in locus F13A01 (sample size $n = 88$) was shown. Most distributed has appeared the allele 7 with cleanness matching 0.3238. On locus F13B ($n = 103$) which is revealed 7 alleles most widespread from 9 and 10 with frequency 0.233 and 0.392 accordingly. In locus TPOX (on sample $n = 100$) is revealed 8 alleles, the greatest frequency matching 0.43 and 0.17 is shown for alleles 8 and 11.

The alleles frequencies distribution of F13A01, F13B, TPOX loci, observed in researched population, is rather similar to those in population groups relating to europeoid race in USA and Europe.

The observable frequencies matching of genotypes investigated loci were checked up on deviation from equilibrium Hardy-Wainberg on criterions χ^2 and G-statistics.

Character of alleles inheriting and the valuation of individual genotypes distribution correspondence of investigated loci is to the equilibrium law Hardy-Wainberg.

A VNTR polymorphism in Human 5'H19 flanking of Japanese and German populations

M. Fukuda¹, E. Naito¹, K. Dewa¹, K. Umetsu², I. Yuasa³, H. Yamanouchi¹

¹*Department of Legal Medicine, Niigata University School of Medicine, Japan*

²*Department of Legal Medicine, Yamagata University School of Medicine, Japan*

³*Department of Legal Medicine, Tottori University School of Medicine, Japan*

In mammals, imprinted genes are preferentially expressed from either the maternal or paternal allele. Several recent observations show that DNA methylation plays an important role in the imprinted inheritance of the gene. Generally, an individual inherits two alleles together from his parents and the origin of each allele can be determined by typing the parents. The ultimate aim of this study is to detect a paternally or maternally derived allele from one person by using the methylation difference in the imprinted region. For the purpose, a useful probe within the region is requisite as the analytic target.

In this study, the forensic utility of the VNTR locus, which is located approximately 7.6 kb upstream of the H19 gene that is maternally expressed, was evaluated. The human 5' H19 flanking sequence was searched from the DDBJ. The allele frequency of the VNTR locus was examined in a total of 199 unrelated Japanese and 171 unrelated German individuals. In the Japanese samples, 7 alleles and 22 genotypes were identified. Their heterozygosity and polymorphism information content (PIC) were 0.749 and 0.669, respectively. By contrast, 9 alleles and 27 genotypes were detected in the German samples. Their heterozygosity and PIC were 0.969 and 0.705, respectively. Thus, the frequency distribution of the two populations showed different profiles (Table 1). In a Japanese case study, this genetic typing was successfully applicable to the personal identification of a decomposed forensic sample. These results indicated that the VNTR in the H19 5'flank is highly polymorphic and useful for personal-identification of both Japanese and German DNA samples. This suggests the VNTR has become a useful probe for the aimed method.

Table 1

Allele(repeat)	Japanese (n = 199)	German (171)
14	–	0.006
13	–	0.020
12	0.060	0.085
11	0.083	0.167
10	0.091	0.023
9	0.472	0.184
8	0.015	0.020
7	0.221	0.436
6	0.058	0.059
Key Words	Imprinted gene, H19-5'flank, VNTR, Japanese, German	

A study on Four Short Tandem repeat systems: African Immigrant, Portugal and Spanish population data

J.J. Gamero^{1*}, J.L. Romero¹, J.L. González¹, M. Carvalho², M.J. Anjos², F. Corte-Real², D.N. Vieira² and M^a.C. Vide²

¹Department of Legal Medicine, Faculty of Medicine. University of Cádiz. Fragela s/n, Cádiz 11003. Spain.

²Institute of Legal Medicine of Coimbra. 3000 Coimbra. Portugal.

Frequency data of short tandem repeat (STR) loci HUMTHO1, HUMVWA₃₁/A, HUMF₁₃A1 and HUMFES/FPS were determined in blood stains obtained from a group of unrelated individuals from Portugal, south west Spain (Huelva, Cádiz and Sevilla), Spaniards of Caucasian origin from North Africa, as well as in the black central West African and Moroccan immigrant populations in Spain. Likewise, we estimated several forensic parameters useful for human identification and paternity testing.

Address:

Joaquín-José Gamero Ph. M.
Dpto. Medicina Legal
Facultad de Medicina
Universidad de Cádiz
Plaza Fragela s/n
11003, Cádiz, Spain.
e-mail: joaquin.gamero@uca.es

Analysis of 15 STR in the Italian Population of Alia

M. Pizzamiglio¹, C.M. Calò², G. Vona², A. Mameli¹ and L. Garofano¹

¹Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italia

²Dipartimento di Biologia Sperimentale, Sezione Scienze Antropologiche, Università degli studi di Cagliari, Italia

A preliminary typing on the Italian population of Alia (Sicily) was carried out.

Situated at 800 metres above sea level, about 80 km from Palermo, Alia today has a population of about 4000 inhabitants. In 1837 Alia was hit by an epidemic of cholera and the population was reduced dramatically. In 1995 an excavation brought to light the remains of about 300 individuals, who had died of cholera. This event attracts the attention of anthropologist and geneticists on Alia population.

Blood specimens were obtained from 50 healthy and unrelated individuals of both sexes, born and living in Alia as well as their parents and grandparents.

The analysed markers in the current study were: THO1, D2S11, D18S51, VWA, FIBRA, D8S1179, TPOX, CSF1PO, D16S539, D7S820, D13S317, D5S818, D3S1358, D19S433, D2S1338. DNA has been extracted through the phenol-chloroform method, and amplified by PCR. Separation and detection of the amplified STR fragment was carried out by use of 377 automated system (Applied Biosystem Division/Perkin Elmer).

All loci, except THO1, meet Hardy-Weinberg expectation. Linkage disequilibrium test, applied to the loci which lie on the same chromosome (D2S1338 e TPOX, D5S818 e CSF1PO), did not show significant values.

PIC (Polymorphism Information Content) and Power of Discrimination resulted generally high, underlining the usefulness of these markers both in the linkage and forensic analysis.

Obtained data were compared with ones of other Mediterranean populations got from bibliography, so that it was possible to analyse the genetic structure of Alia population in relation to the other comparison populations. Some genetic characteristics of Alia population seem to be modelled by the effects of genetic drift and preserved by isolation, as demographic and historical events demonstrate.

The results stressed out the importance of paying attention to the study of subpopulations, that proved themselves to be usefulness in the study of microgeographic variation and in forensic analysis. Moreover, it will be interesting to compare data on present population of Alia with the ones of sub-fossil population died of cholera, that the University of Göttingen (Germany) is carrying on. This will clarify if this dramatic event changed the genetic structure of the population who survived to the cholera.

Allele distribution of fifteen STRs in a population from Extremadura (Central-West Spain)

J. García-Hirschfeld, M.J. Farfán, V. Prieto, M. López-Soto, Y. Torres and P. Sanz

Servicio de Biología, Instituto Nacional de Toxicología, Sevilla, Spain

Our Institute investigates criminal and paternity cases, mainly from Andalusia (Southern Spain) and to a lesser extent from Extremadura, a Central-West Spanish region. An Extremadura population study of fifteen STRs currently used in our analyses would be necessary in order to determine whether the allele frequency distribution differs from that of Andalusia, for which more data is available and is routinely used in our casework.

Here we present allele frequencies for fifteen STRs determined in samples from unrelated individuals from Extremadura using the SGM Plus™ and Power Plex™ 16 multiplex systems. Detection was made by capillary electrophoresis with Applied Biosystems equipment, ABI 310 Prism. Some statistical parameters of forensic interest such as observed/expected heterozygosity, mean exclusion chance, discrimination power and minimum frequencies were determined. Pairwise comparison with an Andalusian population was performed using the Chi-square test for homogeneity.

Dr. Julia García-Hirschfeld, Instituto Nacional de Toxicología,

P.O. Box 863. E-41080 Sevilla, Spain.

Tel. +34 954371233; Fax: +34 954370262; e-mail: biol@sev.inaltox.es

Genetic analysis of the short tandem repeat loci D1S1656, D12S391, D18S535 and D22S683 in the Croatian population

Z. Grubić, K. Štingl and A. Kaštelan

National Referral Organ Transplantation and Tissue Typing Centre, Zagreb, Croatia

Population study was carried out on the sample of 150 unrelated donors from the wider area of the Croatian capital, Zagreb using the short tandem repeat (STR) systems: D1S1656, D12S391, D18S535 and D22S683. After polymerase chain reaction (PCR) samples were loaded on 6% polyacrylamide gel and detected using ALFexpress (Pharmacia Biotech) sequencer. Analysis of polymorphism at D1S1656 locus showed that among 19 observed alleles, the most frequent was allele 15 (17.7%), while the most present allele at D12S391 was allele 13 (13.5%). Nine different alleles were observed at D18S535 locus and predominant was allele 13 (31.1%). At D22S683 locus the highest frequency was found for allele 10 (14.8%). The observed heterozygosities were as follows: 86.2, 87.4%, 74.3%, and 81.2%, respectively. Power of exclusion determined for the individual loci was 0.981, 0.984, 0.932, and 0.985, respectively, and the combined power of discrimination for these loci was 0.999. Testing of the observed genotype distribution for Hardy-Weinberg equilibrium did not reveal any significant deviations. In conclusion, the four STR systems are highly informative and these data can be used in forensic medicine as well as for following chimerism after allogenic bone marrow transplantation.

Address for correspondence:

Dr. Zorana Grubić, National Referral Organ Transplantation and Tissue

Typing Centre, Kišpatičeva 12, 10000 Zagreb, Croatia,

Tel. 00 385 1 23 88 689; Fax: 00 385 1 23 12 684,

e-mail: zgrubic@yahoo.com

Typing of pentanucleotide STR polymorphisms

Y.P. Hou, Q.Ji, J.G.Dong, J.P.Tang, J.Zhang, Y.B. Li, J. Wu and J.Yan

Institute of Forensic Medicine, Sichuan University, Chengdu, PR China

Pentanucleotide tandem repeat markers are interesting for forensic sciences, because they may present less stutter on electrophoretic pattern. We focused on analysis of the DNA sequence for each allele at two pentanucleotide STR loci, D6S957 and D10S2325, in order to understand their structures in the human genome and to construct human allelic ladders, which were necessary for forensic DNA typing. In order to evaluate the forensic applicability of both pentanucleotide tandem repeat loci and to construct a preliminary database, the genotype distributions and allele frequencies in different ethnic groups were investigated. The population samples included Caucasians (Germans) and Asians (Chinese). The Amp-FLP technique was employed for DNA typing. An example of each allele and new alleles were sequenced. Allele determination for each pentanucleotide STR locus was carried out by comparison with the sequenced human allelic ladder made in-house. Both pentanucleotide STR markers provided easily interpretable results. No evidence of deviation from Hardy-Weinberg equilibrium was observed. The power of discrimination at D6S957 locus was 0.883 for Germans and 0.802 for Chinese, while at D10S2325 locus the power of discrimination was 0.953 for Germans and 0.966 for Chinese. In 64 confirmed father/mother/child triplets no mutation event was observed. Using a maximum likelihood method, the mutation rate at both pentanucleotide STR loci was indirectly estimated as 2.5×10^{-5} . These results suggest that both pentanucleotide STR markers are useful for forensic casework and paternity analysis.

*Prof. Yi Ping Hou, Institute of Forensic Medicine,
Sichuan University (West China University of Medical Sciences),
610041 Chengdu, PR China,
Tel. 0086-28-5501549; Fax: 0086-28-5501549,
e-mail: rechtsme@wcums.edu.cn*

A New useful STR Locus for forensic analysis

J. Zhang¹, Y.P. Hou¹, J. Wu¹, Y.B. Li¹ and Y.F. Wang²

¹*Institute of Forensic Medicine, Sichuan University, Chengdu,
PR China*

²*Dep. of Pathophysiology, School of Basic Medicine,
Sichuan University, Chengdu, PR China*

In order to get more available STR loci for forensic DNA analysis in Chinese population, we investigated 10 tetranucleotide STR loci on chromosome 9 selected from GDB (D9S917, D9S254, D9S1121, D9S1118, D9S301, D9S1122, D9S922, D9S252, D9S2026, D9S934). Then we chose some of them for further evaluation of forensic genetics. Many samples were employed for this study, which including 1038 EDTA-blood samples of unrelated individuals and some biological materials from criminal scene, such as stubs, blood stains and remains. Amp-FLP technique was employed for DNA typing. All alleles were cloned in plasmid and sequenced by ABI 310. For each locus, the cloned alleles were amplified and the PCR products were mixed to make an allelic ladder for DNA typing. The Software POPGENE was used to analyze the allele frequencies and heterozygosities of 10 STR loci in Chinese Han population. The Hardy-Weinberg equilibrium was checked using the chi-square test. The results showed that compared with other 9 loci, allele frequencies of D9S1118 had a preferable distribution in Chinese Han population. The amplified segments of D9S1118 were arranged from 141bp to 177bp. Using our sequence results, we named the alleles according to the repeat number of motifs. The most common allele was 14 with frequency 0.210. The exclusion probability was 0.6405, while the discrimination power was 0.9424. Analyzing biological materials from criminal scene showed that typing D9S1118 had a higher successful rate. All of these results suggest that D9S1118 as a marker could be used for forensic DNA analysis in Chinese population.

*Prof. Yi Ping Hou, Institute of Forensic Medicine,
Sichuan University (West China University of Medical Sciences),
610041 Chengdu, PR China,
Tel. 0086-28-5501549; Fax: 0086-28-5501549,
e-mail: rechtsme@wcums.edu.cn*

Polymorphisms of 6 STR loci on chromosome 22 in chinese population

J.P. Tang, Y.P. Hou, Y.B. Li, J. Wu and J. Zhang

Institute of Forensic Medicine, Sichuan University, Chengdu, PR China

In order to develop more STR markers for forensic DNA typing in Chinese population, six STR loci on chromosome 22 were investigated. Amp-FLP technique was employed for genotyping of individuals from Han population samples in North and South China. The Hardy-Weinberg equilibrium was checked using the chi-square test. No significant deviation from Hardy-Weinberg equilibrium could be found at all of six loci. The results showed that the differences of genotype distributions at two loci were significant between two populations, but the distributions of genotypes at other four loci were similar in both populations. Five loci of them showed good polymorphisms in both populations. Their heterozygosities were more than 0.59. The discrimination power and the exclusion probability were more than 0.82 and 0.40, respectively. They were the suitable candidate markers for forensic applications. At another locus, only two alleles were found. The heterozygosity of this locus was less than 0.36. Its discrimination power and exclusion probability were less than 0.62 and 0.12, respectively. It showed that this locus was not good marker for forensic application. A two-step mutation was observed at one of six loci transmitted from father to son in a pedigree.

*Prof. Yi Ping Hou, Institute of Forensic Medicine,
Sichuan University (West China University of Medical Sciences),
610041 Chengdu, PR China,
Tel. 0086-28-5501549; Fax: 0086-28-5501549,
e-mail: rechtsme@wcums.edu.cn*

Spanish Caucasian population genetic data and paternity testing using the PowerPlex® 16 System

J. Puente, C. Cabrero and J.C. Tercero

Department of Human Identification, Pharma Gen S.A., Madrid, Spain

Allele distribution of the thirteen CODIS tetranucleotide STR loci (D3S1358, TH01, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, CSF1PO, vWA, D8S1179, TPOX, and FGA), and two pentanucleotide STR loci (Penta E and Penta D) were determined in a Spanish sample population, consisting in 162 unrelated individuals, using the PowerPlex® 16 System. All loci meet Hardy-Weinberg expectations (HWE). In addition, the pairwise independence testing shows no evidence for association of alleles among the fifteen loci. Statistical analysis of the data also included power of discrimination (PD), probability of exclusion (PE), polymorphism information content (PIC), and typical paternity index. Samples from 133 cases of disputed paternity were analyzed, although the majority of them were deficiency cases (the mother was not tested). In all the 23 exclusion cases, evidence for exclusion was obtained on four or more STR system. Where no exclusion is found, paternity index (PI) values of $>10^6$ are expected in all trio cases (child, mother, alleged father), but $>10^4$ in 85% of the deficiency cases. These results demonstrate that the PowerPlex® 16 System is a highly discriminating test suitable for paternity and identity testing in the Spanish population.

*Address for correspondence:
Jorge Puente, Department of Human Identification,
Pharma Gen S.A., C/Alcarria, 7. 28820 Coslada, Madrid;
Tel. 34 91 674 89 90; Fax: 34 91 674 89 91;
e-mail: tecnico@pharmagen.es*

Codis System in the Basque Country Resident Population Studied with Multiplex Systems

Oscar García¹, Ion Uriarte², Raúl Peñas², Pablo Martín³,
Cristina Albarrán³, Antonio Alonso³

¹Area de Laboratorio Ertzaintza, Sección de Genética Forense,
Bilbao, Spain

²Unidad de Policía Científica, Ertzaintza, Bilbao, Spain

³Instituto de Toxicología, Sección de Biología, Madrid, Spain

Before a new marker system can be introduced into forensic casework, a population database for the relevant population must be established for statistical evaluation of the evidence. Therefore, this report presents allele frequency data in a Basque Country resident population sample ($n > 200$) for 13 STR loci. The loci are: TH01, TPOX, CSF1PO, D3S1358, FGA, VWA, D5S818, D13S317, D7S820, D8S1179, D21S11, D18S51 and D16S539.

Genomic DNA was extracted by standard phenol-chloroform extraction procedure. PCR amplification was performed according to the manufacturer's recommendations using the AmpFISTR Profiler Plus and Cofiler Amplification kit. Samples were denatured for 3 min at 95°C and loaded onto 5% denaturing Long Ranger gels. The gels were run for 3 h at constant voltage (3000 V) on an ABI 377 sequencer. Allele designations were made according to recommendations of the DNA Commission of the International Society for Forensic Genetics. Results demonstrate the assumption of independence within and between the loci analyzed.

Statistical evaluations were performed using a HWE-Analysis software package. Analyses included the possible divergence from Hardy-Weinberg expectations and other parameters of forensic importance: minimum allele frequencies, observed and expected heterozygosities, mean exclusion chance (MEC), polymorphic information content (PIC) and discrimination power (DP). The possible associations between loci were tested using the computer program GDA (Genetic Data Analysis).

The combined power of exclusion is estimated as 99.9988% and the combined power of discrimination is >99.99999%. These 13 STR systems have been shown to be useful tool for personal identification. The allele frequency data can be used for deriving estimates of multiple locus profile frequencies for identity testing purposes using the product rule.

Address for correspondence:

Dr. Oscar García, Area de Laboratorio Ertzaintza, Avda.
Montevideo 3, 48002 – Bilbao (Spain), e-mail: gobiesoi@sarenet.es

Population Genetic Data for 13 STR Loci in a Northeast Colombian (Department of Santander) Population

Clara Inés Vargas¹, Adriana Castillo¹, Adriana María Gil¹,
Adriana Lucía Pico¹, Oscar García²

¹Laboratorio de Genética, Facultad de Salud, Universidad Industrial
de Santander (UIS), Bucaramanga, Colombia

²Area de Laboratorio Ertzaintza, Sección de Genética Forense, Bilbao,
Spain

Before a new marker system can be introduced into forensic casework, a population database for the relevant population must be established for statistical evaluation of the evidence. Therefore, this report presents allele frequency data in a Northeast Colombian (department of Santander) population sample ($n = 103\text{-}399$) for 13 STR loci. The loci are: TH01, TPOX, CSF1PO, D3S1358, FGA, VWA, D5S818, D13S317, D7S820, D8S1179, D21S11, D18S51 and D16S539.

Genomic DNA was extracted by the salting out extraction procedure. PCR amplification was performed according to the manufacturer's recommendations using the AmpFISTR Profiler Plus and Cofiler Amplification kit. Samples were denatured for 3 min at 95°C and loaded onto an ABI 310 sequencer. Allele designations were made according to recommendations of the DNA Commission of the International Society for Forensic Genetics. Results demonstrate the assumption of independence within and between the loci analyzed.

Statistical evaluations were performed using a HWE-Analysis software package. Analyses included the possible divergence from Hardy-Weinberg expectations and other parameters of forensic importance: minimum allele frequencies, observed and expected heterozygosities, mean exclusion chance (MEC), polymorphic information content (PIC) and discrimination power (DP). The possible associations between loci were tested using the computer program GDA (Genetic Data Analysis).

The combined power of exclusion is estimated as 99.9988% and the combined power of discrimination is >99.99999%. These 13 STR systems have been shown to be useful tool for personal identification. The allele frequency data can be used for deriving estimates of multiple locus profile frequencies for identity testing purposes using the product rule.

Address for correspondence:

Dr. Clara Inés Vargas, Laboratorio de Genética,
Facultad de Salud, Universidad Industrial de Santander (UIS),
Carrera 32, N° 29–31, Bucaramanga (Colombia)
e-mail: cvargas@uis.edu.co

Population Data on D7S820, FGA, D1S533 and D9S304 in a Sample of Caucasian-Mestizos from Colombia

Juan J. Yunis^{1,2}, Oscar García³, Sandra Moreno¹, Ion Uriarte⁴, Emilio J. Yunis¹

¹Servicios Médicos Yunis Turbay y Cía. Santafé de Bogotá, Colombia

²Departamento de Patología, Facultad de Medicina e Instituto de Genética, Universidad Nacional de Colombia

³Area de Laboratorio Ertzaintza, Sección de Genética Forense, Bilbao, Spain

⁴Unidad de Policía Científica, Ertzaintza, Bilbao, Spain

Before a new marker system can be introduced into forensic casework, a population database for the relevant population must be established for statistical evaluation of the evidence. Therefore, this report presents allele frequency data in a sample of Caucasian-Mestizos from Colombia ($n = 919-921$) for D7S820, FGA, D1S533 and D9S304 loci.

Genomic DNA was isolated from whole blood by the Wizard Genomic DNA isolation kit (Promega Corporation). PCR amplification was performed according to the manufacturer's recommendations using the Multiplex II kit (Lifecodes Corporation, Stamford, CT). The PCR products were resolved in denaturing gels and detected by silver nitrate staining. Allele designations were made according to recommendations of the DNA Commission of the International Society for Forensic Genetics. Results demonstrate the assumption of independence within and between the loci analysed.

Statistical evaluations were performed using a HWE-Analysis software package. Analyses included the possible divergence from Hardy-Weinberg expectations and other parameters of forensic importance: minimum allele frequencies, observed and expected heterozygosities, mean exclusion chance (MEC), polymorphic information content (PIC) and discrimination power (DP). The possible associations between loci were tested using the computer program GDA (Genetic Data Analysis).

The combined power of exclusion is estimated as 98.24% and the combined power of discrimination is 99.9993%. These 4 STR systems have been shown to be useful tool for personal identification. Therefore, this Colombian population database can be used in identity testing to estimate the frequency of a multiple PCR-based locus DNA profile in forensic cases as well as in paternity testing.

Address for correspondence:

Dr. Emilio J. Yunis, Servicios Médicos Yunis Turbay y Cia. Bogotá, D.C. Colombia, e-mail: emilioyunis@hotmail.com

Population Data on FGA, vWA, TPOX, TH01, Penta E, D18S51, D21S11, D3S1358, D8S1179 and D16S539, D7S820, D13S317, D5S818 In a sample of Caucasian-Mestizos from Colombia

Juan J. Yunis^{1,2}, Oscar García³, Sandra Moreno¹, Cielo Pineda¹, Claudia Rodríguez¹, Ion Uriarte⁴, Emilio J. Yunis¹

¹Servicios Médicos Yunis Turbay y Cía. Santafé de Bogotá, Colombia

²Departamento de Patología, Facultad de Medicina e Instituto de Genética, Universidad Nacional de Colombia

³Area de Laboratorio Ertzaintza, Sección de Genética Forense, Bilbao, Spain

⁴Unidad de Policía Científica, Ertzaintza, Bilbao, Spain

Before a new marker system can be introduced into forensic casework, a population database for the relevant population must be established for statistical evaluation of the evidence. Therefore, this report presents allele frequency data in a sample of Caucasian-Mestizos from Colombia ($n = 247-267$) for Powerplex 2.1 and ($n = 491$) for GammaStar loci.

Genomic DNA was isolated from whole blood by the Wizard Genomic DNA isolation kit or the ReadyAmp DNA isolation kit (Promega Corporation). PCR amplification was performed according to the manufacturer's recommendations using the Powerplex 2.1 and the GammaStar systems (Geneprint Systems, Promega Corporation). The PCR products were resolved in 5% Long Ranger denaturing gels for Powerplex 2.1 and in 4% Acrylamide-Bis-Acrylamide denaturing gel for GammaStar and detected in a Hitachi FMBIO II scanner. Allele designations were made according to recommendations of the DNA Commission of the International Society for Forensic Genetics. Results demonstrate the assumption of independence within and between the loci analysed.

Statistical evaluations were performed using a HWE-Analysis software package. Analyses included the possible divergence from Hardy-Weinberg expectations and other parameters of forensic importance: minimum allele frequencies, observed and expected heterozygosities, mean exclusion chance (MEC), polymorphic information content (PIC) and discrimination power (DP). The possible associations between loci were tested using the computer program GDA (Genetic Data Analysis).

The combined power of exclusion is estimated as 99.9995% and the combined power of discrimination is 99.9999999962%. These 13 STR systems have been shown to be useful tools for personal identification. Therefore, this Colombian population database can be used in identity testing to estimate the frequency of a multiple PCR-based locus DNA profile in forensic cases as well as in paternity testing.

Address for correspondence:

Dr. Juan J. Yunis, Servicios Médicos Yunis Turbay y Cia. Ave 22 # 42-24, Bogotá, D.C. Colombia
e-mail: jyunis@hotmail.com

Allele Frequency Data for 15 STR Loci (AmpFISTR SGM Plus and AmpFISTR Profiler) in the Belgian Population

R. Decorte, A. Gilissen and J.-J. Cassiman

Laboratory for Forensic Genetics and Molecular Archaeology,
Center for Human Genetics, University of Leuven, Belgium

A population study was carried out on 100 unrelated Caucasian individuals of Belgian descent in order to determine allele and genotype frequencies for 15 STR loci for forensic purposes. DNA was extracted from blood by chelex extraction or from buccal swabs with the QiaAmp DNA Kit (Qiagen). In total, 15 STRs (D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S443, TH01, FGA, TPOX, CSF1PO, D5S818, D13S317 and D7S820) were amplified in 2 multiplex reactions (AmpFISTR SGM Plus and AmpFISTR Profiler kit) using 25 µl reactions with cycling parameters according to the recommendations of the manufacturer. Amplified PCR products were size fractionated on the ABI PRISM 310 Genetic Analyzer (capillary electrophoresis) followed by allele designation with GenTyper software. The number of alleles ranged from 5 (TPOX) to 13 (D18S51 and FGA) while expected heterozygosity levels ranged from 63.3% (TPOX) to 88.3% (D2S1338). No deviation from Hardy-Weinberg equilibrium was detected for all loci with an exact probability test (Genepop v3.1). Exact tests for genotypic disequilibrium between pairs of loci revealed no deviations even for the STRs CSF1PO and D5S818 which are localized respectively on 5q33.3–34 and 5q23.3–32. The combined power of exclusion for the 15 STRs was 0.9999992 while the combined matching probability was 1.7×10^{-17} . The two multiplex systems are now in use for routine paternity analysis and their use in forensic cases is under evaluation. Two mutations were observed in the course of 32 paternity cases: one paternal mutation for D2S1338 and one maternal mutation for CSF1PO. Both mutations were the result of a loss or a gain of one repeat unit.

Dr. Ronny Decorte
Laboratory for Forensic Genetics and Molecular Archaeology
Center for Human Genetics
University of Leuven
Herenstraat 49
B-3000 Leuven
Belgium
Tel: 00 32 (0)16 346 077
Fax: 00 32 (0)16 345 997
e-mail: ronny.decorte@med.kuleuven.ac.be

ALU Genetic Diversity in India: population genetic and forensic implications

S.S. Mastana¹, S.S. Papiha², P. Singh⁴, K. Das³, A. Pacynko¹,
P. Fisher¹, N. Malik⁵, M. Das³, P.H. Reddy⁶.

¹Department of Human Sciences, Human Genetics Lab,
Loughborough University, Loughborough, UK.

²Department of Human Genetics, University of Newcastle upon Tyne,
Newcastle upon Tyne, UK.

³Anthropometry and Human Genetics Department, Indian Statistical
Institute, Calcutta, India;

⁴Department of Human Biology, Punjabi University Patiala Punjab,
India;

⁵Department of Anthropology, Raipur University, Raipur, India.

⁶Neurological Sciences Institute Oregon Health Sciences University
1120 NW Avenue Portland, OR 97209-1595. USA

ALU polymorphisms provide a useful tool to population geneticists for understanding the population dynamics that have occurred over time. We report here a study of Six Alu insertion loci (TPA25, D1, APO, PV92, FXIIB and ACE) from 18 endogamous caste and tribal populations (900 samples) representing different geographical regions of India. Overall spectrum of variation in these populations is very interesting at different geographical and cultural levels. High level of insertion frequencies was observed in some highly inbred groups. Average levels of heterozygosities were found to be relatively high in these populations (range 41% to 49.8%). The genetic diversity coefficient G_{ST} among this group of populations was observed to be high. Phylogenetic trees and principal components analysis (PCA) computed from Alu frequencies provide support for socio-cultural and geographical assignment of these populations in Indian population structure. Results are discussed with reference to forensic and paternity implications and human genetic diversity in Indian populations.

Address for correspondence:

Dr. Sarabjit Mastana
Human Genetics Lab.,
Department of Human Sciences
Loughborough University
Loughborough LE11 3TU
UK
Tel: 01509223041
Fax: 01509223941
e-mail: S.S.Mastana@lboro.ac.uk

Paternity testing results – Allelic Distribution of SLPs and STRs in Brazilian Caucasoid Population

L.F. Jobim^{1,2}, M.R. Jobim¹, F. Gamio¹, G. Ewald¹ and L. Fernandes¹

¹Laboratório DNA Reference Ltda, Porto Alegre, Brazil,

www.dnareference.com.br

²Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Brazil

A study of SLPs or STRs allelic frequency in Caucasoid population in Brazil was performed based on 6,000 paternity testing. SLPs (RFLP Nice Probes, Cellmark, UK) were used whenever the case was fatherless and members of the family were available for sampling. STRs in single amplifications (not multiplex) were used when family members were not available and after phenol/chloroform extraction of DNA from bones and teeth from the alleged dead father. In all regular cases, with alleged father and child present for sampling, we used several multiplexed "in house" STRs as well as the ones in the Profiler Plus kit (Applied Biosystems, USA) and after extracting the DNA using the salting out method (Miller, 1988). All STRs were analysed using either an ABI (Applied Biosystems, USA) or an Alpha Express (Pharmacia). The distribution of the most frequent alleles of the STRs used is presented in the table below. Furthermore, the exclusion rate for regular cases in the court was 31.81%. The rate in friendly requests was 23.86%. Results from fatherless cases were analysed regarding the number of individuals tested. Cases after exhumation were also showed.

Prof. Dr. Luiz Fernando Jobim.

Laboratório DNA Reference,

Rua Padre Chagas 140,

Porto Alegre 90580-020, Brazil.

Tel/Fax: +55-51-2221350

e-mail: drjobim@dnareference.com.br

www.dnareference.com.br

LOCUS	N	HETEROZ. %		MOST FREQUENT AELLES							
D8S1179	900	707 0.79	9 0.013	10 0.07	11 0.071	12 0.13	13 0.29	14 0.25	15 0.11	16 0.03	17 0.005
D21S11	898	761 0.85	27 0.02	28 0.16	29 0.21	30 0.22	30.2 0.03	31 0.07	31.2 0.10	32.2 0.10	33.2 0.03
D18S51	900	789 0.88	12 0.13	13 0.11	14 0.17	15 0.15	16 0.14	17 0.11	18 0.07	19 0.05	20 0.02
VWA31	1461	1148 0.79	12 0.002	13 0.004	14 0.09	15 0.12	16 0.24	17 0.24	18 0.20	19 0.08	20 0.02
D3S358	896	710 0.79	14 0.10	15 0.26	16 0.25	17 0.21	18 0.14	19 0.006			
FGA	897	781 0.87	19 0.07	20 0.12	21 0.16	22 0.17	23 0.15	24 0.14	25 0.11	26 0.03	27 0.01
D5S818	894	653 0.73	7 0.01	8 0.02	9 0.04	10 0.07	11 0.034	12 0.33	13 0.17	14 0.02	15 –
D13S317	895	717 0.80	8 0.11	9 0.09	10 0.05%	11 0.31	12 0.28	13 0.10	14 0.03	15 –	
D7S820	896	715 0.80	6 –	7 0.02	8 0.15	9 0.13	10 0.28	11 0.21	12 0.17	13 0.04	14 –
D12S391	358	318 0.89	15 0.05	16 0.03	17 0.09	18 0.16	19 0.16	20 0.15	21 0.09	22 0.09	23 0.08
FES	206	148 0.72	8 0.002	9 0.007	10 0.28	11 0.40	12 0.24	13 0.04			
D8S639	192	138 0.72	23 0.002	24 0.02	25 0.008	26 0.16	27 0.24	28 0.19	29 0.14	30.1 0.08	31.1 0.02
TH01	107	83 0.77	5 –	6 0.15	7 0.17	8 0.13	9 0.16	9.3 0.37	11 –		
CD4	100	70 0.70	4.2 0.04	5 0.29	5.2 0.02	6 0.24	7 –	8 0.02	9 0.04	10 0.27	11 0.04
D1S1656	192	138 0.72	10 0.01	11 0.05	12 0.13	13 0.09	14 0.09	15 0.15	15.3 0.16	16.3 0.05	17.3 0.13
F13A	107	71 0.66	3.2 0.10	4 0.07	5 0.21	6 0.24	7 0.32	8 0.01	9 0.003	10 –	11 0.008

Analysis of Penta D and Penta E STR Loci in a Northern Portuguese Population

D. Abrantes¹, M.L. Pontes¹, G. Lima¹, P.A. Rezende¹, M..J. Pereira¹, and M.F. Pinheiro^{1,2}

¹National Medico Legal Institute – Oporto

²“Abel Salazar” Biomedical Sciences Institute – Oporto-Portugal

Simultaneous amplification and subsequent typing of several STR loci have enhanced successful conclusions of forensic casework.

The Powerplex 16 system is an available kit that can be used to co-amplify sixteen loci, including 13 tetranucleotide STR loci, Amelogenin and two pentanucleotide STR loci (Penta D and Penta E).

The purpose of this study is to report allele and genotype frequency data of Penta D and Penta E in a Northern Portuguese population sample as well as statistical evaluations, including the possible divergence from Hardy-Weinberg expectations and other important forensic parameters.

All PCR product separations were performed by capillary electrophoresis using an ABI Prism 310 Genetic Analyzer.

The high degree of variability obtained using that multiplex, namely the Penta D and Penta E loci, makes it very useful in forensic genetic diagnosis.

Prof. Doctor Maria de Fátima T. Pinheiro – Jardim Carrilho Videira 4050-167 Porto-Portugal; e-Mail: imlporto@ip.pt

OTOMI Amerindian Population (Mexico) Characterised by HumvWA, HumTHo1, HumTPOX, HumCSF1PO, D3S1358, and D19S253 STR-PCR Polymorphisms

E. Piqué¹, N. Borrego¹, C. Brandt², M. A. González¹, G.M. Cruz¹, M. Ortega¹, J. Corbella¹, E. Huguet¹, P. Moreno¹ and M. Gené¹

¹Legal Medicine Department. School of Medicine. University of Barcelona. Spain

²Legal Medicine Institute. Lausanne, Switzerland

The purpose of this study is to report allele and genotype frequency data of a Otomi ethnic group population sample from El Deca, El Buena and San Andrés Daboxhá (Ixmiquilpan Valley, Hidalgo State, Mexico) for six STR-PCR polymorphisms (HumvWA, HumTHo1, HumTPOX, HumCSF1PO, D3S1358, and D19S253).

DNA was extracted from hair root samples by Chelex® method. PCR triplex (HumTHo1, HumTPOX, HumCSF1PO), duplex (D3S1358, D19S253) and singleplex (HumvWA) amplifications were accomplished with fluorescein labelled primers. Genotypes were analysed in denaturing 6% polyacrilamide gel electrophoresis, using a monochrome automated laser fluorescence sequencer. Allele and genotype frequencies were determined. The following allele frequency ranges were found for the six systems:

HumvWA (n = 53): 8 alleles with frequencies ranging between 0.018 (12 and 20) and 0.330 (16).

HumTHo1 (n = 83): 6 alleles with frequencies ranging between 0.006 (8, 9 and 10) and 0.409 (7).

HumTPOX (n = 81): 5 alleles with frequencies ranging between 0.012 (9) and 0.444 (8).

HumCSF1PO (n = 78): 5 alleles with frequencies ranging between 0.006 (9) and 0.481 (12).

D3S1358, (n = 72): 7 alleles with frequencies ranging between 0.006 (13 and 19) and 0.576 (15). D19S253 (n = 71): 9 alleles with frequencies ranging between 0.007 (15) and 0.345 (12).

For all markers the observed genotype frequencies are in good agreement with the expected distribution under the Hardy-Weinberg law.

Dr. Conxita Brandt. Institut Universitaire de Médecine Légale.

Rue du Bugnon 21, CH-1005 Lausanne, Suisse.

Tel 41 21 /314 70 70 Fax 41 21 /314 70 90

e-mail: Conxita.Brandt@inst.hospvd.ch

Population data for nine STR loci FGA, vWA, D3S1358, CSF1PO, TPOX, TH01, D7S820, D13S317 and D5S818 in Japanese

M. Hara¹, A. Kido², K. Saito¹, A. Takada¹, Y. Yamamoto³, H. Kameyama³

¹Department of Forensic Medicine, Saitama Medical School, Japan

²Department of Legal Medicine, Yamanashi Medical University, Japan

³Criminal Investigation Laboratory, Saitama Prefectural Police Headquarters, Japan

Japanese population genetic studies were performed on nine STR loci FGA, vWA, D3S1358, CSF1PO, TPOX, TH01, D7S820, D13S317 and D5S818. Blood samples were obtained from 194 unrelated Japanese individuals living in a central part of Japan, Saitama prefecture. The AmpFISTR[®] Profiler[™] Kit (PE Applied Biosystems) was used to carry out multiplex PCR of the nine STR loci. Genotypings were performed by capillary electrophoresis using the ABI Prism[™] 310 Genetic Analyzer. Thirteen alleles were identified for FGA, nine alleles for vWA, eight alleles were identified for D3S1358, nine alleles for CSF1PO, six alleles for TPOX, seven alleles for TH01, seven alleles for D7S820, eight alleles for D13S317 and eight alleles for D5S818. No significant deviations from Hardy-Weinberg equilibrium were detected in all loci. The statistical parameters for nine STR loci are shown in Table 1. The combined power of discrimination for the nine STR loci was 0.999999990. The results demonstrated that the AmpFISTR[®] Profiler[™] Kit is very useful for personal identification and paternity testing in Japanese population.

Table 1. Statistical parameters for the nine STR loci in Japanese

	EX (P =)	LR (P =)	H. obs	H. exp	DP	PIC
FGA	0.856	0.808	0.872	0.858 ± 0.025	0.965	0.842
vWA	0.664	0.621	0.825	0.787 ± 0.029	0.913	.755
D3S1358	0.583	0.639	0.701	0.724 ± 0.032	0.878	0.675
CSF1PO	0.555	0.607	0.753	0.729 ± 0.032	0.879	0.687
TPOX	0.392	0.267	0.660	0.649 ± 0.034	0.808	0.582
TH01	0.110	0.088	0.753	0.679 ± 0.034	0.834	0.629
D7S820	0.074	0.119	0.696	0.771 ± 0.030	0.916	0.736
D13S317	0.649	0.587	0.789	0.797 ± 0.029	0.929	0.769
D5S818	0.859	0.832	0.825	0.786 ± 0.029	0.916	0.752

EX: Exact test

LR: Likelihood ratio test

H. obs: Heterozygosity observed

H. exp: Heterozygosity expected

DP: Power of discrimination

PIC: Polymorphism information content

Address for correspondence:

Dr. Masaaki Hara, Department of Forensic Medicine, Saitama Medical School, Morohongo 38, Moroyama, Saita 350-0495, Japan
Tel: +81-492-949714, Fax: +81-492-949713, e-mail: haramasa@saitama-med.ac.jp

Portuguese Population Data On Two Pentanucleotide STR loci: Penta E and Penta D

T. Ribeiro¹, L. Viriato¹, C. Vieira-Silva¹, C. Cruz¹, R. Espinheira¹ and H. Geada^{1,2}

¹Genetic and Forensic Biology Department, National Institute of Legal Medicine, Lisbon Delegation

²Department of Legal Medicine, Faculty of Medicine, University of Lisbon, Portugal

Two pentanucleotides – Penta E and Penta D have been studied in 160 unrelated Portuguese individuals to introduce the GenePrint[®] PowerPlex[™] 16 System for paternity investigation studies in our laboratory. DNA was collected in FTA paper, extracted with Chelex and purified with Wizard[®] DNA Clean-Up System.

For paternity investigations, these Portuguese samples were typed with SGM Plus from Applied Biosystems and PowerPlex 16 from Promega Corporation performing 17 STR loci which include the core CODIS loci plus two tetranucleotide loci – D2S1338 and D19S433 and two pentanucleotide loci – Penta E and Penta D. Amplification was performed in a Perkin Elmer GeneAmp PCR System 9600 and electrophoresis was carried out in a ABI Prism[®] 377 DNA sequencer.

The distribution of the observed allelic frequencies are shown in Table 1 for 160 Penta E and 151 Penta D samples.

Table 1. Allelic frequencies for Penta E and Penta D in a Portuguese population

Alleles	5	7	8	9	10	11	12	13	14
Penta E	0.0844	0.1219	0.0219	0.0031	0.1156	0.1250	0.2156	0.0844	0.0375
Penta D	0.0066	0.0033	0.0133	0.2133	0.0900	0.1733	0.1533	0.2266	0.0833
Alleles	15	16	17	18	19	20	21	22	23
Penta E	0.0438	0.0500	0.0344	0.0188	0.0313	0.0063	0.0031	-	0.0031
Penta D	0.0300	0.0066	-	-	-	-	-	-	-

There were 66 and 33 different genotypes observed for Penta E and Penta D loci respectively. The Penta E number of genotypes is considerably higher than the number normally observed with other loci as D21S11 and FGA consisting with a high degree of heterozygosity – $h = 0.8893$ for Penta E and $h = 0.8362$ for Penta D. Only Penta E 10–12, 11–12, 12–12 and 7–12 genotypes have observed frequencies higher than 0.05. Both loci meet HWE and have high power of discrimination (PD) and probability of exclusion (PE) values (PD – Penta E = 0.9771; Penta D = 0.9508 and PE – Penta E = 0.7892; Penta D = 0.6898).

A comparison with an Italian population for Penta E (Garofano et al, Forens.Sci.Int. 1999 (105) 131-136) shows a decrease in allele 13 but mainly in allele 9 in the Portuguese Population, although allele 12 is the most common in both populations. Comparing these results with a small study (19 samples) with African black individuals from Cabo Verde and Angola an increase in alleles 9 and 13 was observed (freq. allele 9 = 0.0294 and freq. allele 13 = 0.1176) although a higher sample number is needed to confirm these results.

Eighty maternal and seventy paternal meioses have also been studied using both Penta E and Penta D loci.

This data indicates that Penta E and Penta D loci are valuable STR loci for forensic purposes in our population.

Dr. Helena Geada, PhD

Genetic and Forensic Biology Department, National Institute of Legal Medicine, Lisbon Delegation, Lisbon, Portugal

R Manuel Bento de Sousa, n^o3, Tel. +351 21 8811800

Fax +351 21 8864493, e-mail: hgeada@mail.telepac.pt

Data analysis of 10 STR loci in a population in the province of Neuquen, Argentina

U. Toscanini, G. Berardi, E. Haas and E. Raimondi

PRICAI – Favaloro Foundation (First Argentine Immunogenetics Centre) – Buenos Aires, Argentina

The population analysed is geographically located at the foot of the Andes Range, in Neuquen, a province in the southwest of Argentina. The most important feature of this population is that it is mainly made up by a mixture of people migrating from the north and centre of the country and aboriginal natives (Mapuche people). The aim of this study was to demonstrate whether this population is in Hardy-Weinberg equilibrium (HWE) for ten short-tandem-repeat (STR) loci and to determine if there exist significant differences in allele and genotype frequencies between the population studied and that living in Buenos Aires.

The analyzed loci were:

SYSTEM	LOCUS	N (chromosomes)
CTT Triplex (Promega Corp.)	CSFIPO, TPOX, THO1	114
Multiplex-I Triplex (Lifecodes Corp.)	D12S1090, D3S1744, D18S849	164
Multiplex-II Tetraplex (Lifecodes Corp.)	FGA, D7S820, D1S533, D9S304	154

DNA was extracted using a non organic procedure from EDTA collected blood from non related men and women from Neuquen. STRs were analysed by silver staining. Genetic data were analysed using the GENEPOP V3.3 and PowerStat softwares.

The population was observed to be in HWE for the analysed loci (heterozygote deficiency and excess test, probability-test). Significant differences between allele and genotype frequencies were found for the D7S820 locus (Genic and genotypic differentiation test) when compared with data of the Buenos Aires population. Paternity and forensic parameters were also calculated. The Combined Power of Exclusion for these ten STR loci was 99.993%. The Combined Matching Probability and Combined Power of Discrimination were 8.8×10^{-12} and 99.999999991% respectively.

These data indicate that the allele frequency estimations can be used to reliably calculate likelihood ratios for paternity and forensic DNA casework.

Address for correspondence:

Prof. Dr. Eduardo H. Raimondi, PRICAI- Favaloro Foundation
Av. Belgrano 1782 – 1er Subsuelo
1093 – Buenos Aires – Argentina
TE: (5411) 4384 5400; FAX: (5411) 4383 1197;
e-mail: eraimondi@ffavaloro.org

Population Data from Chile using the PowerPlex-16

R. Celis¹, E. Aguirre¹, J.A. Lorente², V. Saragoni¹, F. Moreno¹
and H.C. Jorquera¹

¹Molecular Biology Unit. Servicio Medico Legal. Ministry of Justice. Santiago, Chile

²Lab. Genetic Identification. Dept. of Legal Medicine. University of Granada, 18012 Granada, Spain

Short tandem repeat (STR) loci are routinely used for paternity and forensic identity testing. Furthermore, STR loci are useful components of national DNA databanks, such as those in the United Kingdom and the United States. The typing of STR loci is facilitated by the ability to amplify several loci simultaneously in a multiplex polymerase chain reaction (PCR). The 16 STR loci D3S1358, TH01, D21S11, D18S51, PentaE, D5S818, D13S317, D7S820, D16S539, CSF1PO, PentaD, vWA, D8S1179, TPOX, FGA, and the locus amelogenin can be amplified simultaneously using the the PowerPlex 16 kit (Promega Corp. Madison, WI, USA).

This paper presents allele distribution data in the general Chilean population. The data demonstrate that these loci can be useful for providing estimates of the frequency of a DNA profile in forensic identity testing and that a multiple locus profile is extremely rare in all the population.

Whole blood was obtained in EDTA vacutainer tubes by venipuncture from unrelated individuals (N = 323) residing in Chile. Extracted DNA samples were amplified at the 16 loci using the PowerPlex 16 kit (Promega Corp. Madison, WI, USA). Samples were analyzed using the ABI Prism™ 310 Genetic Analyzer (PE Biosystems, Foster City, CA) according to the manufacturer's recommended protocol.

All 15 loci are highly polymorphic in the Chilean sample population with the locus TPOX (67.3%) having the lowest observed heterozygosity, and the locus PentaE (92.9%) displaying the highest heterozygosity. The most discriminating loci were PentaE (PD = 0.983) and FGA (PD = 0.966). The combined probability of exclusion for the 15 STR loci is 0.99999961. There was little evidence for departures from Hardy-Weinberg expectations (HWE) in this sample population. Based on the exact test, the loci that departed significantly from HWE are D16S539 ($p = 0.002$). After employing the Bonferroni correction for the number of loci analyzed, this observation are not likely to be significant. An inter-class correlation test analysis was performed to detect any correlations between alleles at any of the pair-wise comparisons of the 15 loci. A resume of the PD and PE are shown in this table:

Locus	PD (Obs)	PD (Exp)	PE
1 D3S1358	0.88405873	0.88089494	0.49149762
2 TH01	0.89212828	0.90310794	0.53570855
3 D21S11	0.95387339	0.95629693	0.68708958
4 D18S51	0.96501458	0.96831543	0.73361811
5 PentaE	0.98328821	0.98654943	0.82803091
6 D5S818	0.89582466	0.90205337	0.53715336
7 D13S317	0.95340483	0.95562899	0.68325371
8 D7S820	0.90733028	0.90882529	0.55156953
9 D16S539	0.91795085	0.92152643	0.58126074
10 CSF1PO	0.86344232	0.86806648	0.46526042
11 PentaD	0.95168680	0.95664959	0.68774901
12 vWA	0.90207205	0.91023088	0.55517793
13 D8S1179	0.93419409	0.93968115	0.63224998
14 TPOX	0.83756768	0.83824361	0.42261732
15 FGA	0.96647230	0.96878723	0.73539573
Total	>0.99999999	>0.99999999	0.99999961

In conclusion, a Chile database has been established for the loci. All loci are highly polymorphic. The allelic frequencies of these PCR-based loci can be used to estimate the frequency of a multiple locus DNA profile in the Chilean population.

Prof. Dr. med. Jose A. Lorente.
Dept. of Legal Medicine.
University of Granada.
18012 Granada, Spain.
Tel: +34 958 243546
Fax: +34 958 246107
e-mail: jlorente@ugr.es

Spanish Population Data for the 15 STRs Loci included in the PowerPlex-16

C. Entrala, J.C. Alvarez, E. Martinez-Espin, F. Fernandez-Rosado, J.A. Lorente, M. Lorente and E. Villanueva

Lab. of Genetic Identification. Dept. of Legal Medicine.
University of Granada, 18012 Granada, Spain

Short tandem repeat (STR) loci are the most informative PCR-based genetic markers available to date for attempting to individualize biological material. The 16 STR loci D3S1358, TH01, D21S11, D18S51, PentaE, D5S818, D13S317, D7S820, D16S539, CSF1PO, PentaD, vWA, D8S1179, TPOX, FGA, and the locus amelogenin can be amplified simultaneously using the the PowerPlex 16 kit (Promega Corp. Madison, WI, USA).

This paper presents allele distribution data in the Spain population. The data demonstrate that these loci can be useful for providing estimates of the frequency of a DNA profile in forensic identity testing and that a multiple locus profile is extremely rare in all the population. Whole blood was obtained in EDTA vacutainer tubes by venipuncture from unrelated individuals (N=323) residing in Andalucia (Spain). Extracted DNA samples were amplified at the 16 loci using the the PowerPlex 16 kit (Promega corp. Madison, WI, USA). Samples were analyzed using the ABI Prism™ 310 Genetic Analyzer (PE Biosystems, Foster City, CA) according to the manufacturer's recommended protocol.

All 15 loci are highly polymorphic in the spanish sample population with the locus TPOX (66.8%) having the lowest observed heterozygosity, and the locus D18S51 (87.5%) displaying the highest heterozygosity. The most discriminating loci were D18S51 (PD = 0.969) and PentaE (PD = 0.968). The combined probability of exclusion for the 15 STR loci is 0.99999953. There was little evidence for departures from Hardy-Weinberg expectations (HWE) in this sample population. Based on the exact test, the locus that departed significantly from HWE are vWA ($p = 0.0488$). After employing the Bonferroni correction for the number of loci analyzed, this observations are not likely to be significant. An inter-class correlation test analysis was performed to detect any correlations between alleles at any of the pair-wise comparisons of the 15 loci. A resume of the PD and PE are shown in this table:

Locus	PD (Obs)	PD (Exp)	PE
1 D3S1358	0.92269737	0.92278200	0.58299702
2 TH01	0.91326177	0.92153232	0.57932276
3 D21S11	0.96156510	0.96393249	0.71562119
4 D18S51	0.96918283	0.97244149	0.75197184
5 PentaE	0.96892313	0.97153083	0.74728688
6 D5S818	0.87629848	0.87782049	0.48530485
7 D13S317	0.91784972	0.92716219	0.59710511
8 D7S820	0.92269737	0.93174389	0.60817361
9 D16S539	0.90226801	0.91172840	0.55780904
10 CSF1PO	0.83907548	0.86025565	0.45017867
11 PentaD	0.94650277	0.95237783	0.67224651
12 vWA	0.92944945	0.93221428	0.60962035
13 D8S1179	0.92529432	0.93586954	0.62118533
14 TPOX	0.81864612	0.82216391	0.40301804
15 FGA	0.96641274	0.96961134	0.73910229
Total	> 0.99999999	> 0.99999999	0.99999953

In conclusion, a Spain database has been established for the loci D3S1358, TH01, D21S11, D18S51, PentaE, D5S818, D13S317, D7S820, D16S539, CSF1PO, PentaD, vWA, D8S1179, TPOX, FGA. All loci are highly polymorphic. The application of the product rule is valid for estimating the rarity of a multiple loci profile for these 15 loci.

Prof. Dr. med. Jose A. Lorente. Dept. of Legal Medicine.
University of Granada. 18012 Granada, Spain.

Tel: +34 958 243546 – fax: +34 958 246107 – e-mail: jlorente@ugr.es

Kurdish population data for 11 STR loci (ACTBP2, CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D13S317 and D21S11)

I. Shimada^{1,2}, C. Hohoff², S. Rand², B. Brinkmann²

¹Dept. of Legal Medicine, Toyama Medical and Pharmaceutical University, Japan

²Institut für Rechtsmedizin, Universität Münster, Germany

The short tandem repeat (STR) systems D3S1358, VWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820 (as part of the AmpFISTR® Profiler™ PCR Amplification Kit (ABI)) were studied in a Kurdish population sample (950 unrelated individuals mainly from Northern Iraq).

DNA from the buccal swabs was extracted by the Proteinase K / Chelex-100 method and amplified according to the recommendations of the manufacturer (ABI). PCR products were separated by capillary gel electrophoresis on an ABI PRISM 310 Genetic Analyzer (ABI) with laser-induced fluorescence (LIF) detection.

The aim of this study was to establish a database for immigration cases. Since the combined MEC of the Profiler kit was not sufficient for all, especially deficiency, cases (99.94%), a duplex PCR composed of the polymorphic loci ACTBP2 and D21S11 was set up and a combined MEC of 99.998 achieved. No deviation from Hardy-Weinberg equilibrium was observed.

The investigated loci cover 6 of the 8 systems from the German DNA database stored at the Bundeskriminalamt (BKA). These loci possess a combined power of discrimination (PD) of 0.999999996, while the combined PD of all 11 STR systems is 0.999999999994 in the Kurdish population.

Address for correspondence:

Ichiroh Shimada PhD, Institut für Rechtsmedizin, Universität Münster, Von-Esmarch-Strasse 62, D-48149 Münster, Germany, Tel. 00 49 (0)251 8355179; Fax: 00 49 (0)251 8355158, e-mail: shimadai@uni-muenster.de

Allele Frequency Distributions and Other Population Genetic Parameters for 13 STR Loci in a UAE Local Population from Dubai

Farida Alshamali¹, Abdul Qader AlKhayat¹, Bruce Budowle² and Nigel Watson³

¹Crime Laboratory, Dubai Police GHQ., Dubai, United Arab Emirates.

²Laboratory Division, FBI, Washington, D.C.

³Forensic Science Unit, Strathclyde University, UK.

A database of allele frequencies for 13 STR Loci: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, CSF1Po was generated for the UAE locals from Dubai. A sample of 200 unrelated males were typed using the ABI AmpFLSTR® Profiler Plus™ and AmpFISTR® Cofiler™ PCR Amplification Kits and a ABI PRISM™ 377 DNA Sequencer. The observed alleles were tabulated, and the allele frequencies were calculated. Using the exact test, no departures from Hardy-Weinberg expectations were detected. The combined power of discrimination was greater than 0.99999999; the probability of exclusion was 0.999995. Nine of the 13 STR loci were compared with data previously reported on Omanis. The average Fst value over nine loci for Dubaians and Omanis is 0.001. Results are presented. The data support the use of these 13 STR loci for both forensic and paternity testing.

Population Genetic Study of STR Loci using Identifiler KIT (Applied Biosystems)

Anna Barbaro*, Patrizia Cormaci*, Giacomo Falcone**, Angelo La Marca**, Aldo Barbaro***

*Department of Molecular Genetics –
SIMEF -89128 Reggio Calabria-ITALY

**Department of Haematology –
SIMEF -89128 Reggio Calabria-ITALY

***Medico-Legal – Director of SIMEF –
SIMEF -89128 Reggio Calabria-ITALY

The IDENTIFILER is the most recent kit by Applied Biosystem. It coamplifies the repeat regions of 16 STRs repeat loci. A segment of the X-Y homologous gene Amelogenin is also amplified.

By capillary electrophoresis it takes about 30 min to type one sample and up to 48 samples a day can be typed automatically.

In the present study, we analyzed allelic and genotypic distribution of STRs loci in the populations of Reggio Calabria, Catanzaro, Cosenza.

DNA was extracted, by Instant Gene Matrix (Biorad) treatment, from blood samples of unrelated healthy donors (100 per each town), belonging to the population examined since 2 generations at least.

STRs amplification was carried out according to the IDENTIFILER kit protocol using GeneAmp 9600, 9700 and 2400 thermal cyclers (Perkin Elmer). Positive and negative controls were used during the amplifications.

Amplified products were analyzed by capillary electrophoresis on two ABI PRISM 310 Genetic Analyzers (Applied Biosystems) employing ABI softwares (DATA Collection, GeneScan Analysis, Genotyper Fragment Analysis).

For fragment length determination of the products, the internal lane DNA standard (Applied Biosystems) was used for calibration.

Results obtained will be showed in detail. They demonstrate the utility of these STR for human identification.

Our results underscore the importance of the generation of local databases for STRs when these markers are being currently used in forensic casework.

We are grateful to each Italian public and private laboratory that supplied us with blood-samples for this work of typing. In particular we thank Angelo De Biasi and Applied Biosystems for their collaboration.

15 STR loci frequency data and its forensic use in Beijing-han population

Liu Yacheng, Huo Zhen Yi, Tang Hui, Wang Jing, Jiao Zhangping, Yan Jiangwei, Yang Jian, Jia Shuqin, Zhu Xiaojun

Forensic Medical Examination & Identification Centre of Beijing Public Security Bureau

1. Introduction

Short tandem repeats (STR) are useful to forensic science and are characterized by their small range of alleles, their high sensitivity and suitability if the DNA is degraded. In our research, allele frequencies of 15 STR loci (CSF1PO, TPOX, TH01, D5S818, D7S820, D13S317, D16S539, D21S11, D18S51, FGA, vWA, D8S1179, D3S1358, PentaE, PentaD) together with the Amelogenin locus were obtained in a sample of over 200 people from Beijing, China. The results suggest that the 15 loci are excellent markers in identity and paternity test. It is the first time to report the statistical data of all the 15 loci in Beijing-han population.

2. Materials and methods

2.1 Sample collection:

201 blood samples were obtained from unrelated Beijing-han volunteers. DNA was extracted either with 5% chelex 100 or organic reagents.

2.2 DNA typing:

Target DNA (about 2ng) was amplified using Powerplex™ 16 System kit (Promega) following the PCR protocol described in the Technical Manual. The amplified fragments were separated and detected using PE-310 Genetic Analyzer with reference to sequenced ladders. The genotyping results of the 15 loci were obtained through the genotyper software applied by Promega.

3. Results and discussions

The genotype frequencies of the 15 STR loci showed no deviations from HWE expectations based on the χ^2 -test. The study of the genetic mode of 2 generations from 100 families demonstrated that all these loci conform to Mendel genetic law.

The combined matching probability (CPm) and the combined power of exclusion (CPE) of the 15 loci are 0.9999999999999999995 and 0.9999992 respectively, which are both enhanced when compared with the data of 9 STR system that we have already reported. The results suggest that all these 15 loci are excellent markers in identity and paternity testing in forensic science, especially in dealing with degraded or trace evidence when there is no signal in the larger fragments loci while the smaller fragments loci can always give good genotyping results that may help us.

References

1. Pentanucleotide Repeat Loci: Bacher, J. and Schumm, J.W. (1998) Development of highly polymorphic pentanucleotide tandem repeat loci with low stutter. Profiles in DNA 2(2),3.
2. Liu Yacheng, The Allele Frequency Distribution and Implementation in Forensic Science of 9 STR Loci in Beijing-han, China Forensic Science, 1998 (13):210-212
3. Promega TECHNICAL MANUAL Geneprint PowerPlex 16 System
4. Yu chunying, Yang qingen, The Investigation of Polymorphism of 5 STR Loci in Chinese-han, China Forensic Science, 1997.12 21-25
5. Liu Yacheng, The Application of 9 Fluorescent Dye STR Loci in Forensic Science, the Latest Research and Practice in Forensic Science in China, 2000. 10

Population genetic data for eight STR loci in Southern Africa

O. Stefano¹, P. Ricci¹, B. Brinkmann² and C. Hohoff²

¹*Istituto di Medicina Legale e delle Assicurazioni, University "Magna Graecia" di Catanzaro, Italy*

²*Institut für Rechtsmedizin, Westfälische Wilhelms-Universität Münster, Germany*

Population data for short tandem repeat (STR) loci, today's method of choice for human identification and paternity testing in Forensic Genetics, are available for numerous populations from the major ethnic groups. Nevertheless, especially the allele frequency data for Sub-Saharan Africans are extremely generalized and little information is available for particular populations.

The purpose of this work was to study eight STR polymorphisms (TH01, vWA, ACTBP2, FGA, D21S11, D3S1358, D8S1179, D18S51) in two small population samples from Southern Africa (42 unrelated Ovahimbas from Namibia and 72 unrelated black South Africans from the Cape Town area).

Genomic DNA was extracted from oral swabs (Ovahimbas) or blood stored on cotton cloth (South Africans) using the Proteinase K/Chelex method.

Subsequently, the eight STR systems were amplified in a multiplex PCR reaction including the amelogenin locus for gender identification using the MPX-2 kit (Serac, Germany) and the PCR products were analyzed by capillary gel electrophoresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Statistical analyses of forensic parameters were carried out using the software package HWE analysis 3.2 (C. Puers, Münster 1995). No significant deviation from the Hardy-Weinberg equilibrium was observed. ACTBP2 was the most informative locus in both populations, while D3S1358 had the lowest power of discrimination in both Ovahimbas and black South Africans.

Address for correspondence:

Carsten Hohoff, Institut für Rechtsmedizin, Universität Münster, Von-Esmarch-Strasse 62, D-48149 Münster, Germany, Tel. +49 (0) 251 8355145; Fax: +49 (0) 251 8355158, e-mail: hohoff@uni-muenster.de

A Genetic Population Study of Seven Y-Chromosome STR-loci in a Population of Brescia Area (North Italy)

N. Cerri, E. Ponzano and F. De Ferrari

Institute of Forensic Medicine University of Brescia, Italy

Y-chromosomal STR-loci has been extensively investigated in forensic science to an application for male identification and paternity testing. In order to apply a set of useful Y-STR systems and to create an own data-base we have performed a population study in Northern Italy – Brescia Area (Lombardy), the aptotype distribution of seven different systems were investigated in a sample of 51 unrelated males.

DNA extracted the standard procedure with pCIA was typed for the following loci: DYS19, DYS389-I/II, DYS390, DYS391, DYS392 and DYS393.

PCR amplification was performed in a 2400 Thermal Cycler by Perkin Elmer with different amplification conditions and the PCR products were analysed by native polyacrylamide gels or denaturing 6% polyacrilamide gel; setups bands were visualized by silver staining. The results have been compared to other italian and european population.

Prof. Dr. Med Francesco De Ferrari, Institute of Forensic Medicine, University of Brescia, c/o Spedali Civili, Piazzale Ospedale, 1 25100 Brescia, Italy. Tel. ++39/030/3995838; Fax. ++39/030/3995839, e-mail: deferrari@master.cci.unibs.it

Population Genetics of Y-Chromosomal Haplotypes in Asturias (Northern Spain)

P. Nieves¹, B. Martínez-Jarreta¹, R. Hinojal², E. Abecia¹

¹Department of Legal Medicine, University of Zaragoza, Spain

²Department of Legal Medicine, University of Oviedo (Asturias), Spain

Abstract

Y-STR haplotyping has proven to be extremely informative in forensic casework, particularly in cases of rape and other sexual assault as well as kinship testing.

A core set of 10 Y-linked short tandem repeat polymorphisms have been recently described and validated by the forensic and scientific community: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385I, DYS385II, DYS388.

In order to quantify the positive evidence provided by a trace-donor match, or in order to facilitate likelihood calculations in the case of kinship testing, haplotype frequencies inevitably must be known.

The goal of this work was the generation of reliable Y-STR haplotype frequency estimates based on the distribution of these 10 Y-linked short tandem repeat polymorphisms in the population of Asturias (North Spain).

The data base was drawn from 120 fresh blood samples collected from healthy unrelated donors born and living in Asturias. Each locus was amplified individually, except DYS389I/II and DYS385 (two loci each with the same set of primers). Detection of the amplified products was carried out using the Automatic Laser Fluorescent (ALF) DNA sequencer (Pharmacia). The recommendations of the International Society for Forensic Genetic were followed for typing and interpretation.

101 different haplotypes were found in 120 samples (haplotype diversity value: 0.9952) It was concluded that this set of Y-specific STR-loci reveals a high degree of polymorphism in the Asturian population. The haplotype frequency data obtained show the utility of this systems for special cases of paternity and forensic stain analysis.

Genetic analysis of eighteen STR loci on X chromosome in a Japanese population

H. Matsushita, S. Nakamura, T. Nagai, H. Sugie, M. Furukawa and K. Kurihara

Department of Legal Medicine, Kitasato University School of Medicine, Sagami-hara, 228-8555, Japan

Introduction

STR (short tandem repeat) polymorphisms are powerful tools for human identification, paternity analysis and genetic mapping. We have already analysed the AmpFLSTR Profiler Plus STR multiplex system to obtain allele frequency data for a Japanese population, and reported that this system was applicable to routine case work. In the present investigation, we analysed 18 STR loci DXS1227, DXS990, DXS986, DXS987, DXS993, DXS1073, DXS8091, DXS1106, DXS1047, DXS1001, DXS1068, DXS1214, DXS8055, DXS8051, DXS8043, DXS1060, DXS1226 and DXS991 to obtain allele frequency data for a Japanese population living in Kanagawa.

Materials and methods

EDTA-treated blood samples were collected from 130 unrelated Japanese individuals living in Kanagawa, and genomic DNA was isolated by using the Wizard Genomic DNA Purification Kit (Promega). Multiplex PCR amplification of these 18 STR loci were performed using the ABI PRISM Linkage Mapping Set Version 2 Panel 28 (PE Applied Biosystems) according to the user's manual provided by the manufacture. PCR products of 1 µl were mixed with 0.5 µl of GeneScan-400 HD [ROX] size standard (PE Applied Biosystems) and 12 µl of deionized formamide. The fluorescently labeled amplified fragments were electrophoresed using the ABI PRISM 310 Genetic Analyzer in a 47 cm, 50 µm i.d. capillary, filled with performance Optimized Polymer 4 (POP4, PE Applied Biosystems) at 15 kV for 24 min at 60°C, and analysed using GeneScan Analysis Software ver. 2.1. CEPH 1347-02 control DNA and CEPH Genotype Database were used as a reference for allele designation.

Results and discussion

In 130 Japanese subjects, a total 7 alleles for DXS1227, 7 alleles for DXS990, 14 alleles for DXS986, 7 alleles for DXS987, 8 alleles for DXS993, 11 alleles for DXS1073, 8 alleles for DXS8091, 5 alleles for DXS1106, 11 alleles for DXS1047, 7 alleles for DXS1001, 9 alleles for DXS1068, 8 alleles for DXS1214, 5 alleles for DXS8055, 9 alleles for DXS8051, 7 alleles for DXS8043, 10 alleles for DXS1060, 12 alleles for DXS1226, and 9 alleles for DXS991 was observed. The most common alleles were 3 (53.8%), 3 (41.6%), 4 and 11 (17.4%), 1 (43.7%), 3 (42.6%), 2 (47.2%), 2 (54.3%), 2 (78.7%), 1 (25.4%), 1 (27.4%), 1 (34.5%), 1 (35.5%), 2 (90.4%), 3 (42.6%), 1 (34.5%), 2 (22.6%), 9 (20.8%), and 4 (45.2%) for DXS1227, DXS990, DXS986, DXS987, DXS993, DXS1073, DXS8091, DXS1106, DXS1047, DXS1001, DXS1068, DXS1214, DXS8055, DXS8051, DXS8043, DXS1060, DXS1226, and DXS991, respectively. The means of PD (power of discrimination) were estimated as 0.683 (male) and 0.848 (female). From these results, eighteen STR loci on X chromosome were applicable to routine case work.

Address for correspondence:

H. Matsushita, Department of Legal Medicine, Kitasato University School of Medicine, Sagami-hara, 228-8555, Japan,
Tel & FAX: +81 042-778-9026, e-mail: hiroma@med.kitasato-u.ac.jp

Distribution of DYS385 genotypes in several Japanese subpopulations and a Korean population

K. Ago¹, Y. Seo², I. Yuasa³, K. Umetsu⁴, K.S. Park⁵, M. Ago¹ and M. Ogata¹

¹Department of Legal Medicine, Faculty of Medicine, Kagoshima University, Kagoshima, Japan

²Department of Legal Medicine, Miyazaki Medical College, Miyazaki, Japan

³Department of Legal Medicine, Faculty of Medicine, Tottori University, Yonago, Japan

⁴Department of Forensic Medicine, Yamagata University School of Medicine, Yamagata, Japan

⁵Department of Biology, College of Natural Sciences, Sungshin Women's University, Seoul, Korea

DYS385 locus, consisting of two linked short tandem repeat (STR) subloci, is the most polymorphic marker in Y-chromosome. We have reported the distribution of DYS385 genotypes in two Japanese populations, Miyazaki and Kagoshima. In this study, three Japanese populations in Tottori, Tanegashima and Amami-oshima and a Korean population in Seoul were added to the previous data. Tottori, Miyazaki and Kagoshima are located in southwestern part of Japan. Tanegashima and Amami-oshima are islands located on East China Sea.

Genomic DNA was prepared from whole blood according to the standard protocols (116 samples in Tottori, 270 in Miyazaki, 117 in Kagoshima, 158 in Tanegashima, 131 in Amami-oshima, and 94 in Seoul). PCR amplification was performed according to the procedure of Kayser et al. (1997). Electrophoretic separation of the PCR products was performed using 5% native polyacrylamide gel followed by silver staining.

Genotype numbers (GN) and Nei's gene diversity (GD) in each population were as follows: GN = 37, GD = 0.95 in Tottori; GN = 47, GD = 0.96 in Miyazaki; GN = 30, GD = 0.92 in Kagoshima; GN = 39, GD = 0.93 in Tanegashima; GN = 34, GD = 0.95 in Amami-oshima and GN = 38, GD = 0.95 in Korea. Most frequently observed genotypes were: 13–17 were in Miyazaki, Kagoshima and Tanegashima, 10–19 in Amami-oshima, both 13–17 and 10–19 in Tottori and 10–18 in Seoul. Previous reports by Schneider et al. (1998 and 1999) showed that genotypes 10-X were rare in Chinese, Thai and European populations. The genotypes 10-X, especially 10–18, 10–19 and 10–20, were frequently observed in Japanese and Korean populations. The genotype 13–17 was not so frequent in the Korean population as in the Japanese populations. A similarity of the genotypes 10-X and a difference of the genotype 13–17 in distribution between Japanese and Korean populations should be useful databases for the anthropological analysis of the formation process of the Japanese people.

Address for correspondence:

Dr. Kazutoshi Ago, Department of Legal Medicine, Faculty of Medicine, Kagoshima University, Sakuragaoka 8–35–1, Kagoshima 890-8520, Japan,
Tel: +81-99-275-5313; Fax: +81-99-275-5315;
e-mail: ago@m.kufm.kagoshima-u.ac.jp

Y chromosome STR haplotypes in a population from North-East Spain

M. Crespillo, M. Paredes, J.A. Luque; R. Fernández, A. Pifarré, D. Sola, E. Ramírez and J.L. Valverde

Instituto de Toxicología. Ministerio de Justicia. Barcelona. Spain.

Abstract

The aim of this study is to present the distribution of haplotype frequencies for six Y-chromosome short tandem repeat (STR) loci (DYS19, DYS385, DYS389II, DYS390, DYS391 and DYS393) in a population sample of 100 Caucasoid unrelated individuals living in North-East Spain.

A total of 74 different haplotypes were observed among which 67 were unique and 7 were found at least two times. The most common haplotype occurred 12 times. The overall discrimination capacity of the 6 loci haplotypes analysed was 74%, and the gene diversity was 97.04%.

This database study is an essential prerequisite for using Y-chromosomal STR in routine practice.

M. Crespillo. Instituto Nacional de Toxicología.
Ministerio de Justicia, Merced 1, 08002 Barcelona, Spain.
e-mail: biolog@bcn.inaltox.es; Fax: +34-93-3182530

Y-chromosome STR-haplotypes in a Swedish population

G. Holmlund, H. Nilsson, A. Langö-Warensjö, B. Rosén and B. Lindblom

National Board of Forensic Medicine, Institute of Forensic Genetics, University Hospital, SE-581 85 Linköping, Sweden

The Swedish Y-chromosome database of 350 individuals is based on an analysis of fathers and their sons. Individuals with Swedish names coming from different parts of Sweden are included. The Y-chromosomes were typed for the STR markers DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385. An evaluation of 174 chromosomes revealed 134 different haplotypes. Only 20 haplotypes were found more than once. The 5 most frequent haplotypes are shown in the table below. The frequencies of the haplotypes no 1, 2 and 3 are significantly different from the frequencies among the 5529 Y-STR-haplotypes reported to the European database, while the frequencies of haplotype 4 and 5 do not differ from the European population, comparison done by a 2 x 2 table using Yates correction. We also found 3 different haplotypes containing a DYS19 allele 11 not present in the European database. A recombination from allele 26 to 25 in DYS390 was found in one child. This was confirmed by repeated analysis. The results from all the 350 father-son combinations will be reported. The Y-STR polymorphism found in the Swedish population giving a high exclusion capacity will be very useful for testing paternal lineages.

Table 1: The most frequent Y-STR-haplotypes in the Swedish population compared to the European Y-STR database

No	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS385	No/S	Frq/S	No/E	Frq/E	p
1	14	12	28	23	10	11	13	14,14	9	0.052	27	0.005	>99.99
2	14	14	30	24	11	14	14	11,13	5	0.029	8	0.001	>99.99
3	14	12	28	23	10	11	12	14,15	4	0.023	12	0.002	>99.95
4	14	13	29	24	11	13	13	11,14	6	0.035	168	0.030	n.s.
5	14	12	28	22	10	11	13	13,14	6	0.035	66	0.012	n.s.

The number (No) of haplotypes and the frequencies (Frq) for the Swedish population (S) and the European population (E) respectively.

Analysis was done on an ABI377 sequencer using sequenced alleles in the allelic ladders and GS500 for size marker. The STR-markers were amplified in separate PCR-reactions and mixed for analysis in single lanes on the gels.

Address for correspondence:

Ph.D. Gunilla Holmlund, Institute of Forensic Genetics, University Hospital, SE-581 85 Linköping, Sweden.

Tel: 00 46 13 22 30 36, Fax: 00 46 13 13 60 05,

e-mail: gunilla.holmlund@rmv.se

Y-Chromosome STR defined haplotypes in North Portugal

Leonor Gusmão¹, Cíntia Alves¹, Sandra Beleza^{1,3} and António Amorim^{1,2}

¹Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal

²Faculty of Sciences, University of Porto, Portugal

³Institute of Legal Medicine, University of Santiago de Compostela, Galicia, Spain

The Y-specific STR loci, DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS434, DYS437, DYS438, DYS439 and A10, were studied in a sample from a Northern Portuguese population.

Haplotype and allele frequencies of these 14 Y-Chromosome STRs were estimated. In a sample of 161 individuals it was possible to define 150 different haplotypes of which 140 were found only once, 9 were found in two samples and the most frequent haplotype was shared by three individuals.

The observed haplotype diversity value was 0.9991.

In the same sample, a 0.69% increase of haplotype diversity was obtained and the number of different haplotypes rises from 122 to 150, when combining the new markers with the classical set (DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393). When DYS434 is not considered, the haplotype diversity is only slightly affected (0.9990), with the loss of just one haplotype.

Corresponding author:

Leonor Gusmão

IPATIMUP

Rua Dr. Roberto Frias, s/n

4200 Porto

Portugal

Tel: +351 22 5570700

Fax: +351 22 5570799

e-mail: lgusmao@ipatimup.pt

Y-chromosomal polymorphic loci DYS 19, DYS 389 I/II, DYS 390, DYS 391, DYS 392, DYS 393 in a population sample from South-Western Poland

A. Jonkisz, B. Bartnik, T. Dobosz

Medical University, Institute of Forensic Medicine,
Mikulicza-Radeckiego 4, 50-368 Wrocław Poland

In recent years STR – systems from Y chromosome have been studied. These systems are reproducible, sensitive and can be used for forensic applications (male identification). The individual male identification requires knowledge of allelic frequencies.

The goal of this study is to present allelic frequencies of DYS 19, DYS 389 I/II, DYS 390, DYS 391, DYS 392, DYS 393 – systems in South-Western Poland population sample.

DNA was extracted from unrelated male blood samples according to standard phenol-chloroform or chelex pro-cedures. Amplification was performed using fluorescent dye labeled primers. The PCR products were analysed in the ABI PRISM 310 Applied Biosystems sequencer using 310 GeneScan 2.1 programme.

The results were compared to other available data from Poland and other European populations.

Penta, Nona and Decaplex Y-STR Typing Systems: A comparative Study

A. Sala¹, M. Hedman², M. Marino¹, A. Sajantila², G. Penacino¹ and D. Corach¹

¹SHDG y Cát. Gen. y Biol. Mol., Fac. de Farm. y Bioquímica,
Univ.de Buenos Aires, Argentina.

²Department of Forensic Medicine, University of Helsinki, Finland

The analysis of Y-STR haplotypes, that allows patrilineage tracking, and that of mtDNA HVRI and II sequence haplotypes, that permits matrilineage detection, complemented the highly robust autosomal STRs systems used in forensic and kinship identification. In 1994 our lab incorporated the use of the Y-STR Y27H39 (DYS19). Later on, a continuous increasing of available Y-STRs broadens the patrilineage tracking potential. A complete nonaplex Y-STR set, including DYS385, 389 I and II, 390, 391, 392, 393 and DYS19 was investigated in 1995, provided Argentina information to the Charite Reference Database, and supported the forensic casework investigation. For forensic purposes we selected a pentaplex including: DYS19, DYS390, DYS391, DYS392 and DYS393. More recently, a decaplex including 435, 436, 437, 438, 439, A7.1, H4, DYS19, DYS391 and DYS392, became available. It requires a single PCR reaction with remarkable sensitivity. In order to evaluate the penta, nona and decaplex Y-STR systems, unrelated males were analyzed (500, 100 and 104, respectively) by using an automated platform (ABI 310). Discriminative Power was 97.00%, 99.87% and

99.79%, respectively. Although nonaplex and decaplex systems are the most discriminative, the decaplex are the most sensitive and rapid (only one PCR reaction is required). However, its haplotype frequency distribution is not as that supporting the nonaplex system, which also includes the pentaplex data. Then, we concluded that for rapid forensic investigations, in which autosomal STR is required, the pentaplex might be considered; if extra informative data are required, nonaplex should be selected, very especially due to worldwide Internet supported Y-STR Reference Database. It may be suggested to include part of the decaplex Y-STR markers in the Charite Database in order to stimulate the system use.

Y-Haplogroups in 2000 Norwegians

B. Myhre Dupuy, M. Stenersen, A.G. Flønes, R. Wallum and B. Olaisen

Institute of Forensic Medicine, University of Oslo, Norway

Five biallelic markers (YAP, 92R7, SRY-1532, Tat and 12f2) located on the male-specific portion of the human Y-chromosome were studied in 2000 unrelated Norwegian males involved in consecutive paternity cases from a 5 years period. Males from each of the 20 counties in Norway are represented at numbers reflecting the number of habitants. Amplification was performed in a Perkin Elmer System 9600 and fragment lengths, some after enzyme digestion, were analyzed in agarose gels stained with ethidium bromide.

Six haplogroups were detected (1,2,3,4,9, and 16 (according to Pandaya 1998)). Three of them (1,2 and 3) represented about 90% of the population sample.

Haplogroup distribution in the various part of Norway will be presented, aiming at disclosing any variation between the different geographic parts of the country. Haplogroups characterized by these markers have been used for studies in other populations (Zerjal 1997, Lahermo 1999, Helgason 2000, Rosser 2000) and comparative studies will be presented.

Berit Myhre Dupuy, Rettsmedisinsk Institutt,
Rikshospitalet A2-1002, Gaustadalléen 20, 0027 Oslo, Norway,
Tel. 00 47 23071312; Fax: 00 47 23071331,
e-mail: b.m.dupuy@labmed.uio.no

DXS10011: a hypervariable TTTC/GAAA repeat marker on human chromosome Xq27-q28

T. Matsuki, K. Sawazaki, E. Tsubota and R. Iida

Department of Forensic Medicine, Fukui Medical University, Japan

Introduction

DXS10011 was first reported as Human STS UT413 (HUMUT413)¹ on GenBank and formerly registered as DYS384, tetranucleotide repeat marker on human chromosome Y, in Genome Data Base (GDB). We analyzed this locus extensively, confirmed as a hypervariable polymorphic marker localized on human chromosome X and renamed as DXS10011². In this paper we described allele determination method by capillary electrophoresis, the assignment of the chromosomal localization, exact sequence of two types of the tetranucleotide repeat alleles³, and Japanese population genetics data of DXS10011.

Materials and Methods

1) Sample collection and DNA purification: EDTA blood samples were collected from 450 unrelated Japanese (292 male and 158 female) for a population study. DNA was extracted by Chelex-100 method or QIAamp blood kit (QIAGEN, Hilden, Germany). 2) PCR primers: Primer 1 (GAAA strand): 5'-6Fam-CCAGCCAGGGCAACAAGAGTGAA-3'; Primer 2 (TTTC strand): 5'-GCGCGATGGGAGAACGTTTGAAG-3'. 3) PCR conditions: PCR was performed in a total reaction volume of 5 µl containing 1–20 ng genomic DNA, 0.05 mM each dNTP, 0.25 µM each primer, 1.5 mM MgCl₂, 0.25 U AmpliTaq Gold (AB: Applied Biosystems Japan, Tokyo). Amplification was carried out using a GeneAmp PCR System 9700 (AB), preheating for 16 min at 95°C, for 30–40 cycles with denaturation for 30 sec at 94°C, annealing for 30 sec at 55°C, extension for 30 sec at 72°C, and extra-extension for 10 min at 72°C. 4) Analysis of PCR products: Capillary electrophoresis was carried out on denatured polymer POP6 in 47 cm length capillary with size standard of GS-500 ROX by AB PRISM 310 Genetic Analyzer with GeneScan software (AB). Some PCR products from male samples were directly sequenced with a Taq Dye Deoxy Terminator Cycle Sequencing Kit (AB). 5) Determination of chromosomal localization: PCR analysis of DNAs from human-mouse or human-chinese hamster hybrid panel cells was performed using Somatic Cell Hybrid Mapping Panel#2(version 3), Regional Mapping Panel for Chromosome #X (Coriell Cell Depositories, Camden, NJ) and Human monochromosomal hybrid cell DNA (Japanese Collection of Research Bioresources, Tokyo).

Results and Discussion

Chromosomal localization was analyzed by PCR amplification of target sequence in the panel cell DNAs. Sample number NA06318C (human X chromosome DNA) and NA1MR91 (parent human male cell DNA) from Somatic Cell Hybrid Mapping Panel#2(version 3) and JCRB2223 (chromosome #X) from Japanese Collection of Research Bioresources Human monochromosomal hybrid cell DNA were amplified. Other 25 samples including NA06317 (chromosome #Y) and parent cell DNAs were unamplified. Sample number NA12510, NA11172, NA13492, NA07298A, NA10501, NA11100, NA13339 and NA10063 from Regional Mapping Panel for Chromosome #X were amplified, and NA10663, NA11099 and NA09142 were unamplified. These results indicated that the DXS10011 marker is located on human chromosome X and the fine localization is Xq27–q28. Sequenced size and observed size of the DXS10011 alleles and allele frequencies of the DXS10011 from 608 Japanese chromosomes analyzed in Fukui (present study) and 456 in

Yamagata (cited from reference 4) are shown in Table 1. In total 1064 chromosomes for the Japanese population, 36 alleles including 9 inter-alleles with different sequences shown in Fig. 1 were detected. The heterozygosity was 0.998 and probability of discrimination was 0.999 in the total 1064 chromosomes. DXS10011 system is a supervariable and stable marker on the X chromosome, and will be extravaluable for forensic and human genetic analysis⁵).

References

- 1) Matsuki T: DXS10011. Genome Data Base (<http://www.gdb.org>) 1998.
- 2) Matsuki T, Tsubota E, Iida R, and Aoyama M: Chromosomal mapping of DXS10011. *Jpn J Legal Med* 53: 66, 1999.
- 3) Matsuki T: #AB024610 & #AB02461. DDBJ/GenBank/EMBL (<http://www.ddbj.or.jp>) 1999.
- 4) Watanabe G, Umetsu K, Yuasa I, Suzuki T: DXS10011: a hyper-variable tetranucleotide STR polymorphism on the X-chromosome. *Int J Legal Med* 113:249–250, 2000.
- 5) Tsubota E, Sawazaki K, Iida R, and Matsuki T: Parentage testing of deceased father case excluded by DXS10011. *DNA Polymorphism volume 9*, Toyo-shoten, Tokyo, 2001, in press.

Key words

DXS10011, X chromosome, Xq27-q28, STR, TTTC/GAAA tetranucleotide repeats, capillary electrophoresis, Japanese population data

Address for correspondence:

Prof. Dr. med. Takasumi Matsuki, Department of Forensic Medicine, Fukui Medical University, Matsuoka-cho, Fukui 910-1193, Japan, Tel: +81 776 61 3111 ext.2265; Fax: +81 776 61 8108, e-mail: tmatsuki@fmsrsa.fukui-med.ac.jp

Human Y-chromosomal STR haplotypes in an Arab population sample from Syria

Elias¹ and C. Augustin²

¹Inst. of Legal Medicine, University of Damascus, Syria

²Inst. of Legal Medicine, University of Hamburg, Germany

The eight Y-chromosomal STRs DYS385, DYS19, DYS389I and II, DYS390, DYS391, DYS392, DYS393 were analyzed in an Arabic population sample of 100 males from the Damascus region. The Y-STRs were amplified in single-plex-PCR-reactions. Fragment analysis was done either by silver-staining of native polyacrylamide gels or on an automated sequencer.

Among the 100 samples 91 different haplotypes were observed of which 84 were unique. The gene diversity was 99.87%.

Corresponding author:

Christa Augustin, Department of Legal Medicine, University of Hamburg, Butenfeld 34, D-22529 Hamburg, Tel.: 0049 (0)40 428035485; FAX: 0049 (0)40 428033934, e-mail: Augustin@uke.uni-hamburg.de

Y chromosome haplotypes in the Madeira archipelago population

A.T. Fernandes¹, L. Gusmão², L. Pereira^{2,3}, A. Brehm¹, M.J. Pratas^{2,3} and A. Amorim^{2,3}

¹Centro de Ciências Biológicas e Geológicas da Universidade da Madeira

²Instituto de Patologia e Imunologia Molecular da Universidade do Porto

³Faculdade de Ciências da Universidade do Porto

Introduction

The archipelago of Madeira is constituted by two inhabited islands, Madeira and Porto Santo, with 250,000 inhabitants and 5,000, respectively. The islands were initially populated in the 15th century by the Portuguese, but suffered many different influences throughout the centuries. In the 15th and 16th centuries, Madeira was part of the slaves' route, and received many of them for sugar cane plantations. On the early 19th century, there was some British influence with entire families settling in the island. Known for its favourable climate, people from different origins came to islands to recover from diseases such as tuberculosis. In the 20th century, tourism certainly contributed to the settling of new waves of Europeans.

Unlike Madeira, Porto Santo was exposed, during early colonisation period, to attacks by North African pirates.

Here we present a survey on the genetics of the present day Madeira population using Y STRs (DYS19, DYS389I and II, DYS390, DYS391, DYS392 and DYS393) and 11 Y-chromosomal biallelic polymorphisms defining 10 haplogroups. Our aim is to verify the different genetic male input in the present day population of these islands.

Material and methods

We collected blood samples from unrelated male individuals originating from Madeira (N = 95) and Porto Santo (N = 16) with known local ancestors for at least 3 generations back. The DNA extraction was made by chelex method and the STRs were determined by PCR multiplexing, using a Perkin Elmer ABI 310 sequencer. Y biallelic markers were done by PCR with specific primers for each Y marker separated in polyacrilamide gels and visualized by silver staining. We used restriction enzymes to digest the PCR products except in case of YAP.

Discussion

The haplotype frequencies of both populations were compared with other populations and the results discussed.

There is no relevant influence of Africans in the today's male population in contrast to one other study using mitochondrial DNA and showing a high influence of African haplotypes.

We also found that at least some influence from North Europe could have not originated in the Portuguese mainland.

Address for correspondence:

Prof. António Brehm, Centro de Ciências Biológicas e Geológicas, Universidade da Madeira, Campus da Penteadá, 9000 Funchal, Portugal. Tel: 00 351 291 705383; Fax: 00 351 291 705399, e-mail: brehm@uma.pt

A genetic-population study of five Y chromosomal STRs in Centralwest African immigrant in Spain and Southwest Spain populations

J.J. Gamero^{1*}, J.L. Romero¹, J.L. González¹, M.I. Cuesta¹, M. Carvalho², M.J. Anjos², F. Corte-Real², D.N. Vieira² and M^a.C. Vide²

¹Department of Legal Medicine, Faculty of Medicine, University of Cádiz. Fragela s/n, Cádiz 11003. Spain.

²Institute of Legal Medicine of Coimbra. 3000 Coimbra. Portugal.

The major part of human Y chromosome consists of hypervariable sequences that can be used for characterization of individual human Y chromosomes. Y linked loci are of special interest, because they are haploid and paternally inherited. These properties make Y chromosome STRs highly relevant for both population genetics and molecular studies of human evolution.

The Y chromosome polymorphism of six STRs (DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS393) were studied in 120 unrelated individuals from south west Spain and 80 unrelated individuals from the black West African immigrant population. This study has determined the haplotype diversity of both populations confirming the extreme polymorphisms of the haplotypes of Y Chromosome and indicating that these loci are a powerful tool for the forensic identification of male DNA.

Address:

Joaquín-José Gamero Ph. M.

Dpto. Medicina Legal, Facultad de Medicina, Universidad de Cádiz

Plaza Fragela s/n, 11003, Cádiz, Spain; e-mail:joaquin.gamero@uca.es

Population genetics of 7 Y-STR loci in the Pomerania – Kujawy region of Poland

M. Wozniak, T. Grzybowski, J. Czarny and D. Miscicka-Sliwka

Forensic Medicine Institute, The Ludwik Rydygier Medical University in Bydgoszcz, Poland

Microsatellite loci localized on Y chromosome are useful tools for both forensic identification and molecular anthropology. However, due to the Y chromosome uniparental mode of transmission the population calculations require large number of individuals to be haplotyped. This allows for creation of a database large enough to properly assess haplotypes' frequency. We have performed haplotyping of 7 Y-STRs (DYS19, DYS389, DYS390, DYS391, DYS392, DYS393 and DYS385). We have found 136 different haplotypes in 168 unrelated males from the Pomerania – Kujawy region of Poland. Among the haplotypes found 116 (85,3%) were unique in our population sample while 20 occurred more than once. Inter-population comparisons were also performed and showed statistically significant differences in allele frequencies between our population and other populations of European origin while allele distribution in populations from other regions of Poland was very similar.

Population study and validation of the Y-STR pentaplex for use in forensic case work

C. Hallenberg and N. Morling

Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark

With the aim of using Y-chromosomal polymorphic markers in Danish crime cases and deficiency paternity cases, a validation study of a Y-STR pentaplex (1) was established. The Y-STR pentaplex included the systems DYS19, DYS389I/II, DYS390 and DYS393. Here, we present the results of the study, including the haplotype distribution among 200 male Danes and 91 male Eskimoes randomly selected from paternity cases.

Among 93 Danish father-son pairs, two inconsistencies were found in DYS389I/II, while one inconsistency was found in DYS19. The father-son pairs had previously been investigated for at least five autosomal VNTR-systems, and in the VNTR-systems, the paternity indices exceeded 10,000 to 1. No inconsistency was found in the VNTR-systems.

A validation study of the Y-STR pentaplex for stain analysis showed that the pentaplex produced reliable results using as little as 0.05 to 0.1 ng template DNA. Investigations of stains containing mixtures of female DNA and male DNA showed that the Y-STR pentaplex worked satisfactory in all five systems if the amount of female DNA was below 5 ng and if the ratio of female DNA to male DNA was less than 1000 : 1. If the amount of female DNA exceeded 5 ng, a product in DYS393 arising from female DNA was seen. However, it has been reported (2) that changing one of the DYS393 primers solves this problem. If the ratio of female DNA to male DNA exceeded 1000 : 1, artefact-peaks in DYS389II were observed some cases.

Investigations of stains containing mixtures of DNA from two men showed Y-STR results from both men when the amount of one of the men did not exceed 10 times the amount of the other man.

- (1) L. Gusmao, A. Gonzalez-Neira, C. Pestoni, M. Brion, M.V. Lareu, A. Carracedo, Robustness of the Y STRs DYS19, DYS389I and II, DYS390 and DYS393: optimization of a PCR pentaplex, *Forensic Sci. Int.* 106 (1999) 163–172.
- (2) B. M. Dupuy, T. Gedde-Dahl, B. Olaisen, DXYS267: DYS393 and its X chromosome counterpart, *Forensic Sci. Int.* 112 (2000) 111–121.

Address for correspondence:

Charlotte Hallenberg, Ph.D, Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, 11 Frederik V's Vej, DK-2100 Copenhagen Ø, Denmark, Tel: + 45 35 32 61 10; Fax: + 45 35 32 61 20, e-mail: charlotte.hallenberg@forensic.ku.dk.

Polymorphism of two new Y-STR loci in Chinese population

Y.P. Hou, J. Zhang, H. Tang, J. Zhang, J. Wu, Y.B. Li, and J. Yan

Institute of Forensic Medicine, Sichuan University, Chengdu, PR China

Human chromosome Y-specific short tandem repeat (Y-specific STR) markers have useful properties for forensic application. However, there is a need to develop more Y-specific STR markers, because the discriminating power of each STR locus is limited. In the present study, we describe our results on two new Y-specific STR markers, which were initially reported by White et al as Y-GATA-C4 and Y-GATA-A10. The distributions of alleles and haplotypes for both Y-specific STR loci were studied in a Chinese Han population sample. DNA was extracted using the Chelex method. DNA Typing was carried out by Amp-FLP technique. Allele determination was carried out by comparison with the sequenced human allele ladders, which were made in-house. Following the recommendations of the International Society of Forensic Genetics, the allele classification for both Y-specific STR loci was based on the number of repeat motifs. The results show that the haplotype diversity, the power of discrimination and the exclusion probability in Chinese population for both Y-specific STR loci were 0.9090. Standard errors were calculated as 0.0097. The implication from this study is that Y-GATA-C4 and Y-GATA-A10 are useful Y-specific STR markers for forensic DNA typing in Chinese population.

Prof. Yi Ping Hou, Institute of Forensic Medicine, Sichuan University (West China University of Medical Sciences), 610041 Chengdu, PR China, Tel. 0086-28-5501549; Fax: 0086-28-5501549, e-mail: rechtsme@wcums.edu.cn

Forensic Validation Studies on the Y-PLEX™ 6 Kit

P. Martín¹, C. Albarrán¹, O. García², P. García¹, A. Fernández-Rodríguez¹, L. Fernández de Simón¹, M. Sancho¹, and A. Alonso¹

¹Instituto de Toxicología. Secc. Biología. Luis Cabrera, 9. 28002 Madrid. Spain

²Area de laboratorio Ertzaintza. Sección de Biología. Bilbao. Spain

The Y-PLEX™ 6 Kit (Reliagene Technologies, Inc.) is a commercial multiplex PCR system for the simultaneous analysis of six tetranucleotide STR loci (DYS393, DYS19, DYS389II, DYS390, DYS391 and DYS385) with high potential interest in some forensic DNA typing applications. In this study we present the results of some forensic validation studies including the following aspects: (1) evaluation of stutter bands and inter-locus balance, (2) sensitivity studies, (3) specificity studies against different amounts of female DNA, (4) challenge microbial study against 30 DNA samples from different microorganisms, (5) analysis of male/female mixtures, (6) population studies, and (7) analysis of forensic cases previously analyzed with other autosomal and Y-STR systems including mixed samples from rape cases and bone samples from personal identification and parentage testing cases. In conclusion, our data indicate that the Y-PLEX™ 6 Kit system yielded sensitive (up to 250 pg of male DNA), reproducible and balanced typing results. However, some non-specific PCR products amplified from female DNA were observed for the yellow channel. This lack of specificity could be a drawback for some casework applications.

Address for correspondence:

Dr. Pablo Martín Martín, Instituto de Toxicología, Sección de Biología, Luis Cabrera 9, 28002 Madrid, Spain,
e-mail: biologia@mad.inaltox.es

Validation of the X chromosomal STR DXS7424 which is closely linked to DXS101

J. Edelmann¹, S. Hering², E. Kuhlisch³ and R. Szibor⁴

¹Institut für Rechtsmedizin, Universität Leipzig, Germany

²Institut für Rechtsmedizin, Technische Universität Dresden, Germany

³Institut für Medizinische Informatik und Biometrie, Technische Universität Dresden, Germany

⁴Institut für Rechtsmedizin, Otto-von-Guericke-Universität Magdeburg, Germany

X-linked microsatellite markers are powerful tools for parentage testing in special cases, mainly in deficiency paternity cases when the disputed child is female. However, only a small number of X-linked STRs have been comprehensively described for forensic application to date. We analysed the DXS7424 STR (GDB-Goo-577-633).

PCR fragment length measurements and sequencing were carried out using the automated gene analyser A310 (Applied Biosystems). For linkage studies the RH mapping procedure and the pedigree inspection method were applied.

In a sample of 474 unrelated Germans (220 females and 254 males) we found 12 alleles, i.e. alleles 9–20, corresponding to fragments of 147 to 180 bp in length.

The German population which was checked for this STR was characterised by the following features: polymorphism information content (PIC) = 0.776; heterozygosity (Het) = 0.836; mean exclusion chance (MEC) = 0.762. Kinship tests revealed a typical X-linked inheritance. Examination of 300 meioses did not reveal any mutations. Significant deviations from the Hardy-Weinberg equilibrium were not established. Sequencing of randomly selected alleles revealed the regular repeat structure (TAA)_[9–20].

The DXS7424 STR located in the intron 5 of the human Bruton tyrosine kinase (BTK) gene [1] was cytogenically mapped at Xq22.

The RH-mapping procedure carried out for DXS7424 and DXS101 [2, 3] ordered both STRs into the anchor marker interval from DXS990 to DXS1059 corresponding to a genetic localisation of 104.9–121 cM from Xp-tel.

Haplotyping of 280 male individuals was not indicative of any linkage disequilibrium between DXS101 and DXS7424.

Based on our data we conclude: DXS7424 is a highly informative ChrX marker which is closely linked to DXS101. Therefore, this linkage group is qualified for ChrX haplotyping in complicated cases of parentage testing.

1. Caggana M et al. (1997): Fabry Disease: Molecular carrier detection and prenatal diagnosis by analysis of closely linked polymorphisms at Xq22.1. *Am J Med Genet* 71: 329–335
2. Allen RC, Belmont JW (1993): Trinucleotide repeat polymorphism at DXS101. *Hum Mol Genet* 2: 1508
3. Edelmann J, Szibor R (2001) DXS101: A highly polymorphic X-linked STR. *Int J Legal Med* (in press)

Correspondence address:

Dr. rer. nat. Jeanett Edelmann, Institut für Rechtsmedizin, Universität Leipzig, Johannisallee 28, D-04103 Leipzig, Germany,
Phone: 0049 (0)341 9715111; Fax: 0049 (0)341 9715109;
e-mail: edej@server3.medizin.uni-leipzig.de

Y-chromosomal Haplotypes in Baltic males

R. Lessig¹, J. Edelmann¹ and M. Krawczak²

¹Institut für Rechtsmedizin, Universität Leipzig, Germany

²Institute of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff, UK

Abstract (P)

Y-chromosomal microsatellites (STRs) have been established in forensic practice for several years. However, an in-depth evaluation of their population genetic properties requires a large number of haplotypes from different populations. In addition to more than 400 samples from Western Saxony, we therefore analysed the Y chromosomes of 152 males from Lithuania, 145 from Latvia, and 133 from Estonia. These samples were genotyped for the so-called "extended core set" of nine Y-STR marker systems – comprising DYS19, DYS393, DYS392, DYS391, DYS390, DYS389I/II, DYS385 and YCAII.

The extended core set markers, which detect 11 Y-chromosomal STR loci, are easy to handle, sensitive and the genotyping results are reproducible. Seven of the systems are characterized by a tetrameric repeat unit, with one of them (DYS385) representing two loci, one marker (DYS392) comprises a trimeric repeat, and one marker (YCAII) detects two loci containing a dinucleotide repeat. DNA was extracted from dried blood using the QIAamp DNA Blood Kit (Qiagen, Hilden/Germany) or Chelex procedures. PCR was performed as previously described using fluorescence-labelled primers. PCR fragments were analysed by capillary electrophoresis (ABI Prism 310 DNA Sequencer, ABI/PE) in denaturing polymer (POP4, PE). Allele assignment was performed by comparison to a self-established allelic ladder.

Allele frequency distributions and discrimination indices were calculated, and the three populations were tested for genetic differences by means of Analysis of Molecular Variance (AMOVA). A larger genetic difference became apparent between Estonian and both Lithuanian and Latvian males than between the latter two, non-Finno-Ugric speaking populations. The haplotype data have been included into the Y-STR database maintained at the Institute of Legal Medicine, Humboldt-University, Berlin.

Y-chromosomal microsatellites are very useful in practical forensic casework, particularly for paternity/kinship testing and stain analysis. The discrimination indices of ~0.99, as observed for Baltic males, are compatible with data from virtually all populations analysed so far. The most frequent haplotype in the Baltic populations, using the extended core set without YCAII, is also the most commonly observed one in other Europoid populations.

Acknowledgements

We thank Dr. V. Volkson, Centre of Legal Medicine, Riga (Latvia), Dr. M. Väli, Institute of Pathology and Legal Medicine, Tartu (Estonia), Prof. A. Garmus and Dr. J. Rybalko, Institute of Legal Medicine, Vilnius (Lithuania), for the provision of male blood samples.

Address for correspondence:

Dr. med. Rüdiger Lessig, Institut für Rechtsmedizin, Universität Leipzig, Johannisallee 28, D-04103 Leipzig, Germany, Tel. +49 (0)341 9715118, Fax: +49 (0)341 9715109, e-mail: lesr@medizin.uni-leipzig.de

Haplotype frequencies of DYS19, DYS389 I/II, DYS390, DYS391, DYS392, DYS393 and DYS385 STR loci in Barcelona (North-East Spain).

N. Borrego¹, E. Piqué¹, A. Xifró¹, C. Brandt², M. Ortega¹, J. Corbella¹, P. Moreno¹, E. Huguet¹ and M. Gené¹

¹Legal Medicine Department. School of Medicine. University of Barcelona, Spain.

²Legal Medicine Institute. Lausanne, Switzerland

Haplotype frequencies for eight Y-chromosomal short tandem repeat (STR) loci were determined in a population sample from Barcelona (NE Spain). After PCR amplification and denaturing PAGE electrophoresis, DYS19, DYS389 I/II, DYS390, DYS391, DYS392, DYS393 and DYS385 loci were typed. Complete eight Y-chromosomal STRs haplotypes could be formed for 163 subjects, among which 129 different haplotypes were observed. The most common haplotype (14, 13, 29, 24, 11, 13, 13, 11–14) was shared by 9.20% of the sample, while 115 haplotypes were unique. The gene diversity was 0.9897. From the forensic point of view the combined polymorphisms provide a high diagnostic efficiency.

Our group has participated in the Y-STR Haplotyping Quality Assurance Exercise 2000–2001. The alleles at all loci reported were typed correctly according to the published nomenclature and the ISFG guidelines for STR analysis.

Dr. Conxita Brandt. Institut Universitaire de Médecine Légale.

Rue du Bugnon 21, CH-1005 Lausanne, Suisse.

Tel 41 21 / 314 70 70 Fax 41 21 / 314 70 90

e-mail: Conxita.Brandt@inst.hospvd.ch

Population genetics of Y chromosomal STR haplotypes in South Spain (Andalusia)

V. Prieto, Y. Torres, M.J. Farfán, M. López-Soto, J. García-Hirschfeld and P. Sanz

Instituto Nacional de Toxicología, Departamento de Sevilla, Spain

In the last years the increased information on the genetic diversity of male specific markers located in the Y chromosome has provided a useful tool in forensic applications. Although PCR conditions are well established, its applicability in routine forensic casework has been limited up to now by a poor standardization of the techniques used by the different laboratories and the unavailability of sequenced allelic ladders. These difficulties could be overcome by the use of commercial validated methods that ensure accurate and reproducible results.

We present herein a study of 100 unrelated male individuals from an Andalusian population (Southern Spain) using in parallel the Y-Plex™ 6 STR kit (Reliagene Technologies, Inc.) and amplification and typing protocols according to Elmoznino and Prinz (<http://ystr.charite.de>). Different parameters of forensic interest have been calculated combined with 13 autosomic STRs (Profiler Plus and Cofiler).

Mrs Victoria Prieto, Instituto Nacional de Toxicología,

P.O. Box 863, E-41080 Sevilla, Spain.

Tel. 34 954371233; Fax: 34 954370262; e-mail: biol@sev.inaltox.es

Differences in Y-Chromosome Haplotype Frequencies at the Microgeographical Level

M.T. Zarrabeitia¹, J.A. Riancho², P. Sánchez-Velasco²,
P. Sánchez-Diz³, E. Altuzarra¹ and A. Carracedo³

¹Unidad de Medicina Legal, Universidad de Cantabria,
Santander, Spain

²Hospital U. Marqués de Valdecilla. Santander

³Instituto de Medicina Legal. Santiago de Compostela, Spain

Studies of Y microsatellites have usually shown little variation among Western European populations (Roewer et al. *Forensic Sci Int* 2000; 114: 31–43). However, the possibility of population stratification within certain regions with specific historical and demographic characteristics still remains. The clarification of this issue is of importance not only from the perspective of population genetics studies, but also for a proper interpretation of paternity disputes and other forensic cases.

We have analysed Y-specific microsatellites in a small region in Northern Spain to elucidate the possible existence of population sub-structuration.

Population

We studied male subjects living in Cantabria, a region in Northern Spain with a population about 500,000. This region of 5,000 sq.Km is situated between the Cantabrian Sea and the Cantabrian Mountains, and has a flat, well communicated, and densely populated coastal area. On the other hand, it has a mountainous area with several valleys that have traditionally had difficult communications. Their inhabitants have had less opportunity for social and economic interaction with people from other areas. The study group consisted of 182 subjects living either in the coastal area (n = 97) or in two of those valleys, the Liébana valley (n = 44) and the Pas valley (n = 41).

Methods

The following loci were studied: DYS390, DYS19, DYS 389I, DYS389II, and DYS393. DNA was isolated from peripheral blood by the Quiagen method. A pentaplex PCR was carried out as described by Gusmao et al (*Forensic Sci Int* 1999; 106:163–72). The size of amplified fragments was determined in a ABI Prism 310 analyser. The differences in allelic frequencies of isolated loci and the corresponding haplotypes were estimated by Fisher's exact test with SPSS software.

Results

There were significant differences in allele frequencies between the 3 subpopulations studied regarding DYS390 ($p < 0.001$), DYS389I ($p = 0.043$), and DYS393 ($p = 0.004$) systems. However, no statistically significant differences existed in DYS19 and DYS389II allelic frequencies.

As expected, strong allelic associations were found to exist between the loci studied. Among the 182 individuals genotyped, 79 different haplotypes were found. The two most common were present in 29 and 16 individuals, respectively (16% and 9% of the overall population). However, significant differences were also observed among the three subpopulations at the haplotype level ($p < 0.001$). The overall haplotype diversity was somewhat higher in the coastal area (0.95) than in the Liébana and Pas valleys (0.94 and 0.90, respectively).

Conclusion

Albeit preliminary, these results suggest that Y-chromosome microsatellites may show significant population substructuration at the microgeographical level in areas with distinctive demographic features.

Correspondence:

Dr. María T. Zarrabeitia. Unidad de Medicina Legal, Facultad de Medicina, Universidad de Cantabria. Santander 39011, Spain.

Fax: 34-942-201903. e-mail: zarrabet@unican.es

Y-chromosomal microsatellites in the Finns

M. Hedman, K. Höök and A. Sajantila

*Laboratory of Forensic Biology, Department of Forensic Medicine,
University of Helsinki, Finland*

We have evaluated 16 Y-chromosomal microsatellites for forensic purposes. The loci were amplified in two multiplex PCRs, a 9-plex (http://ystr.charite.de./index_gr.html) and a 10-plex (Ch.M. Rutberg and J.M. Butler 2000), with 3 common Y microsatellite loci. Nine of these 16 loci belong to the widely used Y-chromosomal minimal haplotype (DYS19, *DYS389I*, *DYS389II*, *DYS390*, *DYS391*, *DYS392*, *DYS393*, *DYS385a* and *DYS385b*), and the additional 7 loci were *DYS435*, *DYS436*, *DYS437*, *DYS438*, *DYS439*, Y GATA A7.1 and Y GATA H4. The PCR-products were subsequently analysed using capillary electrophoresis.

For population studies and PCR validation purposes DNA samples from 200 Finnish males, 20 female samples and various male animal samples were analysed with both multiplex PCRs. To compare the sensitivity of the 9-plex and the 10-plex reactions, we performed the multiplex PCRs with samples from a dilution series of a known amount of DNA.

Our results show that the 9-plex PCR is more sensitive than the 10-plex PCR. In addition, the 10-plex PCR showed constant, unspecific peaks which were detected in male and in female samples.

When using the set of minimal haplotype markers, our 200 Finnish male DNA samples fell in 85 different haplotypes, 64 (75%) of them occurring only once in our data. When we were using data from 10-plex, we found 72 different haplotypes, 52 (72%) occurring only once. Combining the data, altogether 120 different haplotypes were found, and from these 99 (82,5%) occurred once. In the 9-plex PCR, 26,5% of the Finns had the most common haplotype. Similarly in the 10-plex PCR also 26,5% of Finns shared the most common haplotype. However, when the two marker sets were combined, the most common haplotype was shared only in 13% of the samples.

Power of discrimination values of separate microsatellite loci varied in the 9-plex PCR between 0,448 (*DYS392*) and 0,706 (*DYS385a* and *b* together) and in the additional 7 loci between 0,020 (*DYS436*) and 0,523 (Y GATA H4).

We further divided our samples according to the geographical origin from where the sample donors had their family roots. This resulted in six geographically defined sub-populations (Häme, Karjala, Pohjanmaa, Satakunta, Savo and Varsinais-Suomi), each containing 30 individuals. The most common haplotype in Finns (0,130) was found in four of these sub-populations (10/30 in Savo, 7/30 in Pohjanmaa, 3/30 in Karjala and 2/30 in Häme), but in the sub-populations located in south-western Finland (Satakunta and Varsinais-Suomi) this haplotype was not observed, and no other haplotype was found to be dominating. Altogether, the power of discrimination values of individual loci were the lowest among individuals from Savo (*DYS435* being the only exception).

In conclusion, the 9-plex PCR appears more sensitive and produces no unspecific or female-derived peaks, where as in the 10-plex constant, unspecific peaks were observed in male and in female DNA samples. For forensic purposes the 9-plex PCR is more discriminating among the Finns than the 10-plex PCR. This is mostly due to the fact that in the 10-plex, two loci (*DYS435*, *DYS436*) had very little variation, with only 2 or 3 allele and 98-99% of the samples shared the one most common allele. However, by combining these two multiplex PCR a high resolution Y-chromosomal DNA profile can be achieved for criminal investigations and for establishing family relationships. From population genetics point of view our observations are in accordance with earlier studies that show decreased variation among individuals from Eastern Finland. Our findings also support the theory that only small number of individuals have inhabitant the Savo-area in historical times.

Address for correspondence:

M. Hedman, Laboratory of Forensic Biology, Department of Forensic Medicine, P.O. Box 40, 00014 University of Helsinki, Finland.

Tel. +358 9 191 27466 Fax. +358 9 191 27518

e-mail: Minttu.Hedman@Helsinki.Fi

Optimisation of Y-STR multiplexing combining established and newly described loci

S. Belezã¹, L. Gusmao¹, A. González-Neira², A. Carracedo² and A. Amorim¹

¹Instituto de Patologia e Imunologia da Universidade do Porto (IPATIMUP), Porto, Portugal

²Instituto de Medicina Legal, Universidad de Santiago de Compostela, Galicia, Spain

In the actual days much attention from a number of fields is being paid to the determination of human Y-polymorphisms. There has been an increasing use of compound haplotypes to differentiate populations and to study human migration, the settlement or mating structure of human populations, as well as for paternity testing and forensic casework. Among all of Y polymorphisms, short tandem repeats (STRs) seem to be the most used for forensic genetics due to their high levels of diversity. All of the studies mentioned require the generation of considerable quantities of data on the allelic state of STRs, a process that can be both expensive and slow. Therefore there is a need for the establishment of appropriate methodology that reduce the cost and that speeds up the rate at which Y-haplotype databases are generated. Given the increasingly widespread use of automated sequencers, the development of PCR multiplex systems seems to be the best strategy. Recently, primers for a series of Y-specific STRs have been developed and tested. Our aim was to select some of these novel markers and co-amplify them with the established ones in a multiplex reaction in order to extend Y-databases for forensic and anthropological applications. The markers selected were DYS391/434/437/439, whose size range of the alleles of each marker does not overlap, allowing its use in both monochromatic and polychromatic platforms. For DYS391 we used a new reversed primer designed by our group, which improves specificity and amplifies a smaller PCR-product. All markers were labelled with the same fluorescent dye, TET. However, when amplifying DYS391 a double-peak was observed. This problem was solved by labelling the reverse primer instead of the forward primer like in the case of the other markers. An additional peak appeared also in the range of 360–370 bp. However, this artefact does not lead to errors in typing samples, since it does not fall in the size range of any other marker.

Sequenced allelic ladders were constructed with previously sequenced alleles and reamplification conditions were tested.

This newly constructed tetraplex has proved to be very useful in population genetic studies because all four Y STR markers can be loaded in the same lane of a gel with other Y STR singleplex or multiplexes. Also, this tetraplex can be co-amplify with a pentaplex composed by the markers DYS19/389 I & II/390/393. The pentaplex is a modification of a previous one developed by our group, using a new reverse primer for Y19 in order to prevent an overlap in the size range of the alleles for DYS19 and DYS390. In this way it is possible to label all the systems included in this pentaplex with only one fluorescent dye. These two multiplexes were amplified together with good results for all the nine STRs included.

Tetraplex is also a good system for forensic casework since there was no amplification observed in females and we were able to genotype the YSTRs in mixed stains.

Address for correspondence:

Prof. Dr. António Amorim, IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Rua Roberto Frias s/n, 4200 – Porto, Portugal, Tel. 00 351 22 5570700, Fax: 00 351 22 5570799, e-mail: aamorim@ipatimup.pt

Y-chromosome STRs in populations of Bantu origin from Mozambique: male contribution to the Africa genetic pool and forensic implications

P. Sanchez-Diz, T. De la Fe, B. Quintáns, A. Salas, M. Lareu, A. Carracedo.

Institute of Legal Medicine, Unit of Forensic Genetics, Faculty of Medicine, University of Santiago de Compostela, Galicia-Spain

A set of 7 common Y-specific chromosome microsatellites have been used to type 308 individuals from 16 African population groups from Mozambique. These microsatellites include the DYS19, DYS390, DYS391, DYS392, DYS393, DYS389I and DYS389II systems. The population structure was analyzed and therefore genetic distances and several diversity indexes were computed and compared with other populations around the world. The results obtained in the present study shows that these populations share common characteristics with other available Bantu speaking African populations and clear differences with non-African ones. The combination of the analysis of Y-chromosome STR with other more slowly mutating Y chromosome polymorphisms (biallelic markers) and mtDNA sequences and autosomal data for the same populations will allow us the analysis of the human evolution in paternal lineages in different timescales and lineages. Forensic implications of these results are discussed in depth.

Address for correspondence:

M. Lareu. Institute of Legal Medicine, Unit of Forensic Genetics, Faculty of Medicine, C/San Francisco. 15705. Santiago de Compostela. Spain.

Tel. 00-34-981-582327, Fax: 00-34-981-580336, e-mail: apimllar@usc.es

Sequence structure of 12 novel Y chromosome microsatellites and PCR amplification strategies

A. González-Neira, P. Sánchez-Diz, L. Gusmao, M. Lareu, A. Carracedo

Institute of Legal Medicine, Unit of Forensic Genetics, Faculty of Medicine, University of Santiago de Compostela, Galicia-Spain

In order to be able to decide on the appropriate allele nomenclature and to construct sequenced allelic ladders, 12 novel Y STRs were sequenced in 170 samples from different ethnic groups (African American, Caucasian, Hispanic and Asian). The systems were DYS434, DYS435, DYS436, DYS437, DYS438, DYS439 described by Ayub et. (2000) and GATA A10, GATA 7.1, GATA 7.2, GATA C4, GATA H4, GATA A4 described by White et al. 2000). Complex sequence patterns were found for GATA C4 and DYS437.

A PCR multiplex strategy was implemented for the detection of these systems. Sequenced allelic ladders were constructed and a nomenclature for these new systems is proposed based on the sequence structure and following ISFG recommendations.

GATA A4 and DYS439 are probably the same STR. They have the same STR structure and the alleles are always the same in the same individuals although some differences are found in the flanking region probably due to sequence errors in the Genbank.

Sequence polymorphisms were observed in the GATA C4 and DYS437 STRs. The variation in DYS437 was associated with a specific population group and is very interesting not only for forensic genetics but also for anthropological studies.

Address for correspondence:

M. Lareu Institute of Legal Medicine, Unit of Forensic Genetics, Faculty of Medicine, C/San Francisco. 15705. Santiago de Compostela. Spain
Tel. 00-34-981-582327, Fax: 00-34-981-580336,
e-mail: apimllar@usc.es

Study of 9 new Y-chromosome STRs in a Valencian population (East of Spain): analysis of gene and haplotypes frequencies

Mercedes Aler¹, Eduardo Murcia¹, Marina Gisbert-Griffo¹, María Brión²

¹Institut of Legal Medicine. Valencia. Spain

²Institute of Legal medicine. Santiago de Compostela. Spain

Because of the great interest of the Y-chromosome polymorphisms not only in the forensic field but also in evolutionary studies, the number of described Y chromosome markers has been increased considerably in the recent literature.

During the last few years, new Y chromosome polymorphisms have been described, including binary polymorphisms, microsatellites and minisatellites. Specially, the new Y-STRs described have a great importance in forensic genetics, because of their special characteristics and because of the necessity of developing extensive database including as much number of polymorphisms as possible, and as much number of samples as possible.

In this study 9 recently described tetranucleotide microsatellites have been analysed: DYS434, DYS439, DYS437, Y-GATA A10, Y-GATA A4, Y-GATA A7.2, Y-GATA A7.1, Y-GATA C4 and Y-GATA H4.

Gene and haplotype frequencies have been estimated in the Valencian population, in order to determine highly informative haplotypes, using this new Y-STRs combined with the classical ones.

Address for correspondence:

Mercedes Aler. Institute of Legal Medicine. Valenci. Spain
e-mail: mercedes.aler@uv.es

Haplotype Databases Comprising Eleven Y-Chromosome STRs for Three UK Population Groups

D. Ballard, C. Phillips, C. Thacker, C. Robson and D. Syndercombe Court

Department of Haematology, Barts and The London, Queen Mary's School of Medicine and Dentistry, London, UK

In certain circumstances, analysis with Y chromosome markers provides potential benefits over autosomal STRs, for example paternity cases in which the putative father is deceased and in rape cases. The polymorphism level of Y chromosome STRs however is generally quite low compared to the commonly used autosomal STRs. Discrimination is further reduced as a result of linkage and consequently profiles need to be analyzed as haplotypes rather than independent loci. To improve the current level of discrimination we extended the range of Y-chromosome STRs most commonly used in forensic analysis (1), with the addition of three recently reported STR markers: DYS437, 438 and 439 (2) to give a total of 11 loci. 250 individuals were typed from three racial groups: UK Caucasian, UK Afro-Caribbeans and UK Asians (from the Indian sub-continent). Two PCR triplex reactions were developed and optimized, combining the three new loci with the existing markers: DYS391, 392 and 385. These were used in combination with an established pentaplex combination: DYS19, 389 I/II, 390 and 393 (3). Sequenced allelic ladders were developed for the three new loci. For each racial group allele frequencies are reported for all loci and haplotype diversity values calculated. Intermediate alleles were found in the course of constructing the database and were characterized. We also report an extended family study, which examined the male lineage of a family over 7 generations, with observed mutations in the locus DYS439 and 389 I and II giving a strong indication of their familial relationship.

References

1. M. Kayser, A. Caglià, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. Perez-Lezaun, A. Piccinini, M. Prinz, C. Schmitt, P.M. Schneider, R. Szibor, J. Teifel-Greding, G. Weichhold, P. de Knijff, L. Roewer, *International Journal of Legal Medicine*, 110 (1997) 125–133.
2. Q. Ayub, A. Mohyuddin, R. Qauar, K. Mazher, T. Zerjal, Q. Mehdi, C. Tyler-Smith, *Nucleic Acids Research*, Volume 28 (2000) e8.
3. L. Gusmão, A. Gonzalez-Neira, S. Pestoni, M. Brion, M.V. Lareu, A. Carracedo, *Forensic Science International*, 106 (1998) 163–172.

Correspondence:

David Ballard, Department of Haematology, Barts and The London, Queen Mary's School of Medicine and Dentistry, Turner Street, London, E1 2AD, UK, Tel: 00 44 (0)20 7377 7076; Fax: 00 44 (0)20 7377 7629, e-mail: d.j.ballard@mds.qmw.ac.uk

Y-chromosome STRs DYS385, DYS19, DYS389-I and II, DYS390, DYS391, DYS392 and DYS393 in five African populations

V. Lopes¹, M. Carvalho¹, S. Antunes¹, M.J. Anjos¹, L. Andrade¹, M.V. Santos², F. Corte-Real¹, D.N. Vieira¹, J.J. Gamero³, M.C. Vide¹

¹National Institute of Legal Medicine, Forensic Genetics of Coimbra, Portugal

²Ph.D. Student of University of Coimbra, INML, CNPq/Brazil

³Department of Legal Medicine, Faculty of Medicine, University of Cádiz, Spain

The Y-chromosome has been used to compare the relation between populations, representing a rich source of potential information to trace paternal lineages and providing a record of our relatedness.

Among different populational groups, African populations seem to be very interesting to study, considering the theory's origin of modern humans and the ethnic variability usually existent.

Five male populations from Angola (n = 50), Cap Verde (n = 47), Guinea-Bissau (n = 33), Mozambique (n = 37) and S. Tome and Prince (n = 30) were studied for Y-chromosome STRs DYS385, in addition to the DYS19, DYS389-I and II, DYS390, DYS391, DYS392 and DYS393 previously searched.

DNA was extracted from air-dried blood stains from unrelated males, using chelex procedure, the amplification was made according to Schneider et al. and the detection was carried on ABI 377 DNA sequencers.

The great DYS385 polymorphism, adding a considerable variability to the Y-chromosome haplotype, was confirmed, and the comparisons between the African populations and other data showed an interesting location of insular populations, with an intermediate position between Caucasoid and African continental populations, probably due to the European presence in the colonization of those islands.

Dr. M. C. Vide, Instituto Nacional de Medicina Legal, Delegação de Coimbra, Serviço de Genética Forense, Largo da Sé Nova, 3000-213 Coimbra, Portugal. Tel. 00 351 239 854 230; Fax: 00 351 239 820 549; e-mail: mcvide@ci.uc.pt

Y-chromosome DNA haplotypes in a sample population from Bahia, Brazil

M.V. Santos¹, M. Carvalho², M.J. Anjos², L. Andrade², V. Lopes², F. Corte-Real², D.N. Vieira² and M.C. Vide²

¹Ph.D. Student of University of Coimbra, INML, CNPq/Brazil

²National Institute of Legal Medicine, Forensic Genetics of Coimbra, Portugal

The Y-chromosome polymorphisms have a great value to population genetic studies and evolutionary aspects since these markers show high levels of heterogeneity within and between populations and complement the autosomal STRs and mtDNA matrilineages information.

Y-linked STRs remain stable in a given paternal lineage over many generations so they are useful for identification in forensic cases, namely in cases with male/female stain samples.

In this study we report Y-chromosome haplotypes in a population sample with 68 unrelated males blood donors from Bahia, Brazil. The STRs (DYS19, *DYS389I/II*, *DYS390* and *DYS393*) were determined after PCR multiplexing with an automatic ABI sequencer.

The results and the haplotype diversity were compared with other population data.

*Dr. M. C. Vide, Instituto Nacional de Medicina Legal, Delegação de Coimbra, Serviço de Genética Forense, Largo da Sé Nova, 3000-213 Coimbra, Portugal.
Tel. 00 351 239 854 230; Fax: 00 351 239 820 549;
e-mail: mcvide@ci.uc.pt*

Y-chromosomal haplogroups in male identification: study of a population sample from Portugal (central area)

L. Andrade, F. Balsa, V. Lopes, M.J. Anjos, M. Carvalho, F. Corte-Real, D.N. Vieira and M.C. Vide

National Institute of Legal Medicine, Forensic Genetics of Coimbra, Portugal

The non-recombining portion of the Y chromosome represents a valuable tool for the study of human population history.

Biallelic markers usually arise from unique mutational events (base substitutions, insertions/deletions) and are binary in nature.

Biallelic markers can be useful for illuminating the substructure of a population or the Y-chromosomal origin of an individual, through the definition of paternal lineages which can be related to one another by a single most parsimonious tree; these markers have been widely used for human evolutionary studies and population genetics.

Y-chromosomal biallelic markers have been investigated in a Portuguese population sample (n = 100). By combining the allelic state of 10 biallelic markers (YAP, SRY-8299, 92R7, 12f2, SRY-1532, SRY-2627, Tat, SY81, Mg, LLY22g) we could define the haplogroup to which each sample belonged.

*Dr. M. C. Vide, Instituto Nacional de Medicina Legal, Delegação de Coimbra, Serviço de Genética Forense, Largo da Sé Nova, 3000-213 Coimbra, Portugal.
Tel. 00 351 239 854 230; Fax: 00 351 239 820 549;
e-mail: mcvide@ci.uc.pt*

A new duplex PCR System for YCAII and DXYS156Y microsatellites analysis

I. Sani, U. Ricci, L. Giunti, S. Guarducci, M. Lapini, A. Brusaferrri, A. Lasagni and M.L. Giovannucci Uzielli

Genetics and Molecular Medicine Unit, University of Florence – "A. Meyer", Italy

The scientific interest for the Y chromosome is wide and rising in many branches of Medicine and Biology. In Human Genetics, after the discovery of SRY gene, in the last few years, the identification of new genes involved in the spermatogenesis and other important functions, had attracted the attention to many loci of Y-chromosome. In the same time, the Y chromosome acquired a good place in Anthropology and Population Genetics Research, by using DNA polymorphisms. The Y-chromosome polymorphisms represent an important tool also in Forensic Genetics for different purposes: paternity testing, special problems of Medical Genetics, such as incest causing Autosomal Recessive Diseases, mixed stains and other biological samples in criminal events. The number of Y-chromosome polymorphisms is huge, but in fact, based on their characteristics and database collections, a well defined number of them is used in official protocols of Forensic Genetics International Community. Our Research Group gave a contribution to the European Database for Y-Chromosome STR [1], by using two different protocols, officially proposed. The "minimal" haplotype utilizes DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS385I/II polymorphisms, while an extended haplotype involves also the hypervariable locus YCAII (Mathias et al. 1994).

Kayser et al. [2] suggests to add other polymorphisms such as DXYS156XY, that was also investigated by Karafet et al. [3]. To improve the identification power of the first battery of Y-STR used in our laboratory, we set up a new duplex PCR system for the contemporary analysis of YCAII and DXYS156XY loci.

A new primer pair for the pentanucleotide DXYS156XY locus was designed (GenBank AF257078), to amplify this locus with the dinucleotide YCA-II marker. These primers have been designed on the basis of the annealing temperature, about 45°C. The amplicons are respectively 203–218 bp for DXYS156XY and 147–165bp for YCAII. The forward primers were labelled with a new IR molecule (IRDye™800, LICOR Inc.) and the detection was performed with an IR Automated Fluorescence DNA Sequencer (LICOR-4200).

A sensitivity study was also performed to evaluate the efficiency of this new duplex for stains analysis.

- [1] Roewer L, Krawczak M, Willuweit S, et. al. Online reference database of European Y-chromosomal short tandem repeat (STR) haplotypes. *Forensic Sci Int.* 2001 May 15;118(2-3):106–113.
- [2] Kayser M, Caglià A, Corach et al. Evaluation of Y-chromosomal STRs: a multicenter study. *Int J Legal Med* 1997;110:125–133.
- [3] Karafet T, de Knijff P, Wood E et al. Different patterns of variation at the X- and Y- linked microsatellite loci DXYS156X and DXYS156Y in human populations. *Hum Biol* 1998;70:979–992.

Address for correspondence:

Dr. Ugo Ricci, Genetics and Molecular Medicine Unit, University of Florence – Hospital "A. Meyer", Italy Via Luca Giordano, 13 50132 Florence Italy, Tel. 0039 555662942, e-mail: u.ricci@meyer.it

Y-Chromosome Haplotypes in an Albanian Population Sample

C. Robino, S. Gino and C. Torre

Laboratory of Criminalistic Sciences, Department of Anatomy, Pharmacology and Legal Medicine, University of Turin, Italy

Analysis of microsatellite systems located on the non-recombining portion of the human Y chromosome is a powerful tool in male identification and paternity testing. Y haplotypes can display a remarkable degree of population specificity, therefore detailed population data are required when using these markers in forensic studies.

In recent years Italy experienced a strong migration flow from Albania, as a consequence the number of cases of DNA analysis involving Albanians is constantly increasing.

Seven Y-chromosomal microsatellites (DYS19, DYS385, DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393) were studied in a sample of unrelated Albanian males (n = 70) residing in Italy, by means of two multiplex PCR reactions. Amplified products were identified by capillary electrophoresis on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

These preliminary results allowed to establish the core of a reference database of the distribution of Y-chromosome haplotypes in the Albanian population to be used in routine casework.

Albanians are, linguistically, clearly different from other Indo-European speaking populations, nevertheless both their genetic history and relationship with Balkan and Mediterranean populations were scarcely investigated in the past. As Y-chromosome information on these populations gathers, our data may prove themselves useful to molecular anthropology studies as well.

Dr. Carlo Robino, Dipartimento di Anatomia, Farmacologia, Medicina Legale, Lab. di Scienze Criminalistiche, Università degli Studi di Torino, C.so M. D'Azeglio 52, 10126 Torino, Italy, Tel. 00 39 (0)11 6707731; Fax: 00 39 (0)11 6707732; e-mail: carlo.robino@unito.it

Forensic validation of Y-chromosome STR polymorphisms in Italy: the GE.F.I. collaborative database

A. Caglià¹, S. Presciuttini², M. Alù³, A. Asmundo⁴, L. Buscemi⁵, L. Caenazzo⁶, E. Carnevali⁷, E. Carra⁸, Z. De Battisti⁹, F. De Stefano¹⁰, C. di Nunno¹¹, R. Domenici¹², A. Piccinini¹³, N. Resta¹⁴, U. Ricci¹⁵, and V.L. Pascali¹⁶

¹Lab of Immunohematology, Catholic University, Rome

²Dept. of Biomedicine, University of Pisa

³Dept. of Morphological and Forensic Sciences, University of Modena

⁴Institute of Legal Medicine, University of Messina

⁵Chair of Legal Medicine, University of Ancona

⁶Institute of Legal Medicine, University of Padova

⁷Chair of Legal Medicine at the Hospital of Terni, University of Perugia

⁸Dept. of Cellular Biology and Development, University of Palermo

⁹Institute of Legal Medicine, University of Verona

¹⁰Institute of Legal Medicine, University of Cagliari

¹¹Institute of Legal Medicine, University of Bari

¹²Chair of Legal Medicine, Dept. of Biomedicine, University of Pisa

¹³Institute of Legal Medicine, University of Milan

¹⁴Chair of Medical Genetics, Dept. of Internal Medicine, University of Bari

¹⁵Center of Medical and Molecular Genetics, Hospital "A. Meyer", Florence

¹⁶GE.F.I. president

Laboratories participating to the exercise were asked to type at least 50 unrelated subjects born in their region for eight pre-defined STR loci, namely DYS19, DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393, and DYS385. The organizing committee of the GEFI group (Forensic Geneticists of Italy) sent four blind control samples to each group (two single-profile stains and two admixtures, one male-male and one male-female, respectively). Laboratories were left free to use their preferred experimental procedures. Data from the groups that returned blind-test correct results were included in the database.

Thirteen forensic laboratories contributed a total of 1,176 males from 10 Italian regions, including 68.2% of the Italian population. Individual haplotype data are available on line, at <http://www.mclink.it/personal/MD1696/gefi/>. Data from different laboratories of the same region were pooled. The frequency of the most frequent haplotype in the entire sample ($n = 30$) was 0.026. This haplotype was the most frequent in 8 out of 10 regions, and is also the more frequent in Iberian and Center-European populations (IFYUG on-line database).

A low degree of heterogeneity was demonstrated among Italian regions at the frequencies of Y-chromosome haplotypes. A validated database of Y-chromosome haplotypes is now available for use in Court cases and in whatever forensic instance.

Y-chromosome variation and inter-haplotype mutational distances in 111 unrelated individuals from Tuscany, Italy

S. Presciuttini, C. Toni, I. Spinetti and R. Domenici

Dept. of Biomedicine, Section of Forensic Medicine, University of Pisa, Italy

A Y-chromosome STRs loci database has been prepared for the Tuscany (central Italy) population. Blood samples from 111 unrelated individuals were typed for the following 12 loci: DYS19, DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS439, DYS438, DYS385a, DYS385b. Three multiplex PCR reactions were performed, and the amplified fragments were separated using an ABI Prism 310 (PE Applied Biosystems).

106 haplotypes were unique, 1 haplotype was observed twice and another was observed thrice (or a total of 108 different haplotypes in 111 chromosomes). A comparison with another population sample from Northern Italy, typed at the same loci, was carried out.

We considered the inter-haplotype mutational distances, after excluding marker DYS385, since its dual product cannot be resolved into alleles of different loci. The number of unique haplotype dropped to 90 (81%), whereas 6 haplotypes were counted twice (12 chromosomes, = 11%), 1 haplotype thrice, and, finally, 1 haplotype was observed six times. We computed the mutational distances between all chromosomes paired to all others (6,105 pairs). The distribution of the number of mutational steps was included between 0 (identical haplotypes) and 19. The distribution was bimodal, with a first peak at $m = 4$ mutational steps and a second peak at $m = 11$; this suggests that the sample is composed by at least two clusters of haplotypes, both connected to a number of related haplotypes (small m), but separated one from the other by a large value of m . Interestingly, all haplotypes represented by more than one chromosome were separated by a small number of steps, indicating their probable derivation from the same ancestor haplotype.

Y Chromosome haplotypes for 9 STRs in Tobas, Amerindians from the North of Argentina

G. Berardi¹, U. Toscanini¹, E. Haas¹, L. Gusmao² and E. Raimondi¹

¹PRICAI-Favaloro Foundation (First Argentine Immunogenetics Centre) – Buenos Aires, Argentina

²IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Oporto, Portugal

The purpose of this study is to report the haplotype distribution for nine Y Chromosome STRs observed in the Toba population.

The Toba native community is geographically located in the North of Argentina. Their characteristic feature is that, as they live in an isolated area with almost no interaction with individuals outside their community, their cultural and social traditions remain intact.

We studied forty unrelated male individuals from this population for nine Y Chromosome STRs. DNA was extracted from blood samples which were frozen for almost ten years. The DYS389II, DYS389I, DYS390, DYS19, DYS393, DYS391, DYS434, DYS437 and DYS439 STRs markers were amplified in two PCR reactions: a tetraplex reaction for DYS391, DYS434, DYS437 and DYS439, and a pentaplex reaction for DYS389II, DYS389I, DYS390, DYS19, DYS393. PCR products were detected by silver staining of polyacrylamide gels. These 9 STRs allowed the definition of 18 different haplotypes. Differences were found in the most and least frequent haplotypes when compared to data from the Buenos Aires population. In our opinion, these data may be useful for anthropological and forensic studies.

Address for correspondence:

Prof. Dr. Eduardo H. Raimondi, PRICAI- Favaloro Foundation
Av. Belgrano 1782 – 1er Subsuelo
1093 – Buenos Aires – Argentina
TE: (5411) 4384 5400; FAX: (5411) 4383 1197;
e-mail: eraimondi@ffavaloro.org

A nomenclature for YCA II which is compatible to the ISFG guidelines for STR analysis

U. Schmidt¹, S. Lutz¹ and L. Roewer²

¹Institut für Rechtsmedizin, Albert-Ludwigs-Universität Freiburg, Germany

²Institut für Rechtsmedizin, Humboldt-Universität Berlin, Germany

YCA II is a highly informative dimeric y-chromosomal short tandem repeat (STR) (Mathias et al. 1994, Kayser et al. 1997). Combined with DYS19, DYS389 I, DYS 389 II, DYS390, DYS391, DYS392, DYS393 and DYS385 (= "minimal haplotype") it builds the so called "extended haplotype" as it was defined for the online Y STR Haplotype Reference Database (YHRD, available at <http://ystr.charite.de>). By additional typing of YCA II 20% more of the European Y chromosomes can be distinguished than by the minimal haplotyping alone.

So far there is no agreeable repeat based nomenclature for YCA II. This is probably due to the dinucleotide repeat structure PCR which typically produces a small amount of stutter products two (n-2) or even four (n-4) bases shorter than the corresponding main allele peak. As a consequence sequence analysis of PCR products lead to inhomogenous results.

By cloning of PCR products it was possible to separate "allelic" and "stutter" products. Sequencing of the inserts produced unambiguous results that lead to a consistent repeat-based nomenclature of YCA II. The sequenced samples contained all alleles typed so far in extensive population studies (Kayser et al. 1997) and an additional 135 bp PCR allele.

The repeat-based nomenclature is a prerequisite for further forensic application of this locus, databasing and evaluation (Gill et al., International Society of Forensic Genetics recommendations on forensic analysis using Y-chromosome STRs, in preparation). The revision of the nomenclature in the YHRD should be discussed.

Dr. med. Ulrike Schmidt, Institut für Rechtsmedizin,
Universitätsklinikum Freiburg, Albertstr. 9, D-79104 Freiburg,
Tel: 00 49 (0)761 203 6869, Fax: 00 49 (0)761 203 6858,
e-mail: ulischmi@ukl.uni-freiburg.de

Y-STR typing in forensic analysis

N. von Wurmb-Schwark, S. Petermann and R. Wegener

Institute of Legal Medicine, University of Rostock, Germany

Y-STR polymorphic markers have become an important method in forensic genetics for male identification, especially in rape cases and paternity testing. In those investigations the typing of Y-STRs can lead to more information and thus might be much more suitable than autosomal STR typing alone.

We have investigated the variability of Y chromosome polymorphisms (DYS390, *DYS391*, *DYS392*, *DYS393*, *DYS19*, *DYS385I* and *II*, *DYS389I* and *II* and *YCAII*) in a population sample ($n = 200$) from Mecklenburg-Vorpommern, North Germany. We present the distribution of the different alleles and the amplification/detection conditions for multiplexing and electrophoresis on the *AbiPrism310*.

Using the new established Y-STR typing we reinvestigated rape cases from the years 1999–2001 and repeated paternity analysis where the supposed father was not available.

The cases of rape were chosen under the following conditions: No suspect, sperm traces in vaginal swabs and/or other traces that derived from a male person and thus could possibly belong to the rapist.

We will show the limitation of Y-STR typing (e.g. detection threshold, degrades samples) and compare the information of Y-STR investigation to autosomal STR typing.

Address for correspondence:

Dr. Nicole von Wurmb-Schwark

Institut für Rechtsmedizin, Universität Rostock

St.-Georg-Str. 108, 18055 Rostock

Tel.: 0381/494-9911, Fax: 9902

e-mail: nicole.vonwurmb@med.uni-rostock.de

Comparison of two isolated "Hungarian" Population to Szegedian (Mixed) Population by Y-Chromosomes

Zs. Beer, K. Csete, T. Varga

Six Y-chromosome (*DYS19*, *DYS385*, *DYS389*, *DYS390*, *DYS392*, *DYS393*) short tandem repeat (STR) polymorph systems were typed in two isolated "Hungarian" populations. One of them is from Őrség (Southwest of Hungary populated by Székelys) and the other population is from Targu Mures (a Romanian town populated by Székelys). Székelys are known as a Hungarian people. The allele frequencies of this two populations were compared to the allele frequencies of the mixed Hungarian (Szegedian) population. The results of this comparison show that the Székelys are ethnically different than Hungarians. This is the first evidence that show difference between the Y-chromosome markers of Hungarian and Székely groups. By counting of genetic distance it was concluded that the population from Őrség is genetically closer to the Szegedian population than the population from Targu Mures.

Individual Difference in Drug Metabolism and Disposition: Toxicological Significance of Genotypes and Phenotypes of S-mephenytoin 4'-hydroxylase(CYP2C19)

J. Ikebuchi¹, M. Yamada², Y. Ogura³, Y. Yamamoto⁴, A. Nishimura⁴, K. Yamada³, K. Nishi⁴ and Y. Irizawa¹

¹Department of Legal Medicine, Faculty of Medicine, Tottori University, Japan

²Department of Human Life Science, Seibo Jogakuin Women's College, Japan

³Department of Biochemistry, Faculty of Medicine, Tottori University, Japan

⁴Department of Legal Medicine, Shiga University of Medical Science, Japan

The 4'-hydroxylation of S-mephenytoin has been shown to be mediated by CYP2C19. It is also important in the metabolism of a number of related hydantoins and barbiturates, as well as in that of structurally dissimilar drugs such as omeprazole, proguanil, and citalopram. As a result, large interphenotypic differences occur in the disposition of these drugs, which may affect their toxicity and efficacy. Therefore, we examined the relationship between genetic polymorphism of CYP2C19 and metabolism of omeprazole in order to assess the severity and to predict the outcome of poisoning for the forensic and clinical toxicology. In this study, we prepared the DNA samples from the blood of unrelated healthy Japanese, and developed a rapid and simple genotyping method using a polymerase chain reaction (PCR) based restriction fragment length polymorphism. Genotyping procedures for the identification of CYP2C19 were performed by PCR amplification with use of the allele-specific primers described by de Moraes et al. with minor modifications. PCR products were digested with the restriction enzymes, and were analyzed by polyacrylamide gel electrophoresis. CYP2C19 phenotypes were determined by measuring omeprazole and hydroxyomeprazole concentrations in the serum, collected at 2 hours after omeprazole ingestion, by high performance liquid chromatography described by Marinac et al. with minor modifications. Consequently, the genotypes observed were CYP2C19*1A (wild type: wt), CYP2C19*2(m1), and CYP2C19*3(m2). The omeprazole hydroxylation index of wild-type was -1.15, whereas hetero-type was -0.78, and homo-mutated type 1.22. The genotype of CYP2C19 correlated with the phenotype. These results proved that genotyping assays of drug metabolizing enzymes would play more important role in assessing the severity and predicting the outcome of poisoning for forensic and clinical toxicology.

Address for correspondence:

Dr. Jun Ikebuchi

Department of Legal Medicine, Faculty of Medicine, Tottori University, Nishimachi 86, Yonago 683-0826, Japan

Tel: (81)859 34 8030, Fax: (81)859 34 8076,

e-mail: ikebuchi@grape.med.tottori-u.ac.jp

Gender determination in highly degraded DNA samples

M. Zoledziewska and T. Dobosz

*Medical University, Institute of Forensic Medicine,
Mikulicza-Radeckiego 4, 50-368 Wrocław, Poland*

The main lesions of DNA occurs after death is hydrolytic modification (depurination) and followed this process the degradation to the short fragments (Lindhal 93).

The most useful and known strategy for gender estimation is amplification of short fragments of amelogenin gene 106bp = X, 112bp = Y of size (Sullivan et al 93). Whereas the high sensitive known sex -typing markers are DXZ3, DYZ1 (130bp, 170bp) based on centromeric alphoid repeats (Witt, Erickson 89) which can be almost compared to mtDNA sensitivity (5000 copies for DXZ3 locus and 10-10 000 copies for mtDNA). The known shortest marker used for Y-typing is a 93bp fragment of SRY gene (Santos 98).

After shortening of the X and Y amplified fragments the sensitivity increases but specificity decreases usually. The possibility of contamination of the PCR increases due to high sensitivity. The method chosen is the balance between those factors.

We shortened the fragment of amelogenin gene (exon 6) based on Stone et al. 96 publication. In this method flanked and amplified fragment of X and Y amelogenin gene 78 bp of length in which X and Y sequences differs in seven mutation sites, followed by ligation of oligonucleotides FAM and YOY labeled results in two gender specific products. The fragment we choose can be detect with use of SNaPshot method also when reverse primer is used as an extend and terminate primer. This mean that we can perform single amplification and then a two ways detection. The results were analysed by capillary electrophoresis on ABI 310 Genetic Analyser, with ROX 350 as an internal standard of migration (Applera).

The gender is an valuable information for anthropologists and archeologists especially. The ligation technique of amelogenin fragment and SRY 93 marker gave as good results for 3 – 4 thousand years old bones (Neolithic) from South East Poland as well as for forensic samples in variable ages.

Ultimate shortening of the PCR product in the STR system TH01 – a new perspective in testing of decay forensic samples

A. Lebioda, M. Zoledziewska, E. Kowalczyk, A. Jonkisz and T. Dobosz

*Medical University, Institute of Forensic Medicine,
Mikulicza-Radeckiego 4, 50-368 Wrocław, Poland*

The microsatellite TH01 system was flanked with a new designed primers as short as possible. One from the primers was 6FAM labeled. Analysis of PCR products was performed using capillary electrophoresis with ABI PRISM 310 genetic analyser. The own made allelic ladder contained alleles 6, 7, 8, 9, 9.3 and 10 from 69 to 85 bp of range. 111 samples were examined with use of designed primers and AmpFISTR SGM Plus Kit. The range of TH01 allelic ladder from wide used SGM Plus test is in comparison from 172 to 188 bp.

The obtained results was exactly the same in both tests. The possibility of amplification and analysis as short polymorphic fragments increases the chance of positive DNA-typing from authentic decayed forensic samples.

Mitochondrial DNA Analysis of Ancient Human Teeth from a XVIth Century Archeological Site

A. Hernandez¹, I. Mamely², J.A. Cuellas¹, C. Rodriguez²

¹Instituto Nacional de Toxicología, Delegación de Canarias, Spain

²Instituto Canario de Bioantropología, Cabildo de Tenerife, Spain

Our laboratories are involved in a large-scale study whose purpose is to contrast the hypothesis of Canary Islands peopling, by means of physic-anthropological, genetical and archeological approaches.

The main problems associated with ancient DNA studies are the low yield, the high fragmentation and the contamination with exogenous DNA. In this study we tried to evaluate the viability of our laboratory setup to accomplish mitochondrial DNA isolation and analysis from ancient samples. We present preliminary results of HVI region mtDNA sequencing of several teeth from the Monastery of Los Silos (North of Tenerife Island), an archeological site dated back in the XVIth century, used as graveyard for some time. We compared the mtDNA data obtained from this old population with contemporary mitochondrial lineages, as a preliminary study of the genetic diversity origin in the present Canarian population

We also make some technical considerations about the DNA yield by comparing two variants in the isolation method, and about the mtDNA amplification by comparing two different PCR strategies.

The hand of Lunow – verification of an ancient tale using DNA analysis

M. Klintschar, M. Kleiber

Institut für Rechtsmedizin, Universität Halle-Wittenberg, Germany

In the church of Lunow, a small village in the north-east of Germany, a mummified hand is kept in the sanctuary. According to an ancient tale, the hand belonged to a man who lived in the village some centuries ago: After having been a good father and husband for many a year a strange disease killed his wife and nearly himself. After having recovered from the disease it was as if he had got possessed by the devil: He started drinking and did not look after the farm anymore. When his father reminded him of his duties, the drunken man attacked him with his fists and hurt him badly. When his sister realized what he had done, she cursed him. The man left his house and was never again seen alive: the next morning he was found lying dead in the street. The father of the deceased man survived his ill-begotten son for some weeks only. His daughter buried the father to the right of his son. The next day, when she came to attend to the graves she noticed with terror that the hand of her brother, which had hurt his father so badly when being alive, reached out of the grave. Several attempts to bury the hand were without avail: Every next morning the villagers found the hand of the evil man reached out of his grave. The villagers got afraid and decided to chop the hand off the arm of the deceased. After having accomplished that the horror was over and the villagers could live on peacefully. The hand was kept in the sanctuary of the church, where it is still exhibited as a warning example for the youth.

It is out of question that this ancient legend is beyond scientific reasoning. In the present case however, one additional aspects made the story even less believable: The mummified hand is very slim and could very well be of female rather than male origin. The parson of the community thus approached us and we agreed to try a gender determination of the hand. We were nevertheless not allowed to use bone and had to confine our study to the mummified soft tissues. We therefore extracted 1g of dried muscle using the QIAamp tissue kit (Qiagen, Hilden). Using PCR for the amelogenin locus, the recovered DNA was sufficient to confirm the male origin of the mummified hand. We were therefore able to confirm at least one aspect of an ancient tale of horror.

Address for correspondence:

*Michael Klintschar, Institut für Rechtsmedizin,
Martin Luther Universität Halle-Wittenberg, Franzosenweg 1,
D-06112 Halle(Saale), Germany
Tel. 0049 345 557 1833; Fax. 0049 345 557 1587;
e-mail: michael.klintschar@medizin.uni-halle.de*

Preliminary Molecular Study of Time-Dependent Changes in DNA-Stability in Soil-Buried Skeletal Material

*B. Bachmeier¹, Ch. Kaiser¹, A. Nerlich², R. Penning¹,
W. Eisenmenger¹ and O. Peschel¹*

¹*Institut für Rechtsmedizin and*

²*Pathologisches Institut, Ludwig-Maximilians-University, München, Germany*

The postmortal stability of DNA in various human remains is a major prerequisite for the further identification of individuals. While up to now there exist innumerable studies on these applications in forensic medicine, far less is known about the postmortal decay of the human DNA and its stability over the time. In particular, only few analyses have dealt with the stability of the ancient DNA (aDNA) in human skeletal material that has been entombed for several years. This approach is of particular interest, since any time-dependent destruction of the aDNA offers potentially a new marker for the estimation of the age of any bone sample of unknown age.

In our present study, we used up to now long bones from 7 individuals with known time of death. This material had been obtained from exhumations for medico-legal reasons (one case, 2.5 years postmortem) or during opening and transfer of previous burials (2 cases each 8 years postmortem, and one case 15, 18, 30 and 34 years after death). The skeletal material had been buried in cemeteries of Southern Germany and there was no evidence for major differences in the burial practises. From each of these long bones, a complete transversal section was cut exactly at the center of the diaphysis. This complete section was then divided into three distinct zones: the outer third occupying the part of the slice close to the bone surface, the middle third and the inner third which is close to the marrow-zone. From each zone, the aDNA was extracted using the commercial Genial First DNA method with subsequent phenolization (GEN-IAL, Institut für angewandte Laboranalysen, Troisdorf, Germany). The extracted aDNA was then used to amplify three segments of the b-actin-gene of 150, 507 or 763 base pair length. The gene segments were selected for specificity of the human reaction product so that any cross-reaction with bacterial aDNA could be excluded.

Using this approach, we detected generally significantly better results in the inner and middle zone of the transverse sections, while the aDNA extracted from the outer third of the slices contained either less aDNA or that of worse quality. The latter fact was also seen by the presence of significantly more non-specific bands in the outer third material than in those from the inner or middle thirds. These observations provide substantial evidence that the preservation of aDNA in human bone samples is different in various microanatomic settings. There seems to be better conservation of aDNA in the more central bone regions. Possibly, the outer third is more affected by bacterial aDNA overgrowth. Furthermore, we detected significant differences in the presence of amplifiable aDNA with differing age of the samples. While a specific amplicon was seen for the 150 bp β -actin-product in all individuals tested (even in that 34 years old), the 507 bp b-actin-product was identified in the 2.5 up to 18 year old material and that of the 703 bp β -actin-product only in the 2.5 and in one of the 8 year old cases.

Although our data are as yet preliminary and deserve further investigation by a larger group of samples, we present initial evidence that the testing for the length of aDNA may provide significant information on the postmortal age of a long bones sample. This seems to be based on a gradual destruction of the aDNA after death. Furthermore, we present interesting data indicating that the stability of the aDNA is different depending on the microanatomic location of the biomaterial.

STR analysis of artificially degraded DNA – results of a collaborative European exercise

P.M. Schneider¹, K. Bender¹ and the European DNA Profiling (EDNAP) Group²

¹Institute of Legal Medicine, Johannes Gutenberg University, Mainz, Germany

²Working Group of the ISFG (www.isfg.org)

Degradation of human DNA extracted from forensic stains is the result of a natural process due to the exposure of the stain samples to the environment. Once the average DNA fragment length is reduced to sizes smaller than 300 bp, a loss of genetic information occurs due to the lack of suitable template DNA and the subsequent failure of STR systems to generate any useful or reproducible result. Experience with degraded DNA from casework samples shows that every sample may exhibit different properties in this respect, and that it is difficult to systematically assess the performance of routinely used typing systems to analyze degraded DNA samples. To learn more about the efficiency of STR systems, a standardized reference sample of degraded DNA in sufficient amounts could be quite helpful. A large batch of high molecular weight genomic DNA was prepared at the Institute of Legal Medicine in Mainz from the two human cell lines The DNA samples were degraded under standardized conditions to an average fragmentation size of less than 200 bp, using a combination of physical and biochemical methods, i.e. sonication and treatment with DNase I

Participation was offered to 50 forensic laboratories across Europe, and results were returned from 37 laboratories from 15 countries. The majority of results was obtained using the ABI SGM Plus kit, but other multiplex STR kits such as Profiler/Profiler Plus and PowerPlex were also applied. The results were assessed according to correct allele detection, peak height and balance as well as the occurrence of artifacts and allele dropouts.

A number of common problems were identified based on these results. A strong peak imbalance was observed in heterozygous genotypes in particular for the larger STR fragments and for PCR conditions applying more than 28 cycles. Artifact signals ("pull-ups") occurred due to over-amplification mimicking alleles not present in the sample. Allelic dropout (i.e. the complete loss of one allele in a heterozygous genotype) occurred frequently, and sometimes the smaller of two alleles at a given locus was affected. The use of the Genotyper software for automatic data analysis sometimes prevented the detection of an allele due to the threshold settings of peak height detection, but also prevented the inclusion of artifact bands in some cases.

Based on these observations, strategies can be developed to overcome these problems. These include a careful balance between the amount of template DNA and the PCR cycle numbers, the reaction volume and the amount of Taq polymerase. Furthermore, a careful visual inspection of results both from fragment analysis and from Genotyper is necessary to identify and exclude artifacts.

(The present study was supported by contract SMT 97-7506 from the European Commission in the context of the STADNAP Network Initiative.)

Dr. Peter M. Schneider, Institut für Rechtsmedizin, Am Pulverturm 3, D-55131 Mainz, Tel. +49 (6131) 3932687, Fax +49 (6131) 3933183, e-mail: pschneid@mail.uni-mainz.de

Evaluation of Powerplex™ 16 for typing of degraded DNA samples

B. Glock¹, R.B.K. Reisacher¹, S.O. Rennhofer¹, D. Tröscher¹, E.M. Dauber and W.R. Mayr^{1,2}

¹Laboratory for Molecular Biology, Blood Donation Center of the Austrian Red Cross for Vienna, Lower Austria and Burgenland, Vienna, Austria

²University of Vienna, Medical School, Clin. Dept. for Blood Group Serology, Austria

Introduction

We used the new STR typing kit Powerplex™ 16 on whole blood samples as well as on samples of "cellfree" samples (plasma, fresh frozen plasma = FFP) in order to optimize the reaction conditions and to evaluate the performance of the kit. Subsequently results of pairs of retention samples of blood donations and the corresponding whole blood samples were compared taking into account the optimized Powerplex™ 16 conditions and our previously modified SGM Plus protocol.

Materials and Methods

Optimization of Powerplex™ 16 reaction conditions was either done on recent whole blood samples or on archive plasma samples. Pairs of retention samples (plasma) and the corresponding whole blood samples, which were assumed to be degraded after storage at low temperatures for 1 to 3 years, were used for comparison between the two kits. DNA extraction was carried out on 10µl whole blood or 200 µl plasma using Qiagen DNA Blood Mini kit® employing a modified protocol. Amplification and detection were performed as shown below.

Results and discussion

	Powerplex™ 16			AmpFISTR SGM Plus®	
	Samples	Conditions	Sample Volume	Conditions	Sample Volume
Amplification	Whole Blood	according to protocol	0.5 µl input-DNA in 12.5 µl	protocol + 2 cycles	1 µl input-DNA in 10 µl
	Plasma, FFP	protocol + 2 cycles	10 µl input-DNA in 25 µl	protocol + 2 cycles	20 µl input-DNA in 50 µl
Detection	Whole Blood	2 seconds of injection	0.5-1.0 µl	2-5 seconds of injection	0.5µl
	Plasma FFP	25 seconds of injection	1 µl-2 µl	15-25 seconds of injection	1.5 µl

In a sizing precision study with 10fold injections each of an amplified whole blood sample and the allelic ladder, standard deviations for the different loci varied between 0.0 and 0.10 bp.

Both kits performed well with non-degraded DNA samples, but showed drop out of loci with larger fragment length (> 250 bp) at varying extents with degraded DNA samples. Unfortunately this included the two highly discriminating pentanucleotide loci Penta E and D of the Powerplex™ 16 kit.

Conclusion

Inclusion of further loci, which show amplification products smaller than 250/300 bp, into STR multiplex kits or combination of two different kits might help to increase the information obtainable on degraded DNA samples.

Address for correspondence:

Dr. B. Glock, Laboratory for Molecular Biology, Blood Donation Center of the Austrian Red Cross for Vienna, Lower Austria and Burgenland, Nordportalstrasse 248, A-1020 Vienna, Austria; e-mail: glock@redcross.or.at

Parentage Testing Following an Infanticide Case using Fetal DNA from Archival Fixed Tissues

Ligia Barbarii, D. Dermengiu, Daniela Maxim, Simona Toroiman and D.T. Stefanescu

Institute of Legal Medicine "Mina Minovici", Bucharest, Romania

An infanticide case happened in a small town in January 2000 has been investigated after the dead body of a new-born child was found in a plastic bag in a dustbin. The police investigations and the medical forensic examinations have indicated, without succeeding to prove it, a young woman and her lover, both living in the neighbourhood of the town as the putative parents, and also the woman's husband as the child murderer. DNA analysis was conducted an year later in an attempt to provide independent evidence of the parents identity. DNA typing with PCR using STR provided a reliable method for parentage testing. Fetal DNA was successfully extracted from paraffine embedded tissues proceeded from the autopsy and also from the blood stains from the plastic bag. After some failed attempts and despite the presence of a potent inhibitor of DNA amplification, the microsatellite alleles from the archival fetal DNA could be analysed. Comparison of the fetal DNA with DNA from the putative mother and her husband revealed a 9 loci genotype compatible with both the maternity and the paternity of the fetal remains.

Using SLP's-STR the biostatistical calculations for inclusion could be carried out and very informative Essen-Moler values for the probability of the maternity and the paternity were obtained.

2000), and the tool of choice for diagnosing matrilineal relationships. A number of scientists have studied the mtDNA of Basque populations. Bertranpetit et al., 1995 and Côtte-Real et al., 1996 analyzed the base sequences of the hypervariable segment I (HVS I). All of these studies were based on an underlying assumption of homogeneous population structure in the autochthonous Basque group. The D-loop regions analyzed in this study were the hypervariable segment I (HVS I) and the hypervariable segment II (HVS II) in two autochthonous population samples separated linguistically and geographically (Arratia and Goiberri). Since the HVS II segment has not been previously studied in samples from the Basque Country, its analysis in these populations will provide better information on the variability of mtDNA in the autochthonous Basque population and its usefulness in forensic casework.

Objective

To determine the homogeneity or heterogeneity of mtDNA in the autochthonous population of the Basque Country and to use the information contained in the sequence variations of the mtDNA of these fragments to analyze their phylogenetic relationships with other population groups. To find the power of discrimination between maternal lineages using the combined analysis of HVS I and HVS II segments in the autochthonous population of the Basque Country.

Methodology

We studied sequences of the HVS I and HVS II segments from 55 autochthonous Basques from the Arratia and Goiberri regions. The DNA was extracted from peripheral blood leukocytes using the phenol-chloroform method. The HVS I and HVS II segments were amplified using the primers L15996 and H16401 and L29 to H408 respectively (Vigilant et al., 1991). The M13 segment was added to these primers to sequence the PCR products using the BigDye primer kit (Applied Biosystems). Nucleotide substitutions and insertions/deletions were found using Anderson's reference sequence, followed by computation of match probability and discrimination power values. The genetic structure of the population samples was analyzed by calculating nucleotide diversity, gene diversity and mean number of pairwise differences. The phylogenetic analysis was made following classification into haplogroups previously established for the HVS I segment and after making a new classification to group the haplotypes of the HVS II segment. Genetic distance D was calculated (Rao, 1982; Nei, 1987) yielding phylogenetic trees. To evaluate the capacity of the haplogroups obtained from the combination of HVS I and HVS II segments to discriminate racial groups within populations, discrimination analysis was performed revealing the haplogroups that are most effective in differentiating populations.

Results and conclusions

No genetic substructuring was observed in the two population samples studied. These results are sufficiently indicative of the lack of heterogeneity in the maternal lineages of the autochthonous Basque population. The sequence diversity observed is similar to that of other Caucasoid populations, meaning that the power of discrimination value is also similar. Finally, phylogenetic analyses place the Basque populations among the Caucasoids, without providing evidence for differential characteristics in the mtDNA of this population.

Address for correspondence:

M. M. de Pancorbo, Dpto. de Z.y Dinámica Celular,
Facultad de Farmacia, Universidad del País Vasco.
Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain.
Tel. 00 34 945 013045; Fax: 00 34 945 013756;
e-mail: gcpmagom@lg.ehu.es

Genetic structure of autochthonous Basques through analysis of the HVS I and HVS II regions of mitochondrial DNA

C. Martínez-Bouzas¹, A. Castro^{2,3}, I. Fernández-Fernández^{2,3}, J.L. Rodríguez-Filgueira¹ and M. M. de Pancorbo^{1,3}

¹Dpto. de Z. Dinámica Celular A., F. Farmacia, U. País Vasco, Vitoria-Gasteiz, Spain

²DataGene, Sondika (Bizkaia) Spain

³Instituto Vasco de Criminología, U. País Vasco, San Sebastián-Donostia, Spain

Introduction

Mitochondrial DNA (mtDNA) analysis has proved to be of great interest in human population studies for analyzing phylogenetic relationships and differentiation among individuals (Harpending et al., 1993). mtDNA differs from nuclear DNA in: absence of recombination, maternal inheritance, large number of copies per cell and a mutation rate that is 5 to 10 times higher than that of nuclear DNA (Brown et al., 1979; Ferris et al., 1981).

The non-coding region of mtDNA, known as the D-loop, accumulates many more mutations than the rest of the molecule, making it a useful tool for studying short-term evolutionary phenomena (Varesi et al.,

Mitochondrial DNA Polymorphism in 50 unrelated individuals from North-Italy

L. Caenazzo¹, E. Ponzano¹, S. Presciuttini², P. Arslan³ and P. Cortivo¹

¹Dept. Medicina Ambientale e Sanità Pubblica – University of Padua, Italy

²Dept. Biomedicina – University of Pisa, Italy

³Dept. Sci. Med. e Ter. Diagnostiche – University of Padua – Padua, Italy

The two hypervariable regions HV1 and HV2 of mitochondrial DNA (mtDNA) are becoming more commonly used in forensic applications to differentiate among individuals in a population. The statistical interpretation of the results depends on the population frequency of a particular sequence, or haplotype. For this reason, it is essential to determine the haplotype frequency distribution of HV1 and HV2 in the mtDNA in any population of interest.

We obtained sequence data from 50 unrelated individuals living in Veneto. HV1 and HV2 regions were amplified separately, and the amplification products were sequenced using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystem) on both strands. Analysis was carried out by capillary electrophoresis on an ABI Prism 310 (Applied Biosystem). Sequences were then compared to the Cambridge Reference Sequence using the Sequence Navigator Software.

A total of 48 different mtDNA lineages was revealed, of which 2 were shared by 2 individuals. The sequenced regions included a total of 717 bp, among which 97 nucleotide positions were polymorphic, 70 in HV1 and 27 in HV2, respectively. Polymorphisms were due to base transitions rather than transversions. Some haplotypes showed insertions of 1–2 bps, whereas no deletions were found. Based on the number of unique haplotypes and the number of polymorphic sites, the HV1 region resulted more discriminating than HV2.

L. Caenazzo Dept. Medicina Ambientale e Sanità Pubblica – Medicina Legale Via Falloppio, 50, 35121 Padua – Italy
Tel.: +390498272234 Fax: +390498272214
e-mail: luciana.caenazzo@unipd.it

D-Loop-BASE is online

H. Wittig¹, M. Koecke², K.-U. Sattler² and D. Krause¹

¹Institute of Forensic Medicine

²Institute of Technical Information Systems, Magdeburg Otto-von-Guericke-University, Germany

At the 18th Spurenworkshop held in Magdeburg in 1998 fifteen university institutes of forensic medicine from Germany, Austria and Switzerland agreed to establish a Central-European database of forensically secured mtDNA sequences as a common project at the Magdeburg institute. Since then more than 1600 sequences have been included into the D-Loop-BASE data stock which is now a profound basis for both frequency inquiries for expert opinions and scientific investigations into population genetic matters. Due to the consent agreement signed by the institutes involved it was not possible in the beginning to access the data via the Internet which made it difficult for all parties interested to use this database. Among the number of mtDNA databases in the Internet there has been only one to date offering access to its data which, however, could be used for forensic purposes only to a limited extent.

Together with the Institute of Technical Information Systems at the Magdeburg Otto-von-Guericke-University and the database administration an Internet interface was developed which is available now under www.d-loop-base.de.

Online inquiries for both the frequency of individual sequences and biostatistical parameters are possible now. But individual sequences will not be published. This paper shows the opportunities and limits of data inquiries.

Address for correspondence:

Dr. med. Holger Wittig,
Institut für Rechtsmedizin,
Otto-von-Guericke-Universität Magdeburg,
Leipziger Str. 44,
D-39120 Magdeburg
Tel. +49 391 67 15 812; Fax: +49 391 67 15 810;
e-mail: loop@d-loop-base.de

Sequencing of Mitochondrial HV1 and HV2 DNA With Length Heteroplasmy

E.M. Rasmussen, B. Eriksen, H.J. Larsen, E. Sørensen and N. Morling

*Department of Forensic Genetics,
Institute of Forensic Medicine, University of Copenhagen, Denmark*

Introduction

Sequencing of mitochondrial DNA (mtDNA) with length heteroplasmy due to poly C/G stretches (1) in general give ambiguous results in the DNA region 3' of the homopolymeric region. Molecular cloning of single DNA fragments followed by sequencing may be used in cases with length heteroplasmy (2). These methods are, however, time consuming and presently not of practical use in forensic casework. We have sequenced the DNA regions 3' of two homopolymeric mtDNA regions in HV1 and HV2 using PCR and sequencing primers placed at the junctions between the homopolymeric regions and the 3' parts of both the H and L strands.

Materials and methods

DNA was extracted from blood with phenol/chloroform from 15 samples with length heteroplasmy. Six samples showed heteroplasmy in the homopolymeric region in HV1 consisting of poly C/G beginning in position 16,184, eight samples showed heteroplasmy in the homopolymeric region in HV2 consisting of poly C/G beginning in position 303, and one sample showed length heteroplasmy in both regions. For each region, junction primers complementary to the junction between the homopolymeric region and the 3' part of the DNA strands were designed for both the H- and L-strands. The reverse primers were those used for the initial amplification of HV1 and HV2, respectively. PCR-products were purified by MicroSin S-300 HR columns (APB) and sequenced using the Applied Biosystems BigDye Primer and Terminator Cycle Sequencing kits. The extension products were analysed on either ABI 377- or ABI 310-sequencer.

Results and discussion

Sequencing of DNA regions 3' to the homopolymeric regions with length heteroplasmy with junction primers gave unambiguous sequencing results in both directions in all 32 sequences from the 15 heteroplasmic samples. Thus, the use of the junction primers described here facilitates both forward and reverse sequencing in samples with length heteroplasmy in a way so fast and simple that it may be used in forensic casework.

References

1. Bendall KE, Sykes BC. Length heteroplasmy in the first hyper-variable segment of the human mtDNA control region. *Am J Genet* 1995; 57: 248–256.
2. Lutz S, Weisser H-J, Heizmann J, Pollak S. Mitochondrial heteroplasmy among maternally related individuals. *Int J Legal Med* 1999; 113: 155–161.

Address for correspondence:

Erik Michael Rasmussen, Assistant Professor, Ph.D., Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, 11 Frederik V's Vej, DK-2100 Copenhagen, Denmark, Tel.: +45 35326222, e-mail: michael.rasmussen@forensic.ku.dk.

Mitochondrial DNA polymorphisms in Polish Caucasians – population data and family heteroplasmy

T. Grzybowski, J. Bednarek, M. Wozniak, J. Czarny and D. Miscicka – Sliwka

Forensic Medicine Institute, The Ludwik Rydygier Medical University in Bydgoszcz, Poland

Mitochondrial DNA control region sequences were determined in 430 unrelated Polish Caucasians from the Pomerania – Kujawy region of Poland for both hypervariable regions 1 (HV1) and 2 (HV2). The 215 variable positions found (140 in HV1 and 75 in HV2, respectively) resulted in 326 different haplotypes with a genetic diversity value of 0.996. The most common haplotype in Poles (263, 315.1C, found at frequency of 4,65%) is also the most common sequence in other European Caucasians. During direct sequencing of the HV1 and HV2 in 420 individuals, one person with heteroplasmic point mutation at 16093 (C/T) was encountered. This position is commonly reported as a "hot spot" of heteroplasmy. Extended study was performed on the maternal lineage where this heteroplasmy was observed. The family has three generations and consists of 16 members. DNA samples from blood, buccal swabs and multiple hairs were typed from these individuals. Sequence results showed a rapid segregation of mtDNA variants across generations. For instance, out of ten offspring of a heteroplasmic mother, two were nearly fixed for her minority mtDNA variant. Moreover, the results revealed highly variable patterns of segregation of the sequence variants between different tissues of the same individual. Out of 16 family members studied, 11 had 16093 heteroplasmy in blood and 12 shared this heteroplasmic mixture in buccal swabs, although at considerably different ratios. Interestingly, almost all individuals (except one) were fixed for 16093T in multiple hairs studied. These observations are consistent with a very narrow bottleneck that is commonly proposed as a mechanism of differential segregation of mtDNA variants across generations.

Analysis of mitochondrial DNA with an Infrared Automated DNA Sequencer in a Tuscany Population (Central Italy)

L. Giunti, U. Ricci, I. Sani, S. Guarducci, A. Brusaferrri, A. Lasagni and M.L. Giovannucci Uzielli

*Genetics and Molecular Medicine Unit,
University of Florence – Hospital "A. Meyer", Italy*

The non-coding region of the mtDNA provides a very attractive target for human forensic identification studies. This is especially true in difficult situations, such as reduced amounts of biological samples (hair shafts), or degraded DNA (combusted bodies, exhumed old corps, etc.). In these cases, the d-loop analysis may represent the only one way to get the individual identification. The hypervariable regions HVI and HVII are classically analysed because their high mtDNA variability content. Due to this characteristic, it is of the utmost importance to know the frequencies distribution of the various haplotypes of both hypervariable mtDNA regions, HVI and HVII, in defined populations. In our laboratory, we performed the study of the *d-loop* regions, in 100 Italian unrelated individuals, natives of Tuscany region (Central Italy), by using an IR-based Automated DNA Sequencer (LI-COR 4200). In the first step of our study, we adopted the strategy proposed by Holland et al. [1], Pfeiffer et al. [2] e Szibor et al. [3], especially devised for the analysis of C-stretches sequences.

In a second time, taking advantage of the *long read* characteristics of the IR Automated Sequencer, we adopted a new strategy based on a single fragment Amplification and Sequencing, including the entire HVI and HVII region.

The PCR products are generated by using the same Forward Primer for the HVI region (F15971) with the Reverse Primer for the HVII region (R448), designed by Holland et al. [1]. The PCR product, 1046 bp long, was evaluated from a quantitative point of view on an agarose gel. The sequencing reactions mix are prepared by using the "Thermo Sequenase DYEnamic Direct cycle sequencing kit" or the "Thermo Sequenase Fluorescent labelled primer cycle Sequencing kit with 7-deaza-dGTP" (Amersham Pharmacia Biotech). The Sequencing process was performed on both, Forward e Reverse directions, by using a pair of appropriate detection Primers (F15989 and R381) [1], 5' labelled with the new molecule IRDye™800 (LI-COR Inc.). For the identification of sequences we used the dedicated Image Analysis computer program.

The FASTA format was obtained by using the Chromas program, while the landmark sequence of Anderson [4] was employed for the alignment of sequences, with the Clustalw program (EMBL).

- [1] Holland MM, Fisher DL, Roby RK, Ruderman J, Bryson C, Weedn VW, Mitochondrial DNA sequence analysis of human remains. *Crime Lab Digest* 1995;22:109–115.

- [2] Pfeiffer H, Brinkmann B, Huhne J, Rolf B, Morris AA, Steighner R, Holland MM, Forster P, Expanding the forensic German mitochondrial DNA control region database: genetic diversity as a function of sample size and microgeography. *Int J Legal Med* 1999;112:291–298.
- [3] Szibor R, Michael M, Correct mitochondrial L-strand sequencing after C-stretches. *Int J Legal Med*. 1999;112(5):348–9.
- [4] Anderson S, Bankier AT, Barrell BG, De Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Scheier PH, Smith AJH, Staden R and Young IG. Sequence and organization of the human mitochondrial genome. *Nature* 1981;290:457–464.

Address for correspondence:

*Dr. Ugo Ricci, Genetics and Molecular Medicine Unit,
University of Florence – Hospital "A. Meyer", Italy*

*Via Luca Giordano, 13 50132 Florence Italy, Tel. 0039 555662942,
e-mail: u.ricci@meyer.it*

mtDNA Control Region Polymorphism: Sequence Database and Forensic Applications

A. Rodríguez-Monje, M. Montesino, L. Prieto, E. Rivas and E. García.

Scientific Police. DNA Laboratory. Madrid. Spain.

Since 1998, the Spanish Scientific Police has been analyzing both hypervariable segments (HVR1 and HVR2) of the mitochondrial control region by sequencing analysis. During this period, different sample types (e.g.: blood, saliva, telogen hairs, hair shafts, nails, tissues, bone and dental remains) related to murders, sexual assaults, robberies and identifications of human remains have been studied.

Some problematic findings detected in forensic casework routine involving sequence heteroplasmy, point mutation in primer annealing region and one single nucleotide mismatch between forensic specimen and reference sample are described in this paper. Given that a proper statistical evaluation of the results by using a large database is needed samples from 120 individuals living in Spain have been analysed. Sequence outcomes related to this study are presented in this poster.

Ana Rodríguez-Monje.

Comisaría General de Policía Científica.

Servicio de Analítica.

Laboratorio de ADN. C/Julián González Segador s/n°. 28043 Madrid. España.

Tel. 00 34 91 5822321. Fax. 00 34 91 5822541.

e-mail: erivas@dgp.mir.es

Population data of mitochondrial DNA region HVIII in 150 individuals from Bologna (Italy)

C. Bini, S. Ceccardi, C. Colalongo, G. Ferri, M. Falconi, S. Pelotti and G. Pappalardo

Department of Medicine and Public Health, Section of Legal Medicine, University of Bologna, Italy

Recently S. Lutz et al. discovered within the control region of mtDNA a third hypervariable region located at position 438 to 574. They demonstrated that one fifth of all sequences analysed that were identical in the HV1 and HV2 regions could be distinguished by this additional analysis, so the sequence of this region may be helpful in forensic investigations. Nevertheless it is essential for identification purpose to possess a database containing sequences of a given population.

MtDNA sequence variations at HVIII region in 150 unrelated Italian individuals from Bologna were analysed in order to identify polymorphic positions and to determine their frequencies. Sequence of both strands was performed to reduce ambiguities in sequence determination as recommended by DNA Commission of the International Society for Forensic Genetics.

A comparison of our sequence data with the Anderson sequence showed that nucleotide substitutions predominate over insertions and deletions. Among nucleotide substitutions the most frequent was a T-C transition. Deletions and insertions at CA repeat at positions 514–523 and an insertion of four bases at position 368 were observed.

Prof. Dr. Med. Giuseppe Pappalardo,
Department of Medicine and Public Health,
Section of Legal Medicine, University of Bologna, via Irnerio 49,
40126 Bologna, Italy, Tel 00 39 (0)51 243250;
Fax: 00 39 (0)51 248896, e-mail: labemat@medleg.unibo.it

Polymorphism on D-Loop Mitochondrial DNA: Study of HV1 and HV2 Regions in unrelated Individuals Living in the East of France

V. Troesch¹, I. Clisson^{1,2}, M. Petraud¹, B. Ludes² and E. Jaeck-Brignon¹

¹Laboratoire CODGENE, STRASBOURG, FRANCE

²Institut de Médecine Légale, Université Louis Pasteur, STRASBOURG, FRANCE

Sequence analysis of mitochondrial DNA (mtDNA) and particularly its two hypervariable regions HV1 and HV2 is a very useful tool in forensic genetics and human population studies. For samples containing insufficient DNA such as telogenic hair, bones, old and degraded material, analysis of mtDNA has a better success rate than nuclear DNA due to its greater copy number per cell.

The aim of this study is to present sequence polymorphism in both variable regions (HV1/HV2) from 70 unrelated french individuals living in the east of France in order to establish a database of genotype frequencies. This work extends a study performed previously by Rousselet et Mangin (1998)* on 50 french caucasian individuals. A number of polymorphism sites in HV1 and HV2 regions such as transversion, transition, insertion, deletion and heteroplasmy phenomena will be discussed. The results will be also compared to other Caucasian population databases.

This database will facilitate interpretation of results from mtDNA sequence analysis in forensic caseworks performed in laboratory. As mtDNA analysis usually give a better success rate for delicate evidence samples than nuclear DNA, the usefulness of this technique for exclusion and inclusion in some forensic caseworks will be also approached.

*Rousselet F. Mangin P. 1998. *Int. Legal Med.* 87: 99–116

Correspondence to:

Dr. Estelle Jaeck-Brignon, Laboratoire Codgene,
11 rue Humann, 67000 Strasbourg, France,
Tel. 33/3 90 24 31 67, Fax: 33/3 88 24 00 85,
e-mail: eb-codgene@wanadoo.fr

Mitochondrial DNA variability patterns in Southeast Africa and forensic implications

A. Salas, T. De La Fe, B. Sobrino, M. Lareu, A. Carracedo

Institute of Legal Medicine, Unit of Forensic Genetics, Faculty of Medicine, University of Santiago de Compostela, Galicia-Spain

The first hypervariable region and several restriction fragment polymorphisms in the mitochondrial DNA genome were investigated in 308 individuals belonging to 16 different populations from Southeast Africa, all of them Bantu speaking. A total of 30 available African populations (or with an important African influence) as well as other non-African ones were used for comparison. High diversity values were found for the samples analyzed in the present work in comparison with other African samples. Nucleotide mismatch distributions are rugged and multimodal, which can reflect the scenario of stationary populations rather than expansionary ones. Phylogenetic reconstruction allowed us to infer that the Southeast African populations are distant from other population groups, the ancient ones (Pygmies and !Kung) being the most closely related.

The present results are of special forensic interest because new mtDNA patterns were discovered, which increase our knowledge in mtDNA haplotypes.

Address for correspondence:

*M. Lareu, Institute of Legal Medicine,
Unit of Forensic Genetics, Faculty of Medicine, C/San Francisco,
15705. Santiago de Compostela. Spain
Tel. 00-34-981-582327, Fax: 00-34-981-580336,
e-mail: apimllar@usc.es*

mtDNA analysis in portuguese populations (Central Portugal and Azores Islands): polymorphic sites in control region sequences

M. Carvalho, C. Mendes, H. Antunes, M.J. Anjos, L. Andrade, V. Lopes, D.N. Vieira, M.C. Vide

National Institute of Legal Medicine, Forensic Genetics of Coimbra, Portugal

The polymorphism of the two hypervariable segments (HVI and HVII) of control region of mtDNA was analyzed in a population of 81 unrelated individuals from Central Portugal and 48 from Azores islands, using a fluorescent based electrophoresis sequencing method. Sequences have been obtained with ABI PRISM *Big Dye Terminator and dRhodamine Terminator Cycle Sequencing Ready Reaction Kit s, with amplitaq DNA polymerase FS*, and have been detected with ABI PRISM 377 DNA sequencer.

In our population data of 81 random individuals from Central Portugal we observed 69 polymorphic sites of sequence in HVI region and 44 in HVII region. Nucleotide substitution rather than insertion/deletion (one or two bp) was the majority of variation. The distribution showed a large bias towards transitional changes than transversional changes.

The results were compared with other Caucasian population.

*Dr. M. C. Vide, Instituto Nacional de Medicina Legal,
Delegação de Coimbra, Serviço de Genética Forense,
Largo da Sé Nova, 3000-213 Coimbra, Portugal.
Tel. 00 351 239 854 230; Fax: 00 351 239 820 549;
e-mail: mcvide@ci.uc.pt*

No heteroplasmy at base position 16169 of Tsar Nikolai II's mitochondrial DNA

T. Nagai^{1,2}, T. Okazaki^{1,2}, M. Shimadzu² and V. L. Popov³

¹Department of Forensic Medicine and Science, Kitasato University Graduate School of Medical Science, Japan

²Department of Clinical Hematology, Kitasato University School of Allied Health Sciences, Japan

³Regional Office of Forensic Medicine Examination, Saint-Petersburg, Russia

Peter Gill et al. (Nature Genetics, 6: 130–135, 1994) and Pavel L. Ivanov et al. (Nature Genetics, 12: 417–420, 1996) analyzed the DNA sequence of the hypervariable region (HVR) of mitochondrial DNA (mtDNA) extracted from Tsar Nikolai II's bone, and reported that there was a heteroplasmy (C/T) of cytosine (C) and thymine (T) at base position 16169.

In order to confirm their finding, we analyzed, using the usual method, the mtDNA sequences of Tsar Nikolai II's sweat stain, hair/nail/bone of Grand Duke Georgiy Alexandrovich (Tsar's brother), and a blood sample obtained from Tihon Nikolaevich Kulikovskiy Romanoff (son of Tsar Nicholas II's youngest sister, Grand Duches Olga Alexandrovna) before his death and stored at –80. The following four primers were used:

HVRI: F15989 (5'-CCCAAAGCTAAGATTCTAAT)

R16410 (5'-GAGGATGGTCAAGGGAC)

HVR: F29 (5'-CTCACGGGAGCTCTCCATCG)

R381 (5'-GCTGGTGTAGGTTCTTTG)

We obtained results similar to those reported by Peter Gill et al. and Pavel L. Ivanov et al., except that we did not find heteroplasmy (C/T) at base position 16169, the finding to which they attached the greatest importance in their identification test. The base at position 16169 was C in all samples used in our test. In addition, although we searched for T by restriction enzyme digestion using a primer into which a restriction enzyme recognizing site was introduced, *no T* was found in any of the samples tested.

These results lead us to the conclusion that Tsar Nikolai II' mtDNA has no heteroplasmy (C/T) at position 16169, but does have homoplasmic cytosine. Thus arises the question of whose bone was examined by Pater Gill et al. and Pavel L. Ivanov et al. Who is buried in the grave of the Peter and Paul Cathedral at St. Petersburg?

Finally, the assistance of Mrs. Olga Nikolaevna Kulikovskiy Romanoff, who kindly provided us with Tihon Nikolaevich Kulikovskiy Romanoff's blood sample, is gratefully acknowledged.

Variability of the mitochondrial loci nt 00073 and nt 16519 in populations of Germany, Syrian, Cameroon, Japan, Vietnam and Peru – a study utilizing RFLP- and light cycler technique

U. Szibor^{1*}, R. Schneider-Stock², C. Augustin³, M. Benecke⁴, S. Elias^{3,5}, S. Harada⁶, O. Landt⁷, Pham Hung Van⁸, K. Schmerbach^{1*} and R. Szibor¹

¹Institute for Legal Medicine, Otto-von-Guericke-University Magdeburg, Germany

²Institute for Pathology, Otto-von-Guericke-University Magdeburg, Germany

³Institute for Legal Medicine, University Hamburg, Germany

⁴International Forensic Research & Consulting, Cologne, Germany, forensic@benecke.com

⁵Dept. of Legal Medicine, University of Damaskus, Syria

⁶Institute of Community Medicine, University of Tsukuba, Japan

⁷TIB MOLBIOL, Berlin, Germany

⁸Institute of Medicine, University of Hanoi, Vietnam

*Students of the University of Rostock and Humboldt-University-Berlin, respectively

This study was carried out to evaluate markers which can aid discriminating between ethnic different populations. Such markers are needed in identification of skeletons as well as in general forensic case work. One further goal was to establish the Light Cycler™ technique for application in forensic mt-analysis.

We examined the mitochondrial (mt) loci nt 00073 and nt16519 which show a considerable variability in central European populations. The transitions L00073 A>G and L16519 T>C creates an *ApaI* and an *Hae III* restriction site, respectively. Thus, this polymorphisms can easily be detected by use of PCR based RFLPs (Method I). At the same time we used the innovative Light Cycler™ technique that is based on the principle of melting curve analysis as a rapid tool to detect SNPs (Method II).

Method I

Template DNA was amplified using primers L16517 and H 00097 yielding PCR fragments 189 bp in length. Aliquots were digested with both restriction enzymes separately, electrophorized and silver stained.

Method II

For each SNP two probes (sensor and anchor) were designed. The 3' end of one probe was labelled with a donor fluor while the 5' end of an adjacent probe was labelled with an acceptor fluor. Fluorescence resonance energy transfer occurs only when both probes hybridize to the amplicon. Thus, measurements of melting curves allow to detect amplification of SNP alleles.

We checked samples of 150 Germans, 100 Syrians, 100 Japanese, 100 Vietnamese, 100 Peruvians, and 60 Bantu speaking Cameroonians in regard the nt 00073 and 16519 polymorphisms.

Results

1. High frequencies of the nt A in position L00073, which is conform with the Cambridge Reference Sequence, we found only in the German and Syrian population. This trait is rare or almost completely absent in our population samples from Cameroon, Japan, Vietnam and Peru. The L16519 T>C transition was frequently found in all populations investigated.
2. Haplotyping on loci nt00073 and nt16519 leads to a considerable power of discrimination (PD = 0.73) when used in typing of individuals from the German population.
3. Light Cycler™ technology is a powerful and rapid tool to investigate mtSNPs.

Address for correspondence:

Prof. Reinhard Szibor, Institute for Legal Medicine, Otto-von-Guericke-University Magdeburg, Leipziger Str.44, D-39120 Magdeburg, Germany
reinhard.szibor@medizin.uni-magdeburg.de

Mitochondrial DNA sequencing in "unsolvable cases"

N. von Wurmb-Schwark, D. Lange, D. Meissner and R. Wegener

Institute of Legal Medicine, University of Rostock, Germany

In recent years mitochondrial DNA (mtDNA) sequencing has become of more interest in forensic casework. Due to the high copy number per cell mtDNA is still available even when nuclear DNA is too much degraded to be typed correctly or is only present in extremely small quantities.

The aim of this work was to establish the method and to reinvestigate cases that were unsolved by using nuclear STR typing.

We have sequenced a 439 bp fragment of the HVI and a 433 bp fragment of the HVII region of mtDNA in 100 unrelated individuals from Mecklenburg-Vorpommern, North Germany using the Big Dye Sequencing Kit (Applied Biosystems) with separation and detection on an AbiPrism310 capillary-sequencer.

Next we chose 3 unsolved cases from our institute history:

1. A dead man was found wrapped in plastic sheets in the basement showing considerable putrefaction. Estimated post mortem interval was 1.5 to 2 years. STR typing was not possible from muscle tissue or bones. Inhibiting substances and high degradation were thought to have caused this amplification failure.
2. A couple of bones deriving from an unknown person were found in the forest. An STR typing was possible for 5 loci. However, different extractions and amplification led to different genotypes. Sending the bones for reinvestigation to another institute did not help since they could not detect any signals.
3. A dead body was found showing severe putrefaction. DNA from the bones and muscle tissue was taken for DNA analysis and successfully typed. Years later a paternity analysis was. Now DNA typing of the highly degraded material led to different genotypes. Some would have excluded some included the unknown person as the missing son of the family.

We will show the possibilities and the limitation of mtDNA sequencing compared to STR typing and discuss difficulties and trouble shooting.

Address for correspondence:

Dr. Nicole von Wurmb-Schwark

Institut für Rechtsmedizin, Universität Rostock

St.-Georg-Str. 108

18055 Rostock

Tel.: 0381/494-9911

Fax: 9902

e-mail: nicole.vonwurmb@med.uni-rostock.de

The occurrence of heteroplasmy in intra- and related individuals

C. Turchi, L. Buscemi, M. Pesaresi, M. Paoli and A. Tagliabracci

Istituto di Medicina Legale, Università di Ancona, Italy

Knowledge of the significance of the condition of heteroplasmy is of crucial importance for forensic application of mitochondrial DNA, since nucleotide substitutions may be found inside maternal lineages and in biological material belonging to the same individual. With regard to this phenomenon, studies carried out until now have shown its significantly different frequency, to the extent that further investigations must be carried out.

The aims of the present study were:

- a) study the frequency of heteroplasmy in individuals related in maternal lineage and in various tissues from the same individual (blood, saliva, hair shafts, hair roots, fingerprints);
- b) definition of a sequencing protocol to improve analytical techniques and able to overcome possible false or uncertain base calling in mtDNA sequencing, which could be misinterpreted.

Sequencing was carried out on:

- fresh blood from 30 mother-child pairs, regarding cases of questioned paternity coming to our laboratory;
- 46 samples of fresh blood collected from 23 patients subjected to bone marrow transplantation and from their sibling donors;
- blood, saliva, hair shafts, hair roots, fingerprints belonging to laboratory personnel and to a suitable number of medical students.

The study was carried out by amplifying the two hypervariable regions using Taq-cycle-sequencing and capillary electrophoretic techniques. In order to verify sequencing accuracy, to reduce the percentage of "base-calling" error, and to exclude the possibility of artefacts caused by the background noise of the electropherograms, both forward and reverse strands of the two regions were sequenced in all samples.

Prof. Dr. Adriano Tagliabracci,

Istituto di Medicina Legale, Policlinico, Torrette,

I-60020 Ancona (Italy),

tagliabr@popcsi.unian.it

De novo Mutations at D3S1358, D8S1179 and D18S51 loci, emerged during Paternity Testing: confirmation of biological paternal lineage by using a panel of Y-chromosome STR

U. Ricci¹, N. Cerri², I. Sani¹, M. Franchi², S. Mascadri², F. De Ferrari² and M.L. Giovannucci Uzielli¹

¹Genetics and Molecular Medicine Unit, University of Florence – "A. Meyer" Hospital, Italy

²Institute of Forensic Medicine – University of Brescia, Italy

The analysis of Y-chromosome polymorphisms acquires a growing value in the Forensic Genetics practice, for various purposes. Here we report the role of the Y-chromosome DNA markers for the confirmation of biological paternity in three cases of mutations of Autosomal Polymorphic loci in the paternal meiosis. In the first part of the study we performed the analyses of a battery of Autosomal STR: TPOX, D2S1338, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317, D16S359, D18S51, D19S433 and D21S11, by using two different protocols and instruments. We used an Automated DNA Sequencer IR-based (LI-COR Inc.) with a protocol previously described [1] and an ABI Prism® 310 Genetic Analyser (Applied Biosystems) with the AmpFISTR Profiler Plus PCR Amplification kit and SGM Amplification kit, according to the manufacture's recommendation.

In each of the three unrelated families, we identified a genetic incompatibility between the alleged father for one of the DNA STR analysed: respectively, D3S1358, D8S1179 and D18S51. In two cases, the mutation is represented by the deletion of a single repeat, in the child in comparison to the alleged father sequence. In the third case, we observed a mutation for D18S51, but it was impossible to well understand the mechanism of the mutation, because of the haplotype of the mother and the alleged father.

Given the sex of children, all males in the three families, we decided to use a battery of ten Y-STR (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS385I/II, DXYS156Y and YCAII), as a different control for the biological paternity.

We performed this study by using a PCR multiplex amplification protocol and the characterisation of the alleles was achieved with the IR-based Automated DNA Sequencer (LI-COR 4200). We also used specific allelic ladders and positive controls in part provided by the Italian Collaborative Project of the Ge.F.I Group (Fiuggi 2), and in part by the European Y-Users Group.

All ten Y-chromosome polymorphic markers showed a complete compatibility between the child and the alleged father, in each of the three families. We are able to confirm, once again, the utility of the Y-chromosome polymorphic markers in paternity testing with a male child, and underline the role of the Y-chromosome markers to well establish the mutational event in the paternal meiosis.

[1] Ricci U, Sani I, Guarducci S, Biondi C, Pelagatti S, Lazzerini V, Brusaferrri A, Lapini M, Andreucci E, Giunti L, Giovannucci Uzielli ML, Infrared fluorescent automated detection of thirteen short tandem repeat polymorphisms and one gender-determining system of the CODIS core system. *Electrophoresis* 2000;21:3564–3570.

Address for correspondence:

Dr. Ugo Ricci, Genetics and Molecular Medicine Unit, "A. Meyer" Hospital, Via Luca Giordano 13, I-50132 Florence, Italy, Tel. 0039 55 5662942, e-mail: u.ricci@meyer.it

Presence of two mutations between father/child in two cases of paternity testing

C. Brandt-Casadevall¹, M. Gené², E. Piqué², N. Borrego², Gehrig C¹, N. Dimo-Simonin¹, P. Mangin¹

¹Institut universitaire de Médecine légale, Lausanne, Switzerland

²Departament de Medicina legal, Universitat de Barcelona, Spain

The presence of mutations and specially step mutations (loss or win of a tandem repeat) is well known and relatively frequent. We report here two cases of paternity testing presenting two different mutations between father and child.

In the first case two mutations were observed at the VWA locus: mother 16–17 / child 15–16 / alleged father 17 and at the D5S818 locus: mother 11 / child 11–12 / alleged father 11–13.

The second case also showed two inconsistencies with the following phenotypes for the VWA locus: mother 14 / child 18 / alleged father 14–19 and the D5S818 locus: mother 11–12 / child 11–13 / alleged father 12.

The two cases were examined with more than 15 PCR-based loci and 4 RFLP-Southern probes and the probability of paternity for every one was > 99.999% (including the mutations in the calculation).

It is interesting to notice that in both cases the mutations were present at the same two loci. Furthermore in the first case a double-step mutation, which is quite rare, was observed at the VWA locus between father and child. In the second case, a single-step mutation between father and child and an inconsistency between mother and child were observed.

In spite of the fact that the mutation's rate for the different loci used in the forensic analysis is reported in the literature, the presence of a double mutation in a paternity testing is not common. The double mutation (father/child) rate observed in the two laboratories concerned shows a value of about 0.7% of all paternal meiosis (N = 294). Therefore, when working with STRs, we believe that a "three exclusion rule" should be used when issuing a judgement of non-paternity.

Dr. C. Brandt-Casadevall
Institut universitaire de Médecine légale
Rue du Bugnon 21
1005 Lausanne
Switzerland
Tél.: 41 21 3147070
Fax: 41 21 3147090
e-mail: Conxita.Brandt@inst.hospvd.ch

Meiotic mutation rates of mini- and microsatellites in a Spanish population sample

M.J. Farfán, M. López-Soto, V. Prieto, Y. Torres, J. García-Hirschfeld and P. Sanz

Servicio de Biología, Instituto Nacional de Toxicología, Sevilla, Spain

The knowledge of mutation rates of DNA markers used in forensic genetics is important for the interpretation of results. Although rare, transmission incompatibilities in paternity testing or single mismatches in DNA profiles obtained during forensic investigations may occur due to germline or somatic mutation.

Here we present an estimation of meiotic mutation rates of six minisatellites and fifteen STRs deduced from the results obtained in the course of the filiation analyses carried out in our institute. At the six minisatellites, we observed 12 mutational events out of 2036 meioses, leading to a calculated overall mutation rate of 5.9×10^{-3} . Only 3 mutations out of approximately 3000 meioses were found at the fifteen STRs under study, leading to an overall mutation rate of approximately 10^{-3} . According to the stepwise mutation model, in these three cases the mutations could be due to the gain or loss of one repeat. In order to confirm this point, sequencing of the mutated and parental alleles would be necessary.

*Dr. M.J. Farfán, Instituto Nacional de Toxicología,
P.O. Box 863, E-41080 Sevilla, Spain.
Tel. +34 954371233; Fax: +34 954370262; e-mail: farfan@cica.es*

Quantification of fluorescent STR genotyping results for chimerism control after bone marrow transplantation

P.M. Schneider¹, S. Kreiter², G. Derigs², K. Kolbe²,
N. Winkelmann², J. Lummer¹, B. Stradmann-Bellinghausen¹,
C. Rittner¹

¹*Institute of Legal Medicine, Johannes Gutenberg University, Mainz, Germany*

²*Dept. of Haematology, III. Medical Clinic, Johannes Gutenberg University, Mainz, Germany*

Engraftment of donor stem cells after allogeneic bone marrow transplantation can be genetically monitored by PCR typing of DNA polymorphisms. Successful engraftment with complete chimerism, and presence of the donor's genotype in the bone marrow has to be demonstrated, and the presence of the patient's alleles has to be excluded. Detection of patient's alleles provides evidence for an incomplete chimerism or for a relapse of malignant disease. For the present study, we have developed an approach to quantify the ratio of donor chimerism using mock mixture experiments. The usefulness of our approach is demonstrated in typical cases where the donor chimerism could be monitored over periods of more than one year after transplantation (Tx).

For the mock experiments, genomic DNA samples from two healthy siblings were mixed to represent donor chimerism between 5 and 50%. PCR was carried out using the ABI AmpFISTR Profiler PCR Kit as recommended, and STR fragments were separated using the ABI Prism 310 Genetic Analyzer. Based on the peak areas (in rfu) as provided by the fragment analysis software, relative genotype proportions were calculated across five informal autosomal loci including the amelogenin locus. The mean value \pm s.d. was calculated across these five loci to compensate for PCR-related artifacts. These mock experiments showed an excellent correlation of the genotype proportions calculated from the mixtures with the expected values.

For the patient study, blood and/or bone marrow samples as well as blood samples (prior to Tx) or mouth swabs (after Tx) were available. Some of these patients were treated according to an experimental protocol using incomplete hemoablative conditioning to use the graft-vs-leukemia effect for elimination of residual malignant cells. In these cases, the semiquantitative analysis proved to be quite helpful for prospective monitoring the treatment, as FACS-separated mononuclear cells from the recipient (CD3-positive T-cells and CD3-negative cells) could be genotyped and quantified separately.

Thus DNA typing of STR loci followed by semiquantitative analysis of donor/recipient chimerism is suitable both for retrospective as well as for prospective monitoring of bone marrow transplantations even among first-degree siblings.

*Dr. Peter M. Schneider, Institut für Rechtsmedizin,
Am Pulverturm 3, D-55131 Mainz,
Tel. +49 (6131) 3932687, Fax +49 (6131) 3933183,
e-mail: pschneid@mail.uni-mainz.de*

STR Mutations in Paternity Investigations During One Year Studies

H. Geada^{1,2}, L. Viriato², C. Vieira-Silva², C. Cruz, T. Ribeiroz and R. Espinheira²

¹Department of Legal Medicine, Faculty of Medicine, University of Lisbon

²Genetic and Forensic Biology Department, National Institute of Legal Medicine, Lisbon Delegation, Portugal

Genetic inconsistencies detected during paternity investigation studies can add complexity to the analysis and resolution of these investigations. Since STR systems are nowadays the most commonly used systems to solve a paternity case, the chance of detecting mutations increases as STRs have mutation rates higher than conventional genetic markers.

During last year, paternity investigation cases in our Laboratory were performed using SGM Plus and Powerplex 1.2. At the end of the year we have introduced Powerplex 16. More than 500 paternity investigation cases have been studied and 15 mutations have been detected in several loci in 3% cases (Table1).

Table 1. Mutations and mutation rates detected in several loci

Loci	D3S1358	D8S1179	D18S51	D13S317	FGA	D16S539	D2S1338	D7S820	CSF1PO	D19S253
Nº Mut	1	3	1	1	1	2	2	1	2	1
Meiosis	689	689	687	683	689	689	543	683	675	643
Mutation Rate %	0.15	0.44	0.15	0.15	0.15	0.30	0.37	0.15	0.30	0.16

Meiosis studied are maternal and paternal meiosis. In all these cases there is overwhelming evidence in favor of paternity or maternity. The mutations encountered during this investigation have the following characteristics:

- The majority of mutations observed involved the addition or deletion of a single repeat unit from the allele in question
- The number of additions and deletions detected was equivalent
- In D8S1179, one paternal genetic inconsistency involved the deletion of two repeat units (12 → 10)
- A null allele was assumed in D2S1338 loci in a maternal exclusion due to non-matching homozygous patterns (20 → 18)
- In the 15 genetic inconsistencies detected, 3 were from maternal origin and 12 from paternal origin which indicates a paternal mutation rate higher than a maternal one.

With the increase of STR loci number and due to mutation rates detected in every loci we can expect to find cases with two or even three genetic incompatibilities that are non-exclusion cases. In this study we have detected a case with two paternal genetic inconsistencies in D7S820 and CSF1PO when studying 22 different loci.

Moreover, we have also detected three cases with a multi-banded pattern – two with maternal origin in TPOX and one with paternal origin in D18S51. A vWA genotype difference in one case was also detected when Powerplex 1.2 and SGM Plus were performed in 1406 different samples (vWA – 16 – 17 and 16, respectively).

In all genetic inconsistencies cases 19 to 22 loci were studied and two paternity probabilities were always calculated – without the mutation and with the mutation rate. As a guideline for exclusions we only consider an exclusion case based on at least four incompatibilities.

When using Powerplex 1.2 and SGM Plus, 85% of paternity cases investigated have $W > 99,9999\%$ and 60% of cases have $W > 99,9999\%$. As a criterion for non-exclusion cases we have $W > 99,9999\%$ including the mutation system in statistical calculations.

Dr Helena Geada, PhD
Genetic and Forensic Biology Department,
National Institute of Legal Medicine, Lisbon Delegation,
Lisbon, Portugal, R Manuel Bento de Sousa, n°3, 1150-219
Tel. + 351 21 8811800, Fax + 351 21 8864493
E-mail: hgeada@mail.telepac.pt

Analysis of Paternity Index of 164 Paternity Trios DNA-typed by either 10 STR or 4 RFLP loci

E. Raimondi, U. Toscanini and E. Haas

PRICAI – Favaloro Foundation

(First Argentine Immunogenetics Centre) – Buenos Aires, Argentina

In the past few years short-tandem-repeat (STR) typing has become the method of choice for many, if not most, laboratories to perform paternity testing. RFLP is a very important tool for forensic and paternity testing but, since STR analysis is technically simpler and a considerable number of STR loci are now available, RFLP analysis is being less and less used. We began with STR typing by 1996 (6 loci). Up to that time RFLP and HLA typing was the methodology applied in our laboratory for paternity testing. Gradually, in the past four years, there was a transition from RFLP to STR typing, though not complete. Currently, we analyse most paternity cases by STR typing and we perform RFLP and HLA typing mainly in incomplete cases in which our 18-locus STR battery is not enough to reach conclusive results.

The aim of this study is to show that a set of carefully chosen and well-known STR loci may provide as reliable results as RFLP typing does. We analysed the Paternity Index (PI) and Residual Paternity Index (RPI) obtained in 67 nonexclusion cases and 22 exclusion cases, typed by ten STR loci (Table I). Results from a similar number of cases (61 non-exclusions, 14 exclusions) typed by four RFLP loci (D12S11, D17S79, D4S163, D7S467 [Nice Paternity™ probe]) were analysed the same way. Cases were all true trios from Buenos Aires metropolitan area. Cases in which only one locus showed a difference between child and alleged father (probably mutations) were excluded from this study (10 cases). DNA was extracted by non organic procedure from EDTA collected blood. STR typing was performed by silver staining of amplified DNA (two triplex and one tetraplex reactions) and RFLP typing by using alkaline phosphatase-labelled probes. PI was calculated for the trios and also for child and alleged father without the mother and were assigned one out of six categories: 1) <1,000; 2) 1,000 to 10,000; 3) 10,000 to 100,000; 4) 100,000 to 1 million; 5) 1 million to 10 millions; 6) >10 millions. Frequency tables were local.

Table I.

STR MULTIPLEX	LOCUS
CTT Triplex (Promega Corporation)	CSFIPO, TPOX, THO1
Multiplex I Triplex (Lifecodes Corporation)	D12S1090, D3S1744, D18S849
Multiplex II Tetraplex (Lifecodes Corporation)	FGA, D7S820, D1S533, D9S304

Analysis of PI showed that for STR typed trios the value was always >1,000. One (1.5%) of the trios analysed by RFLP typing yielded a PI <1,000 (PI = 992). The highest value for PI was 53,655,218 for STR and 578,015 for RFLP. When PI was calculated for duos (same child and alleged father without the mother), PI was <1,000 in 15 cases (22.4%) for all STRs and in 34 cases (58.6%) for all RFLPs. The highest PI for duos was 341,876 and 36,125 for STR and RFLP respectively.

PI was also calculated for STR-typed trios considering either six or seven loci. When using six STRs (CTT plus Multiplex-I) PI was >1,000 in 46% cases, while when using seven STRs (CTT plus Multiplex-II) it was >1,000 in 75% cases.

Exclusion cases always had RPIs <1,000 for both trios or duos using either STR or RFLP typing except for one RFLP case which yielded a RPI of 3,676. The number of loci that excluded the alleged father was between 3 and 8 out of the ten STR loci and between 1 and 4 for RFLP loci (cases with only one exclusion were confirmed with more probes).

In our opinion, analysing these ten STRs loci for paternity testing in our laboratory may lead to conclusive results for all trio cases, sometimes yielding higher PIs than those expected with four RFLPs probes and a high probability of excluding non biological fathers. In cases where the mother cannot be typed, more than ten STRs should be analysed to get similar results.

Address for correspondence:

Prof. Dr. Eduardo H. Raimondi, PRICAI-Favaloro Foundation

Av. Belgrano 1782 – 1er Subsuelo, 1093 – Buenos Aires – Argentina

TE: (5411) 4384 5400; FAX: (5411) 4383 1197;

e-mail: eraimondi@ffavaloro.org

Statistical pattern analysis of D1S8o alleles in Northwestern Russians and other populations using COLLAPSE software

A.G. Smolyanitsky¹, N.N. Khromov-Borisov^{1,2}, I.B. Rogozin^{3,4}, J.A.P. Henriques⁵, V.L. Popov¹ and G.I. Zaslavsky¹

¹Forensic Bureau of Leningrad District, Saint Petersburg, Russia

²Biological Institute, St.-Petersburg University, Saint Petersburg, Russia

³Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk, Russia

⁴National Center of Biotechnology Information, National Institute of Health, Bethesda, Maryland, USA

⁵Center of Biotechnology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Polymorphism at the locus D1S8o was studied in 298 unrelated habitants of the Northwestern region of Russia that includes cities Saint Petersburg, Petrozavodsk, Arkhangel'sk, Novgorod, Pskov and surrounding districts. The sample distribution obtained was compared with published data on the nine samples: Muscovites (N = 120), Finns (N = 140), Alaskan Natives (two samples: North Slope Borough, N92, and Bethel Wade Hampton, N = 109), American Caucasians (N = 718), Southeastern (N = 247) and Southwestern (N = 162) Hispanics, Orientals (N = 204) and African Americans (N = 606). The great statistical problem in such kind of analysis is the so-called problem of multiple comparisons (or multiple hypothesis testing). It requires special adequate approaches. For instance, commonly prevalent procedure of the pairwise comparisons based on the analysis of separated contingency tables $R \times 2$ (or $2 \times C$) is misapplied and can lead to false conclusions. For this purpose a new statistical technique – Similarity Pattern Analysis (SPAN) and corresponding COLLAPSE software were used and their validity and applicability were demonstrated with the above data sets. The main features of the method are the following. First, to measure the similarity (homogeneity) between any pair of populations in the study, the relevant sufficient statistics, Kastenbaum-Hirotsu squared distance (KHi^2), is used. Second, the method is based on the Homogeneity Principle, which permits to combine (to collapse) similar subsets of the sample distributions into distinct (locally homogeneous) groups (homoclusters). The procedure can be stepwise where at each step a pair of the most homogeneous distributions is collapsed. Such a procedure permits to reveal one, the most likely (optimal) version of the similarity pattern, among many others, which are less probable. Third, the discrimination among homo- and hetero-clusters is based on the so-called Chi-square Reduction Principle, according to which the corresponding statistics, chi-square reduction, as a measure of intra-cluster homogeneity should be kept non-significant after each step of collapsing. Evaluated similarities and/or dissimilarities appeared to be rather reasonable and interpretable. For instance, similarity (that is the absence of statistically significant difference) observed between Northwestern Russians (NWRus), Finns and Muscovites could be explained by their geo-

graphical vicinity and historical and cultural mutual interactions. Respectively, they can be combined into one homocluster. The differences between two Alaskan populations appeared to be significant at the level $\alpha = 0.05$, but not at $\alpha = 0.01$. The same non-significant differences at $\alpha = 0.01$ were observed between American Caucasians, NWRus and Muscovites and such low level of the above similarities seems could be anticipated. The differences among all other populations were much more significant. Nonetheless, despite of the prevailing heterogeneity and complexity of the similarity/dissimilarity pattern observed, it is possible to partition the above samples into three clusters. A single population of African Americans presents the first one: it differs drastically from all others mainly due to extremely high proportion of allele 34, which is very rare in other populations. In the second cluster two populations, Southwestern Hispanics and Orientals, are combined. The heterogeneity within this cluster is highly significant, but both are even more dissimilar to the remainders. The third cluster consists of other seven populations among which some sub-clusters can be regarded. The results were compared with those obtained using other related procedures and software. Such a comparison shows that the SPAN methodology and COLLAPSE software provide adequate and fruitful approach to forensic population studies.

The program is available from the FTP-site:

<ftp://bionet.nsc.ru/pub/biology/dbms/COLLAPSE.zip> or under request from the authors: v_sidorov@mail.ru, nikita@NH8333.spb.edu, rogozin@ncbi.nlm.nih.gov, pegas@dna.cbiot.ufrgs.br. See also website: <http://www.ufrgs.br/bioinf/>.

Address for correspondence:

Dr. Andrew Smolyanitsky,
Forensic Bureau of Leningrad District, Shkapinstr. 36/40,
Saint Petersburg, 198092, Russia,
Tel.: +7 (812) 251 4000, Tel./Fax: +7 (812) 251 1600,
e-mail: v_sidorov@mail.ru

Evaluation "in house" criteria for STR analysis in immigration casework

P.H. van Eede, S. Keller and G.G. de Lange

Central Laboratory Blood Transfusion Service (CLB), Dept. of Immunogenetics, P.O. Box 9190, 1006 AD Amsterdam, the Netherlands

Predefined criteria, concerning strategy of testing, exclusions, the calculation of the one and two parental indices (I) and consequences of mutations, are evaluated in 156 one parent-child combinations and 105 two parent-child combinations, out of 28 immigration families.

The amplification of the isolated DNA from buccal swabs was performed using the multiplex STR systems CTTv, FFFL and PowerPlex 16 (Promega) and with the SGM+ (Applied Biosystems). Together the multiplex systems amplified 21 different loci.

The analysis of PCR products was performed on the ABI 310 analyzer, based on capillary electrophoresis (Applied Biosystems).

This evaluation shows that in 20 parent-child combinations three or more mismatches were observed and could be reported as excluded. In 10 other cases, only one- or two-mismatches were seen and mutations were assumed and corrected I value were calculated. In 136 parent-child combinations no exclusions were seen and the indices were calculated.

After typing for 21 STR systems, 1 out of 156 combinations could not be reported as included or excluded, because the calculated I value was less than 1000.

The influence of the mutation correction in casework was tested by simulating in 126 one-parent cases, one mismatch. In one-parent combinations an inclusion is reported, when the calculated I value is 1000 or more. As a result of the correction, using an average mutation correction factor of 1/100, in 13% of the one-parent combinations the I value appeared to be less than 1000.

The predefined criterion in two-parent casework for non-exclusions is resolved on $10E4$. Testing 105 two-parent combinations, the calculated I value ranged from $10E7$ to $10E21$. Therefore the criteria in two parent-child combinations seem not to be very meaningful and have to be considered critically.

A combination of the multiplex STR systems, used in this evaluation, appeared to be successful in analysing immigration casework. However to be decisive in all cases, without reducing the quality of the results, more highly informative systems are needed.

Correspondence:

P.H. van Eede, Central Laboratory Blood Transfusion Service, Dept. of Immunogenetics P.O. Box 9190, 1006 AD Amsterdam, the Netherlands, Tel: 31 20 5123179, e-mail: PH_van_Eede@CLB.NL

A method to help the detection of false homozygous samples at D17S5 locus

S. Pelotti, G. Ferri, C. Colalongo, A. Abbondanza, M. Falconi and G. Pappalardo

Department of Medicine and Public Health, Section of Legal Medicine, University of Bologna, Italy

As observed by several Authors the PCR amplification of D17S5 presents difficulties because when the allele sizes differ significantly in a heterozygous individual, the small allele amplifies more efficiently, resulting in misclassified homozygote.

Here we describe a method to check the single-banded samples for the correct evaluation of this polymorphic region.

A forward primer that anneals within the D17S5 repeat sequences was designed and utilised for PCR with Horn's reverse primer.

Amplification of heterozygous controls consisting of large and small alleles (e.g. 2-12) with this primer pair produced a ladder like set of fragments whose number was linked to the number of repeats of the largest allele even if heavier fragments could not be clearly resolved on agarose gel.

Single-banded samples with a small number of repeat (1 to 4), showed in some cases the ladder profile with a number of PCR fragments higher than the expected: this suggested the presence of another allele hidden by the drop-out.

Even if the defective allele can not be definitely assigned, the method allows to distinguish real or false homozygotes and may be suitable to avoid mistyping in population studies and in forensic caseworks.

Prof. Dr. Med. Giuseppe Pappalardo, Department of Medicine and Public Health, Section of Legal Medicine, University of Bologna, via Irnerio 49, 40126 Bologna, Italy, Tel 00 39 (0)51 243250; Fax: 00 39 (0)51 248896, e-mail: labemat@medleg.unibo.it

The use of DNA analyses for subtyping Aend or Bm in ABO blood group system

Y. Itoh¹, K. Satoh^{1,2} and R. Kobayashi^{1,3}

¹Department of Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan

²Medico-Legal Section, Criminal Investigation Laboratory, Metropolitan Police Department, Tokyo, Japan

³Department of Microbiology, Tokyo Medical University, Tokyo, Japan

Introduction

In 1990, Yamamoto et al elucidated the molecular genetic basis of the three major alleles encoding transferases of the ABO blood group system. Since then, rare A or B subtypes such as A₂, A₃, A_x, A_{el}, A_m, B₃, B_x, B_{el} and cis-AB have been analyzed. It is not known at present, however, whether these sequence data can be accepted for subtyping or not. The purpose of this study is to achieve an evaluation of the sequence for subtyping. In this paper, we analyzed genomic DNA of Aend and Bm subtype. We found that these subtypes did not possess any specific mutation.

Material and method

- 1) DNA: Saliva samples obtained from Japanese ABO subtype individuals was used for DNA preparation. Genomic DNA was extracted by the phenol-chloroform method.
- 2) Sequencing: PCR products of exon 6 or exon 7 regions were sequenced using ABI Prism 377 sequencer and BigDye Terminator cycle sequencing v 2.0 Ready Reaction.

Results and discussion

Our data showed that both sequences of the Aend allele from 2 of Aend and 3 of AendB samples and sequences of the Bm allele from 2 of Bm and 1 of ABm samples were identical to those of A₁ and B alleles, subdivided A₁₀₂ and B₁₀₁ alleles, respectively. Incidences of Aend and Bm phenotypes are infrequent, while those of sequenced data are frequent. These subtypes did not possess any specific mutation. Although some of A₂, A₃, A_x, A_{el}, A_m, B₃, B_x, B_{el} and cis-AB were reported to show differences from the original A₁ and B sequences, their constant mutation such as A₁ and B alleles were not found. There might be two types among subtype. One is to show specific mutation, and the other did not show any mutation. In conclusion, ABO blood group subtyping based on the sequence data is not always accepted.

Address for correspondence:

Asist. Prof. Dr. Y. Itoh

Department of Forensic Medicine, Juntendo University School of Medicine, Hongo, Tokyo 113-8421, Japan.

Tel: +81-3-5802-1051

Fax: +81-3-3814-9300

e-mail: yitoh@med.juntendo.ac.jp

Microsatellite instability in mononuclear cells from non-tumorigenic human tonsils and its use in forensic evaluation

R. Kobayashi^{1,2} and Y. Itoh²

¹Department of Microbiology, Tokyo Medical University, Tokyo, Japan

²Department of Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan

Introduction

Microsatellites are highly polymorphic and scattered more or less throughout the genome. The detection of polymorphic microsatellite DNA sequences has become extremely important for personal identification in forensic science. However, microsatellite instability (MSI) has also been reported to be a landmark of some tumors of the colon and rectum. The goal of this study was to investigate whether MSI existed or not in normal individuals and to evaluate whether the MSI had a bad effect on the results of forensic studies.

Materials and Methods

THo1 was selected as a microsatellite locus and typed from cells isolated from human non-tumorigenic tonsils. These cells were diluted by limiting-dilution to 1 cell per 2 micro liters for each PCR amplification. The THo1 was typed from these single cells by semi-nested PCR. The additional primer (THo1Ff: cca ttg gcc tgt tcc tcc ctt att) was designed in THo1 locus for semi-nested PCR. First- and second-round amplifications consisted of a 45-sec denaturation step at 94°C, a 30-sec annealing step at 60°C, and a 30-sec extension at 72°C. Prior to second-round amplification, THo1Ff was labeled with ³²P and the PCR products were resolved on a 6% polyacrylamide gel with 8.3 M urea at 1,400 V for 4 hours and the gel was exposed to X-ray film for 16–96 hours. The M13mp18 sequence ladder was used as a size standard.

Results and Discussion

One out of 178 cells (0.56%) examined by semi-nested PCR had a 4-bp shorter than the major band. Studies presented here indicate that the typing data from single cells may represent not only the slippage event of DNA polymerase during PCR amplification but may also reflect somatic mosaicism in the individual. However, the existence of the mutated cells in this proportion may have no effect on forensic examination.

Address for correspondence:

Asist. Prof. Dr. Y. Itoh

Department of Forensic Medicine, Juntendo University School of Medicine, Hongo, Tokyo 113-8421, Japan.

Tel: +81-3-5802-1051

Fax: +81-3-3814-9300

e-mail: yitoh@med.juntendo.ac.jp

DNA Archiving on FTAR paper: Photosensitizer initiated attacks as models of aging

L.H. Seah¹ and L.A. Burgoyne²

¹Malaysian Department of Chemistry, Petaling Jaya, Malaysia

²School of Biological Sciences, Flinders University of South Australia, Adelaide, Australia

Abstract

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 for 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 FTA[®] 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.

Correspondence Address:

Dr. Seah Lay Hong, Jabatan Kimia Malaysia, Jalan Sultan, 46661 Petaling Jaya, MALAYSIA

SE33 (HumACTBP2): Native gel electrophoresis versus denaturing capillary electrophoresis, and population data

S. Stadlbacher, E.M. Dauber, B. Glock, W.R. Mayr

University of Vienna, Medical School, Clinical Department for Blood Group Serology, Austria

The aim of the study was to compare the results obtained by electrophoresis under denaturing conditions with the results of native horizontal gel electrophoresis on the STR locus SE33 (HumACTBP2) in an Austrian Caucasoid population sample.

135 unrelated individuals were tested on the ABI Prism 310 capillary electrophoresis instrument with a commercially available kit (genRes Kit STR System HumACTBP2, Serac) and with our standard method, a native gel electrophoretic system (Schwartz et al. 1993).

In the smaller allelic size range, each allele of the same size typed by denaturing electrophoresis corresponded to a single allele in the native gel electrophoresis. In the allelic size range larger than 267 bp (> allele 20.2) results obtained by the ABI Prism 310 could not be associated with one distinct allele typed by native gel electrophoresis. This might be due to the sequence polymorphism which is limited to the alleles larger than 267 bp, whereas only length polymorphism is observed for the smaller alleles at this locus (Rolf et al. 1997).

Allele frequencies and further statistic data obtained by denaturing electrophoresis are shown below (n = 135):

Allele name	Allele frequency	Allele name	Allele frequency
12	0.007	22.2	0.019
12.2	0.004	23.2	0.033
13	0.007	24.2	0.044
13.2	0.004	25.2	0.052
14	0.026	26.2	0.037
15	0.037	27.2	0.093
16	0.052	28.2	0.041
17	0.074	29.2	0.067
18	0.070	30.2	0.088
19	0.070	31.2	0.037
20	0.052	32.2	0.015
20.2	0.011	34	0.004
21	0.022	34.2	0.007
21.2	0.015	35.2	0.004
22	0.004	36.2	0.004
Rate of heterozygosity: 0.932		Matching probability: 0.013	
Power of exclusion: 0.861		Power of discrimination: 0.987	
Polymorphism information content: 0.95		Typical paternity index: 8.44	

References:

Rolf B, Schürenkamp M, Junge A, Brinkmann B (1997) Sequence polymorphism at the tetranucleotide repeat at the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2) locus, *Int J Leg Med* 110:69–72

Schwartz DWM, Jungl EM, Krenek OR, Mayr WR (1993) Typing for STR-loci by electrophoresis on rehydratable polyacrylamide gels: phenotype and allele frequency of SE33 and TC11 in an Austrian population sample, *Advances in Forensic Haemogenetics* 5, Springer Berlin, Germany

Address for correspondence:

Simone Stadlbacher, Universität Wien-Universitätskliniken, Klinische Abteilung für Blutgruppenserologie, A-1090 Wien, Währinger Gürtel 18-20, Austria, Tel: +43-1-40400-5320, Fax: +43-1-40400-5321, e-mail: simone.stadlbacher@univie.ac.at

Distribution of MN genotypes developed by PCR-SSCP analysis

N. Nakayashiki¹, J. Kanetake¹, Y. Sasaki¹, I. Yuasa², A. Miyoshi³, M. Hashiyada⁴ and Y. Aoki¹

¹Department of Legal Medicine, Iwate Medical University School of Medicine, Japan

²Department of Legal Medicine, Tottori University Faculty of Medicine, Japan

³Department of Forensic Medicine, Fukuoka University School of Medicine, Japan

⁴Department of Forensic Medicine, Tohoku University School of Medicine, Japan

Abstract

Genotyping of MN blood system was performed by means of PCR-single strand conformation polymorphism (PCR-SSCP) analysis. A DNA fragment including exon 2 of glycophorin A was specifically amplified. After polyacrylamide gel electrophoresis of the fragment followed by silver staining, five band patterns corresponding to each MN allele, M^G, M^T, N¹, N² and rare N^V, have been observed from Japanese samples; M^G > N¹ > M^T > N² in order of decreasing frequency. In this study, we will show the distribution of MN alleles not only in several Japanese groups but also in Chinese and German populations.

Nori Nakayashiki, PhD

Department of Legal Medicine,

Iwate Medical University School of Medicine,

19-1 Uchimaru, Morioka 020-8505, Japan,

Tel. 00 81(0)19 651 5110 ext.3383; Fax: 00 81 (0)19 622 2826,

e-mail: nnakaysk@iwate-med.ac.jp

Allele 14 of vWA is characterized by 3'-flanking nucleotide substitutions and a TTAT insertion

A. Tamura, M. Iwata, H. Tsuji, H. Nishio, K. Matsui, T. Miyazaki and K. Suzuki

Department of Legal Medicine, Osaka Medical College, Takatsuki, Osaka, Japan

Intron 40 of von Willebrand factor (vWF) gene is known to harbor three different STRs, which were separately reported by Kimpton et al. (vWA), by Peake et al. (vWF-P), and by Ploos van Amstel et al. (vWF-A). We report nucleotide substitutions and a tetranucleotide insertion observed in a single vWA allele.

The three STRs were typed en bloc and separately by electrophoresing their PCR products. Sequencing was performed on an ABI PRISM 310 Genetic Analyzer.

Sequencing of *en bloc* amplified products spanning the three STRs revealed single nucleotide substitutions at five separate positions and a tetranucleotide insertion. The positions of the substitutions were numbered as nt 1761, 1849, 2122, 2180, and 2192 according to the sequence reported by Mancuso et al. The two upstream sites were found to be located between vWA and vWF-P, and the other three between vWF-P and vWF-A. A TTAT tetranucleotide was inserted between nt 2057 and nt 2058. Analysis of amplified products including various vWA alleles showed these substitutions and insertion occurred exclusively in allele 14 of vWA. Allele 14 is reported to be ancestral to modern vWA alleles by comparing the repeat structure with the other vWA alleles and those of various primates. This study showed allele 14 differed markedly from the other alleles in the sequences intervening between the three repeats in addition to its repeat structure.

Corresponding address:

Akiyoshi Tamura, Department of Legal Medicine,

Osaka Medical College, 2-7 Daigakumachi, Takatsuki-shi,

569-8686, Japan.

Telephone number: 0726-83-1221 ext.2642

FAX number: 0726-84-6515

Rare Alleles Distribution: Importance of Primer Location and Incidence in Population

P. Garofano¹, L. Albonici¹, F. Donato², F. Biondi², M. Pizzamiglio², L. Garofano² and V. Manzari¹

¹Cattedra di Patologia Generale, Dipartimento di Medicina Sperimentale e S. B., Università di Roma "Tor Vergata", Italia

²Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italia

Different alleles in STR are accounted by repeated sequences duplication so that alleles differ by a standard number of nucleotides. Rare alleles are instead derived by insertion or deletion of a different number of nucleotides so that they lay in between usual alleles. Their formation is therefore less frequent than that of canonical alleles. Moreover deletion and insertion can appear both in repeated and flanking sequences, so that primers location in flankings is determinant on their identification. In this paper we report the analytical strategies we have used and implications we have found while studying different rare alleles obtained from casework in Italian population which has shown some interesting profiles. It is basic to know whether the case is of an occasional mutation or whether the allele is genetically transmitted. It must be studied if the probability predictivity can be assumed to be that of the diffusion of the allele in general population, or if two distribution panels in general population and in the specific population has to be utilized: diffusion of an unusual allele can be much higher in a region than in general population.

"Obviously impossible" – The application of the inheritance of blood groups as a forensic method. The Beginning of paternity tests in Germany, Europe and the USA

M. Okroi¹ and P. Voswinckel¹

¹Institut für Medizin- und Wissenschaftsgeschichte, Medizinische Universität zu Lübeck, Germany

When a lecture about "Blood group diagnosis as a forensic method" was given at the evening of the 24th January 1924 at a meeting of the medico-legal society of Berlin nobody in the auditorium could foresee that this evening would be the beginning of the serological paternity

tests. The lecturer was Fritz Schiff who was the head of the bacteriological laboratory at the municipal Hospital im Friedrichshain in Berlin. In his speech he explained the principles of those tests to the audience: If the blood groups of mother and child are known, then, according to the Mendelian law, one can predict that the assumed father cannot belong to certain blood groups: It is "obviously impossible" that a sued father of AB could be the true father of a child with the blood group O. Schiff concluded with guarded optimism that at least in some paternity cases it would be possible to exclude the assumed father as the real father.

This idea found its wide spreading inside the scientific community. Already in September of the same year at the 13th Congress of the German Association of Forensic and Social Medicine at Innsbruck/Austria, Georg Strassmann gave a full account of the first experience with the serological paternity tests at court in Germany. Just a few weeks before that meeting at Innsbruck, Strassmann and Schiff performed the first investigations. But their results did not lead to an exclusion of the sued fathers. It took two more years until Schiff was able to report the first paternity tests, leading to juridical consequences. Then only one year later in Germany in 385 paternity cases blood group tests were requested. In 44 cases an exclusion of the assumed father being the real father was possible. While many courts esp. in South Germany accepted those tests, the supreme court for civil law cases of Prussia denied the reliability of blood group tests. Especially its president, judge Leonhard was a fundamental enemy of paternity tests. At the 11th October 1927 the supreme court published a resolution in which it claimed that blood group diagnostic proving nonpaternity in the present state of research were not a reliable investigation. This resolution unleashed a nation-wide dispute about the reliability of this method. Also inside the medical profession the tests were controversially discussed. The Coexistence of three nomenclatures for blood groups and the incorrect use of blood grouping technique supported the confusion. Mistakes in the laboratories led to wrong results and were misunderstood as exceptions of Mendelian law. In order to put an end to this dispute, the Ministry of Public Health installed a committee consisted of anthropologists, serologists and forensic scientists. In March 1930 this committee finished its work and concluded in its declaration that the blood group test is an objective and reliable method to prove nonpaternity. The Ministry appointed Schiff to the highest court expert of Prussia.

The medical journals in Europe recognised with great interest the development in Germany. Since the mid of the twenties Schiff's articles had been summarized and printed in English and American journals. Schiff's article *medico-legal significance of blood groups* in *The Lancet* found a strong international echo. This article started a European wide discussion inside the national medical societies about the establishment of blood tests for proving nonpaternity. In 1931 the Italian government permitted the blood group investigation at court, referring to the results of Leone Lattes, the Italian pioneer of forensic serology, and Fritz Schiff. One Year later the first application of blood tests led to juridical consequence in a case of maintenance at Dublin. Not until 1935 the blood tests were permitted in the USA. Due to the efforts of Alexander Wiener the New York State as one of the first established the blood group investigation in cases of maintenance.

PCR based diagnosis of Enterovirus and Parvovirus B 19 in paraffin embedded heart tissue

A. Baasner, R. Dettmeyer, M. Graebe and B. Madea

Institute of Legal Medicine, University Bonn, Germany

The incidence of virus-induced lethal courses of myocarditis is still unclear. While enteroviruses are the most common agent of myocarditis, parvovirus B19 is also known to be highly cardiotropic. Enteroviruses belong to the family Picornaviridae. Their genome consists of a single \pm strand RNA molecule. Parvovirus B19 is the only known human pathogen virus of the family Parvoviridae. Parvovirus B19 genome consists of a linear single-stranded DNA molecule. In our investigation enterovirus RNA and parvovirus B19 DNA could be specifically isolated and demonstrated from formalin-fixed material. For the amplification of enterovirus cDNA a seminested PCR was performed. Primers were designed to amplify a 150 bp (first round) and a 110 bp (second round) fragment of the 5'untranslated region conserved among all enteroviruses. For the analysis of parvovirus B19 a 102 bp fragment of the 5'untranslated region was amplified. Control PCR amplification to verify the presence of amplifiable nucleic acid extracts from each sample was performed using cyclophilin primers (enterovirus) or F13B primers (parvovirus). Altogether myocardial samples from 60 autopsy cases were taken from different regions and investigated with the polymerase-chain-reaction. Enteroviruses could be detected in more than 20 cases. PCR revealed single cases with myocardial infection due to parvovirus B19. In the myocardial sample of one case, both enteroviruses and parvovirus B19 were found. Our results emphasize the importance of modern molecular biological methods in cases of sudden death even when histological examination revealed no serious findings in heart muscle tissue.

Dr. Anne Baasner,
Institut für Rechtsmedizin, Universität Bonn,
Stiftsplatz 12, D-53111 Bonn
Bonn, Germany, Tel. 00 49 (0)228 738356; Fax: 00 49 (0)228 738339,
e-mail: a.baasner@uni-bonn.de

Forensic evaluation of tetranucleotide STR instability in lung cancer

G. Peloso¹, P. Grignani¹, R. Rosso² and C. Previderè¹

¹Dipartimento di Medicina Legale e Sanità Pubblica, Università di Pavia, via Forlanini 12, 27100 Pavia, Italy

²Dipartimento di Patologia Umana ed Ereditaria, Sezione di Anatomia Patologica, Università di Pavia, via Forlanini 14, 27100 Pavia, Italy

A correlation between STR instability and several human cancers has been reported in many studies. For example, microsatellite instability (MSI) and loss of heterozygosity (LOH) have been detected at different rates in gastric, colorectal and renal cancer, using mono-di-tri-tetra and pentanucleotide STR markers.

The evaluation of the genetic instability can be important in forensic medicine for a reliable interpretation of the genetic profiles. Tetranucleotide STR loci are, in fact, routinely used in forensic DNA studies and currently employed for genetic identification and paternity testing in particular cases where fixed paraffin-embedded tissues are the reference samples for the analyses.

Many times during the last years, we were asked to determine whether a cancer biopsy originated from a particular individual; consequently we tried to evaluate the incidence of the genetic instability in these particular human tissues in order to avoid incorrect DNA typing results to be obtained.

Our preliminary investigation was conducted on a small sample consisting of 24 patients with a lung cancer diagnosis (adenocarcinoma, squamous-cell carcinoma, small-cell carcinoma). Neoplastic tissues were selected on formalin fixed paraffin embedded stained sections under microscopic control to avoid the contamination of nonneoplastic cells. The paired histological normal tissue derived from lymph node sections of the same patient.

10 μ m sections of each paired normal and tumoral tissue were cut, transferred into an Eppendorf tube and incubated o.n. at 58°C in a lysis solution containing Proteinase K which was subsequently inactivated at 95°C. Aliquots of the extracts were added to the PCR tubes and amplified for the 9 STR loci and the sex determination marker amelogenin contained in the AmpFI STR Profiler Plus Amplification kit (Applied Biosystems, CA), according to the manufacturer's specifications. The PCR products were processed by capillary electrophoresis through an ABI Prism 310 Genetic Analyser (Applied Biosystems, CA).

Our results show the presence of genetic instability in some neoplastic tissue. In general allele drop out rather than loss of heterozygosity is observed, with loci D3S1358, D5S818 and D13S317 preferentially involved. In a case of small-cell carcinoma we observed LOH for the D5S818 and D13S317 loci and a multi-allele pattern was seen for the D3S1358 locus. These results seem to confirm previous data on LOH for markers on chromosomes 3 (3p), 5 (5q), 13 (13q) and 17 (17p) in small cell carcinoma and on chromosome 3 in adenocarcinoma of the lung. These results suggest that these STRs should not be evaluated in forensic DNA analyses regarding this particular malignancy or alternatively a different set of STRs according to the type of cancer should be used.

Address for correspondence:

Dr. Carlo Previderè, Dipartimento di Medicina Legale e Sanità Pubblica, Università di Pavia, via Forlanini 12, 27100 Pavia, Italy, Tel. +39 0382 507810, Fax. +39 0382 528025, e-mail: provide@unipv.it

DNA STR Typing for Forensic Use. Two methods and two Instruments in comparison: IR-based Sequencer and UV-based Sequencer

U. Ricci¹, M. Klintschar², I. Sani¹ and M.L. Giovannucci Uzielli¹

¹Genetics and Molecular Medicine Unit, University of Florence – Hospital "A. Meyer", Italy

²Institute of Legal Medicine, University Halle, Germany

We recently reported the use of an IR automated fluorescence mono-laser sequencer (LICOR-4200) for the analysis of 13 autosomal STR systems (TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317, D16S359, D18S51, D21S11) and the X-Y homologous genes Amelogenin system [1]. These systems represent, together, the core of the Combined DNA Index Systems (CODIS). The protocol we prepared is based on four new independent multiplex PCR reactions, and on the direct labeling of the Forward Primer of every primer pair, with a new molecule (IRDye™800). We standardized two tetraplex systems (MU1: AME, vWA, FGA, D16S359 and MU2: D3S1358, TPOX, TH01, CSF1PO) and two triplex systems (MU3: D8S1179, D21S11, D18S51 and MU4: D7S820, D5S818, D13S317). The exact characterisation of the alleles was performed by using ladders used in earlier collaborative projects and constructed in our laboratory, with specific sequenced alleles. We used this protocol to solve paternity testing and for genetic population study of a Tuscany population.

Moreover, we used the four multiplexes to analyse forensic samples (blood, saliva, sperm, and bone) for identification purposes. A sensitivity study demonstrated that the test can detect an average of 10 pg of undegraded human DNA.

Here we report a new study to compare the use of the same four multiplex systems for forensic samples on the Infrared Automated Sequencer in comparison with an UV-based Automated Sequencer by using its specific commercial kit.

Twenty six degraded forensic samples were extracted with Quiagen kit and analysed with the AmpFISTR® Profiler Plus™ PCR Amplification kit and ABI Prism® 310 Genetic Analyser (Applied Biosystems). Furthermore eight genomic DNA samples were typed.

Afterwards, the same samples were typed with the four multiplex systems, and the IR-based DNA Automated Sequencer. The comparison between the two different methods and instruments, showed identical results.

Our data confirm again the possibility to use the four multiplex PCR, in combination with an IR-based Automated Sequencer use, to analyse the whole set of CODIS loci for forensic purposes. This protocol was compared with the more widespread method based on the UV System and commercial-available kits, with overlapping results.

[1] Ricci U, Sani I, Guarducci S, Biondi C, Pelagatti S, Lazzarini V, Brusaferrri A, Lapini M, Andreucci E, Giunti L, Giovannucci Uzielli ML, Infrared fluorescent automated detection of thirteen short tandem repeat polymorphisms and one gender-determining system of the CODIS core system. *Electrophoresis* 2000;21: 3564–3570.

Address for correspondence:

Dr. Ugo Ricci, Genetics and Molecular Medicine Unit, University of Florence – Hospital "A. Meyer", Italy, Via Luca Giordano, 13 50132 Florence Italy, Tel. 0039 555662942, e-mail: u.ricci@meyer.it

Genes and Laws: The genetically informed societies

Zs. Kozma¹ and I. Bajnóczky²

¹Department of Medical Experts, Institute for Forensic Experts, Kaposvár, Hungary

²Department of Forensic Medicine, University Pécs, Hungary

The presentation explores the ethical, medico-legal and legal ramifications of human genetic investigations based on the results of forensic and clinical human genetics.

The questions like the health insurers' use of genetic information, its possible impact on genetic discrimination, about the gene therapies, the cloning, the need and the mode of genetic testing in cases of adoption, the use of clinical and/or legal human DNA databases in the criminal or civil procedures are just few but very relevant building stones of the modern medico-legal genetic era.

In our work we also focus on the current state of the Hungarian laws covering the above mentioned fields at the same time as some other European jurisdictions. The emerging role of the European Council in the international regulations especially its European Convention on Human Rights and its amendments will be highlighted.

Finally we outline those potential problems to be encountered in the enforcement of any global treaties concerning the medico-legal genetic problems. We try to answer that question also to what extent – if necessary at all – can the Law control the possibly existing "genetic discrimination".

Address for correspondence:

Dr. Zsolt Kozma, Department of Medical Experts, Institute for Forensic Experts, Kaposvár, Po.Box 383, Hungary, H-7400

Tel: 00 36 20 3274884, Fax: 00 36 82 415224,

e-mail: zsoltkozma@free-mail.hu

Sixteen loci STR multiplex genotyping with use of 12 cm plates on an ABI Prism 377™ DNA Sequencer

G. Lago, G. Zignale, G. Vespi, G. Sechi, G. Iacovacci, M. Serafini and A. Berti

Raggruppamento Carabinieri Investigazioni Scientifiche,
via Aurelia 511, 00165 Roma, Italy.

Genotyping of 15 STR loci plus Amelogenin by use of commercial kit Powerplex™16 (Promega Co), on an ABI Prism™377 DNA Sequencer (Applied Biosystem), is generally carried out on 36 cm plates. We demonstrate that genotyping of samples amplified with Powerplex™16 can be accomplished efficiently with use of 12 cm plates.

To guarantee sufficient accuracy, different conditions were tested in over 300 runs in which 12 cm plates were used. This let us affirm that for correct genotyping, two modifications to the standard procedures are necessary: Acrylamide concentration must be increased in the gel solution in order to obtain efficient separation while the run must be performed at lower voltage so as to produce sharper bands particularly for 300–400 bp fragments.

The modifications made in the system lengthen the run by up to almost 4 hours on 12 cm plates compared to the 3 hours required for the standard protocols. There are two advantages to performing electrophoresis, genotyping 16 loci per lane, with 12 cm plates rather than 36 cm plates with ABI Prism™377 DNA Sequencer: first gel casting is more practical; secondly and more importantly, the same gel can be used for up to three consecutive runs because the lower voltage of the run exposes the gel to lower stress, maintaining sensitivity, separation, and correct allele determination.

Address for correspondence;

Cap. Dr. G. Lago – Sezione Biologia – Reparto Carabinieri
Investigazioni Scientifiche

Rome, Italy, via Aurelia 511, 00165 Roma, Italy.

Tel. +39 6 66394644 +39 6 66394665, Fax +39 6 66394737

e-mail: gplago@tiscalinet.it

Evolutionary aspects of the gene for the classical enzyme polymorphism, ACP1

L. Rudbeck and J. Dissing

Research Laboratory, Institute of Forensic Medicine,
University of Copenhagen, Copenhagen, Denmark

The human gene for the classical polymorphic marker, red cell acid phosphatase (ACP1), contains an unusual construction, two alternative exons (E3F and E3S) interspaced by a short 41bp non-coding sequence (I3F), too short to function as a normal intron. During processing of the primary RNA transcript either E3F and I3F or I3F and E3S are removed and the alternative exon is spliced into the mature mRNA. This results in the expression of two isoforms (fast and slow) of the enzyme in human tissues. This expression is allele dependent, i.e. allelic nucleotide substitutions in the E3F-I3F-E3S region influence the splicing result leading to an increase or a decrease of the ratio between the two isoforms. The fast and slow isoforms have different enzymatic properties and may have different physiological functions. There is much evidence that this low molecular enzyme may in fact function *in vivo* as a protein phosphotyrosine phosphatase (LMPTP). The LMPTP gene is highly conserved through evolution and has been found all the way back to yeast's, however, these express only one isoform. The ability to generate two functionally different isoforms by alternative splicing may be of evolutionary significance. To shed light on when in evolution this ability occurred we have analysed various species for the presence of two LMPTP isoforms and we have also detected and analysed the LMPTP gene in some of these species. Two LMPTP isoforms similar to the human fast and slow isoforms were observed in bovine and porcine cells. In both species the LMPTP gene contains a fast and a slow specific exon similar to the human E3F and E3S, but the intron between them is 6bp longer (47 bp) than in humans. Also fish was found to express two LMPTP isoforms with different properties; this was observed in species ranging from the evolutionary young (60 million years) plaice to the 350 million years old shark. However, so far we have not been able to detect and analyse the LMPTP gene in fish.

Correspondence to:

D. Sc. Jørgen Dissing, Research Laboratory,
Institute of Forensic Medicine, University of Copenhagen,
Frederik V Vej 11, DK-2100, Copenhagen, Denmark.

Phone: +45 35326125, FAX: +45 35326150,

e-mail: joergen.dissing@forensic.ku.dk

The regional pattern of μ -opioid receptor (MOR1) mRNA in human brain a real-time PCR assay

J. Becker, P. Schmidt, M. Fitzenreiter, F. Musshoff and B. Madea

Institute of Forensic Medicine, Friedrich-Wilhelms-University Bonn, Germany

The ability to monitor the amplification process online has created new opportunities for the semi-quantitative analysis of RNA and DNA. In this study a reverse transcription / real-time PCR (LightCycler) assay was established to semi-quantify the mRNA levels of the human μ -opioid receptor relative to the housekeeping gene beta2-microglobulin.

Total RNA was isolated from 9 selected regions (thalamus, caudate nucleus, hypothalamus, ventral tegmentum, hippocampus, amygdala, frontal cortex, nucleus accumbens, putamen) of 5 human brains.

The relative amounts of MOR1-mRNA were calculated for each sample by expressing the level of μ -opioid-receptor-mRNA as a percentage of beta2-microglobulin-mRNA levels, that were determined from separate PCR reactions of aliquots of cDNA for different brain regions from each case.

In the present study relatively high levels of μ -receptor expression were detected in the thalamus, the nucleus accumbens and the amygdala, whilst low levels were detected in the putamen. This pattern correlates with the reported distribution of the MOR-receptor protein.

The semi-quantitative PCR method was successfully applied to measure MOR-mRNA-levels in post mortem human brain and should be useful in further studies on steady state mRNA-levels in the brains of addicts died due to opiate overdose.

Dr. Jutta Becker, Institut für Rechtsmedizin, Universität Bonn, Stiftsplatz 12, D-53111 Bonn, Germany, Tel. 00 49 (0)228 738327; Fax: 00 49 (0)228 738339; e-mail: jutta.becker@uni-bonn.de

D1S1171: a new highly variable short tandem repeat polymorphism located on chromosome 1

B. Reichenpfader¹, E.P. Leinzinger¹ and M. Klintschar²

¹*Department of Forensic Medicine, Karl-Franzens University Graz, Universitätsplatz 4, 8010 Graz, Austria*

²*Institut für Rechtsmedizin, Martin Luther-Universität Halle-Wittenberg, Franzosenweg 1, 06097 Halle/Saale, Germany*

Abstract

This study reports the evaluation of the STR locus D1S1171 (GDB: 312934) for forensic purposes, which was investigated by PCR amplification and native polyacrylamide gel electrophoresis in 141 unrelated Austrians.

No deviations from Hardy-Weinberg expectations were observed. The mean exclusion chance (MEC) was 0.677, the discriminating power (DP) was 0.951 and the observed heterozygosity rate was 0.853. An allelic ladder consisting of ten sequenced alleles (96–132 bp) was constructed. Sequence analysis revealed a GAAA repeat motif. According to the number of tetranucleotide repeats the smallest allele was designated 9 and the largest allele 18.

Introduction

Individual differences are based on repetitive DNA sequences in the human genome. In case of short tandem repeat loci (STRs) these sequences consist of di- to pentameric repeats (Jeffreys et al. 1985, Edwards et al. 1991) with fragment lengths usually smaller than 300 bp. These properties make STRs highly suitable for forensic purposes such as stain analysis and paternity testing. As these loci are abundant in the human genome (Weber 1990) and only a small number has been evaluated up to now, the main interest should focus on the evaluation of new, more efficient STRs. The aim of this study was to determine the forensic parameters of the tetrameric repeat locus D1S1171 (GDB ID 312934) which have not been investigated so far.

Materials and methods

DNA was extracted from blood samples of 141 unrelated Austrians as described (Klitschar and Neuhuber 2000). Primers were selected from the GDB entry ID GDB 312934. Amplification and typing was performed according to Reichenpfader et al. (1999). Sequencing was done on the capillary electrophoresis system ABI Prism 310 Genetic Analyzer. Statistical analysis was performed as described (Klitschar et al. 1998).

Results and discussion

The hypervariable region of D1S1171 consists of a varying number of tetranucleotide GAAA repeats which were used for allelic designation from 9 to 18. The ten sequenced alleles with fragment lengths from 96 to 132 bp were used to construct an allelic ladder. A total of 34 genotypes was found in the 141 Austrian probands tested. No significant deviations from Hardy-Weinberg expectations were observed. The heterozygosity rate was 0.853, the mean exclusion chance (MEC) was amounted to 0.677 and the discriminating power (DP) was 0.951.

The fragment length of D1S1171 distinctly below 150 bp should render this locus as suitable for typing severely degraded stains. The polymorphism of the locus proposed in this study is higher than that of other STRs commonly used in forensic practice (Edwards et al. 1991, Urquhart et al. 1994). From the uninterrupted repeat length of this locus it can be concluded that the mutation rate should be comparable to that of other STRs (Brinkmann et al. 1998). Since typing the D1S1171 locus proved to be easy and reliable, we suggest this locus as a useful tool for both paternity testing and stain analysis.

References

- B. Brinkmann, M. Klitschar, F. Neuhuber, B. Rolf, *Am J Hum Genet* 62 (1998) 1408–15
- Edwards, A. Civitello, H.A.Hammond, C.T.Caskey, *Am J Hum Genet* 49 (1991) 746–56
- M. Klitschar, U. Ricci, N. Al Hammadi, B. Reichenpfader, A. Ebner, ML Uzielli (1998) *Forensic Sci Int* 97, 37–45
- M. Klitschar, F. Neuhuber, *J Forensic Sci* 45 (2000) 669–73
- A.J. Jeffreys, V. Wilson, S.L. Thein, *Nature* 314 (1985) 67–73
- B. Reichenpfader, R. Zehner, M. Klitschar, *Electrophoresis* 20(3) (1999) 514–7
- A. Urquhart, C.P. Kimpton, T.J. Downes, P. Gill, *Int J Legal Med* 107 (1994) 13–20
- J.L. Weber, *Genomics* 7 (1990) 524–30

Address for correspondence:

Mag. Barbara Reichenpfader, Institut für Gerichtliche Medizin, Karl-Franzens Universität Graz, Universitätsplatz 4, 8010 Graz, Austria, Tel. 0043/316/380 4356; Fax 0043/316/380 9655; e-mail: barbara.reichenpfader@kfunigraz.ac.at

Application of Restriction Landmark Genomic Scanning to Analysis of Postmortem Interval

T. Sawaguchi, A. Kitamura, R. Shimada, A. Fujiwara, and A. Sawaguchi

Department of Legal Medicine, Tokyo Women's Medical University, Japan

Introduction

Restriction Landmark Genomic Scanning (RLGS) is a two-dimensional electrophoresis method with high accuracy that has been recently developed in Japan. The purpose of this study is to investigate the possibility of a forensic application of this method, particularly for estimate of postmortem time interval.

Materials and method

The mice (C57/B6) were killed under anesthesia using diethylether and kept for 0 hours, 4 hours, 8 hours, 12 hours and 24 hours at 4°C. Genomic DNA was extracted from the liver tissues of these mice, using the Blin et al method. Methylation sensitive restriction enzyme (EagI) and methylation insensitive restriction enzyme (Eco52I) were used for DNA digestion as the landmarks in the process of the so-called RLGS-M method. In the polyacrylamide gel electrophoresis of this study, EagI or Eco52I was used as a landmark and Pst I and Pvu II were used as restriction landmark in the second dimension of electrophoresis.

First, to prevent non-specific labeling, alpha-thio dCTP, alpha-thio dGTP, ddATP and ddTTP were used as substrates and DNA polymerase I was used for blocking.

Second, Genomic DNA was cleaved by Not I.

Third, the end cleaved by Not I was labeled by alpha-32P dCTP and alpha-32P dGTP as substrates and by the profiling activity of Sequenase ver. 2.0.

Fourth, the DNA previously cleaved by Not I was treated by Pst I.

Fifth, the DNA following treatment by restriction enzymes was electrophoresed in 0.8% Seakem GTG Agarose disc gel (60 cm in length x 2.4 cm in diameter) containing 5% sucrose. 2 X Boyer buffer containing 100mM tris-acetate(pH8.0), 40 mM sodium acetate, 3 mM EDTA-2Na, and 36 mM sodium chloride was used for electrophoresis. The conditions of electrophoresis were 100V for 2 hours and 200V for 24 hours. Sixth, after the first dimensional electrophoresis the disc gel was treated by Pvu II. Seventh, the disc gel was connected with 5% polyacrylamide gel (480 cm x 410 cm x 1 mm) containing 50mM tris HCl, 62mM boric acid, and 1mM EDTA-2Na, and electrophoresed in 1xTBE buffer under the condition of 150V/90mA for 24 hours. Eighth, autoradiography was carried out after second-dimensional electrophoresis.

Results

The rate of methylation was 21.56% (0 hours), 20.28% (4 hours), 20.93% (8 hours), and 21.76% (12 hours). The RLGS method could not be applied to the materials under a 24-hour postmortem time interval.

Discussion

The gene activation reflected in the sensitivity of methylation seemed to be changed slightly by a postmortem time interval.

Reference

T. Sawaguchi, A. Kitamura, and X. Wang: Progress in Forensic Genetics. 8:422-425, 2000.

Address for correspondence:

Dr. T. Sawaguchi, Dept. of Legal Medicine, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan, Tel & Fax: 81-3-5269-7300, e-mail: tsawagu@research.twmu.ac.jp

A critical review for DNA polymorphic markers and blood group markers in paternity testing

A. Sawaguchi, X. Wang and T. Sawaguchi

Department of Legal Medicine, Tokyo Women's Medical University, School of Medicine, 8-1 Kawada-Cho, Shinjuku-Ku, Tokyo, Japan

In recent years, genomic DNA analysis technology showed a truly dramatic development in the area of biological research. DNA polymorphism is widely used in areas such as criminal investigation, human identification and paternity testing. We compared probability of paternity examined 16 DNA polymorphic markers and 20 conventional blood group markers in 50 cases of disputed paternity in our laboratory. Samples were obtained from Japanese individuals in disputed paternity casework. A comparative study of usefulness was used 16 DNA polymorphic markers (D1S80, HLADQA1, LDLR, GYPA, HBGG, D7S8, GC, D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820) and 20 conventional blood group markers (ABO, MNSs, Rh, Duffy, Kidd, Lewis, GC, Hp, BF, IF, PLG, C1R, C2, C6, C7, EsD, AcP, PGM, 6-PGD, HLA) for 50 cases of disputed paternity. The calculation of paternity probability used the Essen-Moller's formula and Bayes's theorem, and the probable genotypes of the deceased putative father was deduced by Komatu based on Bayes's theorem from the genotypes of the widow and the genotypes of their children. The mean paternity probability was calculated by StatView J 5.0 software.

The mean probability of paternity confirmation thus obtained was 0.9955 and 0.9242 for 16 DNA polymorphic markers and 20 conventional blood group markers in 39 cases of confirmed paternity. The mean rate of exclusion thus obtained was 0.3655 and 0.1591 for 16 DNA polymorphic markers and 20 conventional blood group markers in 11 cases of excluded paternity.

These results demonstrated that DNA polymorphic markers are an extremely effective method for paternity testing. Significantly high probability was obtained with DNA polymorphic markers in these paternity cases. We are planning to examine more than 16 loci using DNA polymorphic markers in paternity testing.

A. Sawaguchi, Prof. Dr., Department of Legal Medicine, Tokyo Women's Medical University, School of Medicine, 8-1 Kawada-Cho, Shinjuku-Ku, Tokyo, Japan, Tel: 81-3-5269-7300; Fax: 81-03-5269-7300; e-mail: akikosa@research.twmu.ac.jp

Exhausted Primer Method for Multiplex PCR

M. Kane^{1,2}, A. Nishimura¹ and K. Nishi¹

¹Department of Legal Medicine, Shiga University of Medical Science, Japan

²Forensic Science Laboratory, Shiga Prefectural Police Headquarters, Japan

In the case of an imbalance from locus to locus, reducing the number of PCR cycles and amplification using less template can improve the balance among loci. In this study, the primer concentration is set at minimum required to the detectable yield and we examine the efficiency of amplification among loci.

In order to obtain even PCR products, the primer of higher efficiency of amplification is exhausted during early PCR cycles, the remaining PCR cycles employ to the production of lower efficiency locus.

Generally, the smaller loci show greater amplification yield than the larger loci. However, in initial experiments of CSF1PO, TPOX, TH01, vWA and Amelogenin loci, the amplification yield of TH01, vWA loci were less than those of the others. We found that each primer concentration could play a significant role in locus-to-locus balance. Further the elimination of artifact bands may be needed to determine optimum PCR conditions. In addition, the performance of three different polymerases were compared including AmpliTaq, AmpliTaq Gold and Stoffel Fragment (more thermostable by approximately two-fold than AmpliTaq).

Address for correspondence:

Masateru Kane

Department of Legal Medicine,

Shiga University of Medical Science, Seta

Tsukinowa-cho, Otsu 520-2121, Japan

Tel.; +81-775-48-2200; Fax: +81-775-48-2200,

e-mail: nishi@belle.shiga-med.ac.jp

Multiplex PCR using New Designed Very Short Fragments of TH01, TPOX, CSF1PO, and vWA loci

K. Tsukada, K. Takayanagi, H. Asamura, M. Ota, H. Saito and H. Fukushima

Department of Legal Medicine, Shinshu University School of Medicine, Japan

Short tandem repeats (STRs) are very useful tools for identifying individuals in forensic science and criminal investigations. Because the fragment size of STR is smaller than that of RFLP-VNTR, it is suitable for amplification of DNA extracted from degraded materials. While multiplex PCR systems consume more template DNA than multiplex PCR systems, they are capable of detecting many STR loci in a single analysis. This makes multiplex PCR a highly useful tool for criminal investigations.

Many STR loci are used to identify individuals in forensic science and in criminal investigations. In this study, we examined the TH01, TPOX, CSF1PO, and vWA loci, which have more alleles than others. If the amplified STR fragment size is greater than 200 bp, it is difficult to detect STR fragments that are amplified from a highly degraded DNA template.

We designed a new pair of primers that reduce TH01, TPOX, CSF1PO, and vWA loci fragment sizes, compared to the known protocol. These loci can be detected in the range of 72 bp to 150 bp amplifying multiplex PCR. In this experiment, we performed multiplex PCR using DNA extracted from 20-year-old blood stains. Electrophoresis was performed on an ABI 310 Genetic Analyzer, and alleles were determined by GeneScan 2.1 software.

Kazuhiko Tsukada

Department of Legal Medicine,

Shinshu University School of Medicine,

Asahi 3-1-1, Matsumoto, Nagano 390-8621, Japan

Tel 08(0263)373218, FAX 08(0263)373084,

e-mail: phosphoy@po.cnet-na.ne.jp

Investigation of DNA Extraction from Hair Shafts

K. Takayanagi, H. Asamura, K. Tsukada, M. Ota, H. Saito and H. Fukushima

Department of Legal Medicine, Shinshu University School of Medicine, Japan

While human hair shafts can serve as important forensic evidence for identification, DNA typing, even with mitochondrial DNA (mtDNA), is difficult due to the small quantity and the degradation of DNA or water-soluble eumelanins, which inhibit Taq DNA polymerase in polymerase chain reaction (PCR).

We describe three methods for extracting DNA from hair shafts, including the Phenol/Chloroform method, the Nal treatment method, and the Silica-beads method.

Following amplification of the mtDNA control region (D-loop) HV1A (15997-16262) using a FAM-labeled forward primer, the mtDNA amplification rate and efficiency were investigated by comparing fluorescence peak heights from electrophoresis with a 310 Genetic Analyzer. Due to extractions from different lengths of hair shafts and variations in template volume, nearly all fluorescent peak heights observed in DNA recovery by the Nal treatment method and Silica-beads method were higher than that by the Phenol/Chloroform method. With the degraded sample, amplification from the template by the Phenol/Chloroform method produced no results. In contrast, high fluorescent peak heights adequate for sequencing mtDNA were obtained from our two methods, confirming that the Nal treatment and Silica-beads methods are valid, reliable means of extracting and amplifying mtDNA from hair shafts.

Dr. Kayoko Takayanagi
Department of Legal Medicine,
Shinshu University School of Medicine,
Asahi 3-1-1, Matsumoto, Nagano 390-8621, Japan
Tel 08(0263)373218, FAX 08(0263)373084,
e-mail: kayoko@sch.md.shinshu-u.ac.jp

Intragenic haplotypes and molecular evolution of the human α 2-HS glycoprotein (AHSG/fetuin) gene

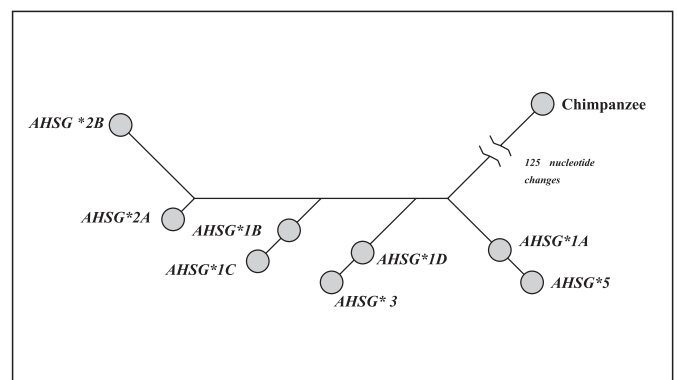
M. Osawa^{1*}, I. Yuasa², J. Henke³, M. Kaneko¹ and K. Umetsu¹

¹Department of Forensic Medicine, Yamagata University School of Medicine, Japan

²Department of Legal Medicine, Tottori University School of Medicine, Japan

³Institut für Blutgruppenforschung, Germany

Alpha2-HS glycoprotein (AHSG/fetuin) is a glycoprotein found in human plasma. AHSG is polymorphic with two common alleles and many variants. To examine the intragenic haplotypes and their diversity at this locus, a contiguous genomic DNA sequence (10.3 kb) was analyzed in 40 chromosomes, and haplotypes were determined for 309 subjects. Judging from the aligned nucleotide sequences in human and chimpanzee AHSG, it was concluded that the type 1 allele is older and has evolved into four major suballeles. The type 2 allele was generated from one major branch of the type 1 allele. AHSG*3 and *5 variants were each found to have a single nucleotide change in exon 7, resulting in an amino acid change from Arg₂₉₉ to Cys and from Asp₂₅₈ to Asn, respectively. The AHSG gene was found to show a low mutation rate and no apparent recombination events. Furthermore, the detected substitutions were nonhomogeneously distributed at this locus. In particular, four nonsynonymous substitutions were concentrated in the carboxyl-terminal domain.



Forensic applications of denaturing High Performance Liquid Chromatography: determination of age at death, gender determination and human identification

P. Cathala^{1,2}, E. Baccino², M. Claustres¹ and A.F. Roux¹

¹Laboratoire de génétique moléculaire,
Centre Hospitalo-Universitaire, Montpellier, France

²Service de médecine légale,
Centre Hospitalo-Universitaire, Montpellier, France

Introduction

Denaturing High Performance Liquid Chromatography (dHPLC) is a new efficient tool for genomic analysis. The separation is based on the Ion-Pair-Reversed-Phase-HPLC technology in denaturing or non-denaturing conditions (denaturation is obtained by heat and by a gradient of acetonitrile).

This presentation introduces 3 different forensic applications of this new technology.

Age determination by mutations detection

Introduction

Accurate estimation of age at death is an important problem in forensic sciences. The macroscopic methods (using teeth and bones) which are commonly used provide results with too wide a confidence interval in individuals older than 45. Several publications have shown that various mutations accumulate in mitochondrial DNA during ageing. According to these results, we are developing a new and original method to determine age at death by mutations detection. Actually, when used in denaturing conditions, dHPLC provides a rapid, automated scanning method for mutations, even when the nature and location of the mutation is unknown.

Materials and methods

Mitochondrial DNA is extracted from forensic autopsy tissues (iliopsoas, liver, kidney, putamen and heart). A total of 100 individuals representing a wide age spectrum will be included in this study. Two commercial extraction kits are compared. DNA is first amplified in 13 fragments of 1–2 kb [Nucleic Acids Res. 2000 Oct 15;28(20):E89]. Each PCR fragment is then digested into smaller fragments (90–600 bp) which can be separated in the dHPLC system. The separation is performed with a Transgenomic WAVE[®] at different temperatures to ensure the complete mitochondrial genome screening. We are studying the qualitative and quantitative differential accumulation of mutations with age among the various tissues. Our first results will be presented.

Gender determination

Used in non denaturing conditions, the WAVE[®] can separate the 2 sex-specific alleles of the amelogenin locus. The separation of the X-specific and of the Y-specific allele is performed in less than 10 minutes without any preparation of the PCR product.

Human identification (separation, purification and sizing of STR)

In non denaturing conditions, dHPLC gives strict and reproducible size-based separation of DNA fragments up to 2000 bp. This allows separation and purification of short tandem repeats (STRs) DNA fragments. For example, the different STR alleles of HUMTH01 can be separated by dHPLC with a good resolution (>1) in less than 14 minutes and their size can be determined with accuracy and precision. Other STRs have been studied like the F13A01, vWa31 and FES/FPS loci.

Conclusion

dHPLC is a new automated, and fast, sizing method when used in non denaturing conditions and is a powerful mutation detection tool in denaturing conditions. These applications might represent a great interest in forensic genetics.

Philippe Cathala,
Laboratoire Génétique Moléculaire et Chromosomique,
Institut Universitaire de Recherche Clinique,
641 av du Doyen G. Giraud, 34093 Montpellier Cedex 5,
France; e-mail: Philippe.Cathala@igh.cnrs.fr;
Tel: 33 (0)4 67 41 53 60; Fax: 33 (0)4 67 41 53 65

Natural radioactivity and human mtDNA mutations

L. Forster^{1,2,3}, P. Forster^{1,2}, H. Willkomm⁴, B. Brinkmann¹

¹Institute for Legal Medicine, University of Münster, Germany

²Molecular Genetics Laboratory, McDonald Institute for

Archaeological Research, University of Cambridge, UK

³Alleppey, Kerala, India

⁴Institute for Pure and Applied Physics, University of Kiel, Germany

A 10-kilometre stretch of coast in Kerala (India) contains the world's highest level of natural radioactivity in a populated area, offering a unique opportunity to identify "radiogenic" DNA mutations and to compare them with "evolutionary" mutations. We have sampled DNA from a total of 988 individuals from 247 native families (covering 791 mtDNA transmissions).

Two thirds of the samples were taken from the radioactive coastal strip, and one third from the 3km-distant, non-radioactive hinterland as a control population. We sequenced the control region of their mitochondrial DNA, and found both point mutations and homopolymeric length changes between mothers and their offspring. In each mutation case we confirmed maternity with a probability of >99.15% by typing nine autosomal loci.

Our study reveals three main results:

- The families living in the radioactive area have 7 times ($SD \pm 1.02$) more new point mutations than the control families.
- Strikingly, the new mutations primarily affected nucleotide positions previously identified as hypervariable in evolutionary studies, raising the possibility that evolutionary point mutations in human mtDNA are largely the direct or indirect result of radioactivity.
- Of central importance to medical, forensic, and evolutionary geneticists is the finding that none of the point mutations attained fixation in any individual, resolving the perceived conflict between "evolutionary" and "pedigree" mtDNA mutation rates.

Corresponding author:

Dr. Peter Forster

Molecular Genetics Laboratory

The McDonald Institute for Archaeological Research

University of Cambridge

Downing Street, Cambridge

CB2 3ER, England

Tel.: +44-1223-339330, Fax: +44-1223-339285

e-mail: pf223@cam.ac.uk

Study of Spanish Public Awareness Regarding DNA Databases in Forensic Genetic

J.J. Gamero¹, M^a.C. Vide², J.L. González¹, J.L. Peralta¹
and J.L. Romero¹

¹Department of Legal Medicine, Faculty of Medicine.

University of Cádiz. Fragela s/n, Cádiz 11003. Spain.

²National Institute of Legal Medicine.

Department of Coimbra. 3000 Coimbra. Portugal

The information obtained from the DNA genetic analysis may furnish a large quantity of data which could result in discriminatory consequences in such areas as health, insurance, employment and the law. Among those rights that may be affected, the following are to be pointed out: the right to privacy, freedom, equality and to non discrimination. Bearing this in mind, the low level of protection afforded to databases that store personal information concerning health must be highlighted, along with the need for regularization within the field of forensics (draft bill for a law regulating DNA data bases in Spain, 1999). There seems to be certain ignorance with regard to the basic norms that regulate databases containing private/personal information as well as to the protection that information of this type must necessarily be given. Due to the situation, the development of educational projects leading to increased public awareness of the implications DNA databases may have for society is highly desirable. We believe that the opinion survey to be carried out among the population as well as the subsequent analysis in ethical terms of the results, may serve to reveal the degree of information that society has with regard to DNA databases. Furthermore, this study may also help in the elaboration of recommendations regarding the creation of databases related to forensic genetics. In the case of forensic genetics, we believe that the results of such a survey may serve not as help with the preparation of a new draft bill for a law regulating DNA databases in Spain (as we consider that this is not a responsibility of ours) but, as stipulated in the abovementioned draft, in the elaboration of subsequent regulations where all type of details related with databases are taken into account.

Address:

Joaquín-José Gamero Ph. M.

Dpto. Medicina Legal

Facultad de Medicina

Universidad de Cádiz

Plaza Fragela s/n

11003, Cádiz, Spain.

e-mail: joaquin.gamero@uca.es

The development of integrated case and laboratory information management systems for forensics laboratories

J. Nolan¹, P. Mee², R. Castelnovo³, G. Firpo⁴ and F. Denimal⁵

¹Applied Biosystems, 3833 North First Street, San Jose, CA 95134 USA.

²Applied Biosystems, Kelvin Close, Birchwood Science Park North, Warrington, WA3 7PB, UK

³Applied Biosystems, Via Tiepolo 18, 20052 Monza (MI) Italy

⁴Applied Biosystems, Parc Tecnologic del Valles, Avda Universitat Autònoma, 3A Baixos 08290 Barcelona Spain

⁵Applied Biosystems, 25, Av de la Baltique B.P. 96 91943 Courtaboeuf, Cedex, France

Throughout the world the workload and throughput of forensics laboratories is increasing significantly. Amongst the factors behind this are; the establishment of national DNA databases, increases in the amount of case work required by the criminal and civil authorities and the availability of higher throughput technology for the analysis of the samples.

As a result of these factors and the increasingly tight regulatory framework in which these laboratories must operate, many workers are recognising the need to enhance the data management systems used to process and store the data.

The requirements of such a system include, centralised data management, facilities for sample and workflow management and tracking, sophisticated security and access control, integration of instrumentation and results analysis software, scalability and adaptability.

A variety of technical approaches are open to labs seeking to develop such a system.

This presentation will assess those options, looking at the practical and technical issues associated with each.

We will present, using case studies, the approach we have taken to fulfill these requirements drawing from our experience in implementing forensics data management solutions in a variety of different forensics and paternity testing laboratories. Our solutions are built upon a robust underlying LIMS system, which serves to address the technical issues faced by the forensics community and provides a robust and flexible platform for the future developments.

Applied Biosystems are a leading supplier of genetic analysis technology and data management solutions to the life sciences community.

Address for correspondence:

Paul Mee, Applied Biosystems, Kelvin Close,
Birchwood Science Park North, Warrington, WA3 7PB, UK,

Tel +44 (0) 1925 282454

e-mail: paul_mee@eur.appliedbiosystems.com

Swiss Federal DNA Profile Information System

M. Strehler, Adelgunde Kratzer and W. Bär

Institut für Rechtsmedizin, Universität Zürich-Irchel, Switzerland

On 1 July 2000, the Swiss Federal Council (Swiss Government) set the regulation for the DNA Profile Information System that is operational since 1st of August 2000. The regulation contains a specified catalogue of crimes that regulate the admittance of DNA profiles from suspects. Buccal swabs are taken from suspects by the police and for stains the C.D.S. swap box is usually used. The samples are transferred to the responsible DNA laboratory at the Institutes of Legal Medicine at Basel, Berne, Geneva, Lausanne, St. Gallen and Zurich. The police anonymizes all samples with a unique identification number (PCN). At the same time they transfer the name of the suspect, his/her fingerprints as well as other relevant information to the AFIS services of the federal police at Berne. The forensic genetic laboratories transmit the DNA profiles to the DNA Coordination Unit at the Institute of Legal Medicine at Zurich that imports the profiles into the CODIS database. Daily automated searches are performed and so-called hits – identical DNA profiles between stains or stains and persons – are reported to the AIFS services by only providing the relevant PCN numbers. The AFIS services link the PCN numbers with the corresponding names and crimes and inform the investigating police unit about the hit. By this procedure a strict separation of DNA data and other personal data is achieved.

Eleven DNA systems are analyzed: D3S1358, VWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, FGA (FIBRA) and AMELOGENIN.

Approximately 500 DNA profiles are entered monthly into the database and by the end of April 2001 approximately 4500 profiles from suspects and 400 profiles from stains were stocked. So far 90 person-to-stain and 47 stain-to-stain hits were observed. The largest group of crimes linked by hits were burglaries followed by offences like endangering life and against the sexual integrity.

A federal DNA law is in preparation and should harmonize the legal situation for the whole country and replace the actual regulation that only legalizes the procedure until the end of 2004.

Address for correspondence:

Prof. Dr. med. W. Bär, Institut für Rechtsmedizin,
Universität Zürich-Irchel

Winterthurerstrasse 190/Bau 52, CH-8057 Zürich,
baer@irm.unizh.ch

ForumDNA, a customdesigned Laboratory Information Management System

C. Karlsson and S. Holgersson

National Laboratory of Forensic Science, S-581 94 Linköping, Sweden

ForumDNA is a Laboratory Information Management System (LIMS) developed to support DNA-analyses and is integrated with a case-handling system. The systems are customdesigned by Ida Infront AB in collaboration with the National Laboratory of Forensic Science in Sweden. The platform is called iPax 2 and the database is an object database. The system has several advantages in comparison with manual handling, e.g. in terms of quality assurance, the simplicity by which information is collected as well as time saving aspects.

New cases are registered electronically as well as all material, reference samples and questioned samples. When a DNA-analysis is to be performed an order is created in LIMS specifying e.g. extraction method, typing method and priority level. During the analysis new objects are created e.g. positive control, negative control, batch, quantification result and typing result. The objects in the database have references to each other and can be displayed in a tree-structure. During analysis the order is the central object and it has a lifecycle determining how the sample is to be processed. Batches of samples with the same method are suggested by the system. If needed, the batch can be changed or rearranged manually. After each step in the analysis a batch is reported and individual decision can be made on each sample e.g. repeat the amplification, make a new quantification and stop the analysis. When a DNA-analysis is finalised an electronic message is send to the reporting officer. A summary of all results in the case can then simply be created including all samples, their orders, quantification and typing results. When all orders in the case have been finalised a statement is written, all materials are included in the report automatically, and the case is closed.

Address for correspondence:

MSc Carina Karlsson, SKL, S-581 94 Linköping, Sweden.
Tel. +46 (0)13 24 14 05; Fax: +46 (0)13 14 57 15,
e-mail: bio@skl.police.se

"Cytochrome b and mitochondrial DNA control region analysis of domestic animal hair in forensic casework"

M.M. de Pancorbo^{1,3}, A. Castro^{2,3}, I. Fernández-Fernández^{2,3}, González C¹, Martínez-Bouzas¹ and N. Cuevas²

¹Dpto. de Z. Dinámica Celular A., F. Farmacia, U. País Vasco, Vitoria-Gasteiz, Spain

²DataGene, Sondika (Bizkaia) Spain

³Instituto Vasco de Criminología, U. País Vasco, San Sebastián-Donostia, Spain

Introduction

Biological traces that appear at the scene of a crime or on the body of the victim may be of human, animal and/or vegetable origin. Among

those of animal origin, household pets are a common source, with pet hair being one of the most frequent traces found. Consequently, it is necessary to have laboratory methods capable of identifying traces from pets and domesticated animals in general.

Savolainen et al. (J. Forensic Sci. 1997; 42(4) : 593–600) have developed a basic method of sequencing the HVI region of mitochondrial DNA from *Canis familiaris* using single hairs as template, with a discrimination capacity of one in ten individuals.

The species from which a biological trace has come can be identified by analyzing a short fragment of the cytochrome b (cytb) gene sequence of the mitochondrial genome. This gene contains species-specific information and has been used in phylogenetic as well as in forensic investigations in a number of studies. Parson et al. (Int J Legal Med 2000; 114: 23–28) have confirmed the usefulness of cytb analysis in identifying the biological origin of casework specimens.

Objective

Due to the homology between certain segments of the hypervariable region in a number of animal species, the aim of the present study is to show that in order to identify an animal hair, it is first necessary to determine the species of origin by cytb analysis, before proceeding to identification through analysis of the HVI region.

Methodology

A number of specimens were analyzed of canine, feline and bovine origin, among others. The samples analyzed were saliva from control animals and traces of hair from forensic casework. Cell lysis was performed using proteinase K and SDS. The DNA was purified using the phenol-chloroform method. Lysis of the hair samples lasted as long as necessary to dissolve the hair, adding proteinase K every 24 h. PCR amplification of 358 pb of the cytb was done under the conditions described by Parson et al. (2000). PCR amplification of the HVI region was performed according to the method described by Savolainen et al. (1997). All the samples were sequenced using the dRhodamine Terminator kit (Applied Biosystems) method in an automatic ABI Prism 310 DNA sequencer. The sequences were aligned using the Clustal program and they were also used to identify the biological origin of the samples by aligning to the cytb gene sequence entries using the program BLAST.

Results and Conclusions

By analyzing the fragment of the HVI segment of mitochondrial DNA, animals of the same species can be distinguished, but this method cannot clearly establish differences between different species. For this reason, we first analyzed cytb to determine the species of origin of each sample. This procedure made it possible to classify each sample correctly according to its biological species. In conclusion, given the possibility of obtaining PCR product of different species using the same primers to amplify the HVI segment of mitochondrial DNA, differences found in the HVI region could indicate the existence not only of different individuals, but even of different species. For each sample, cytb fragments and HVI should be analyzed at the same time to obtain more accurate information showing whether the nucleotide differences found merely indicate that the specimens are from different sources of the same species, or whether the samples are from different species. This information could be of great interest for solving criminal cases.

Address for correspondence:

M.M. de Pancorbo, Dpto. de Z.y Dinámica Celular, Facultad de Farmacia, Universidad del País Vasco. Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain. Tel. 00 34 945 013045; Fax: 00 34 945 013756; e-mail: gcpmagom@lg.ehu.es

The First Criminal Case In Estonia With Dog's DNA Data Admitted as Evidence

A. Aaspõllu^{1,2} and M. Kelve²

¹Forensic Service Centre, Tallinn, Estonia

²National Institute of Chemical Physics and Biophysics, Tallinn, Estonia

A body of a young woman was found in the field near a small village. The forensic examination suggested that the woman was attacked by a dog (or dogs). Dog's hairs and possible saliva stains were recovered from the coat of the woman. The reference hairs and saliva samples were collected from six different dogs living in the neighbourhood.

The StockMark kit for Dog Parentage Verification and Identification (Applied Biosystems) was used to analyze the crime scene and reference samples. The data obtained were used to establish these dogs who were not included in the case.

The analysis of the nuclear DNA was not successful in case of some of the hair samples. Next a segment of the mitochondrial DNA hyper-variable (HV) I region was analyzed. The primers were used to generate a 140 bp and a 125 bp fragments which were sequenced.

As the result of the study the link was established between the evidence material and the suspects (dogs).

Dr. Anu Aaspõllu, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia.
Tel.: +372 6398384; Fax: +372 6398382;
e-mail: anu@kbfi.ee

Primer separation of STR-multiplex amplified samples can avoid appearance of allelic drop outs in capillary electrophoresis

Th. Lederer¹, G. Braunschweiger², P. Betz¹ and S. Seidl¹

¹Institut für Rechtsmedizin, Universität Erlangen, Germany

²Serac, Serologische Reagenzien GmbH, Bad Homburg, Germany

Capillary electrophoresis is a widely used technique for the analysis of PCR-amplified short tandem repeat (STR) polymorphisms in forensic casework analysis. Reproducibility of fragment sizing and high sensitivity due to fluorescent based laser detection can be seen as two of the major advantages of this method. Nevertheless, analysis of micro-specimen containing limited amounts of DNA may lead to allelic drop out phenomena. These may be based on non-amplification during PCR, but could also be the consequence of missing detection during electrophoresis.

In this study, the effect of primer separation of *genRES*[®] MPX-2 (nine locus multiplex, Serac, Germany) amplified samples prior to capillary electrophoretic analysis has been investigated. Six samples containing limited DNA amounts were analysed. Sample purification (QIAQUICK PCR purification kit, Qiagen, Germany) led to an increase of the relative fluorescent signal intensity by a factor of 3.8 ± 0.8 . This effect was observed for specific allele signals as well as for the internal lane standard LS 500 ROX. In contrast, the application of higher sample volumes (3 μ l instead of 1 μ l) led to a decrease of standard signal intensities up to 20–40%, depending on whether samples were purified (20%) or not (40%). Nevertheless, signal intensity of allele peaks were slightly enhanced by a factor of 1.7 ± 0.5 . Generally, the effect of increasing sample volume was more pronounced when purified samples were used. In addition, increase of injection time showed a linear increase of signal intensity between 3s and 10s. Higher injection times may influence electrophoretic fragment separation and, therefore, lead to broad signals which may not sized correctly.

Optimization of sample preparation (purification, applied volume) and increase of injection time may intensify signals up to about 12-fold. As a consequence, appearance of allelic drop out phenomena can be significantly reduced or even be totally avoided.

Dr. rer. nat. Th. Lederer, Institut für Rechtsmedizin der Universität, Universitätsstr. 22, 91054 Erlangen,
Tel.: +49-9131-8522272,
e-mail: thomas.lederer@recht.imed.uni-erlangen.de

Differences in DNA preparation from various tissues and fluids of human corps

T. Dobosz, A. Lebioda, J. Kawecki, J. Jagielski, B. Swiatek

Medical University, Institute of Forensic Medicine,
Mikulicz-Radeckiego 4, 50-368 Wrocław, Poland

The various human body fluids and tissues were tested and compared as source for DNA preparation. In accessible literature only Bär et al./1988/ analysed efficiency of various human body tissues as potential source for DNA preparation, but authors focused mainly on the stability of DNA and tested only selected tissues. The two wide known methods of DNA macro preparation are most frequently used in the moment: the phenol ("organic") method and the "non toxic" salt method. The purpose of this paper is the comparisons of two above mentioned methods used for extraction of DNA from almost all fluids and tissues of the human body. The various body fluids (at volume from 1 to 10 ml) and tissues (at weight from 1 to 10 g) were used for DNA preparation using above mentioned methods. The purity of DNA was examined spectrophotometrically. The minigel analysis were performed in 2% LGT Agarose /Sigma/ and 1x TBE buffer, and examined on transilluminator with ethidium bromide staining. From obtained data arise, that the best source of human DNA is a testis or an ovary, despite of age of the person which corps were examined. It is possible to obtain up to 10 times more of DNA than from the same weight of whole blood. Examination purity showed, that the best pure DNA may be obtained from brain, liver and pituitary gland. In the other hand, the liver tissue have high activity of nucleases and is difficult to obtain long fragments of DNA from it. Testing of relative viscosity shown, that the best source of high polymorphic DNA seemed blood. Obtained data shown, that the efficiency of both used methods of DNA preparation are similar.

Bär W., Kratzer A., Machler M., Schmid W. (1988) A postmortem stability on various human organ tissues. *Adv. Forens. Haemogen.* 2, 392–395.

Discrimination of monocytic twins (and clones) on the DNA level

D. Schlieper¹, A. Ehlich² and M. Benecke³

¹Institute of Biochemistry, University of Cologne, Germany

²Institute for Genetics, University of Cologne, Germany

³International Forensic Research & Consulting, Cologne, Germany

Recent DNA typing methods (RFLP, STR, RAPD) do not allow discrimination of monocytic twins. To overcome this restriction, we suggest the use of variable DNA sequences of bone-marrow derived memory B lymphocytes that are likely to be different even in monocytic twins. Since memory B cells are transported in the blood stream, they can be found in blood stains on crime scenes and checked for a match to the cells of a living pair of twins. The size of the antibody repertoire has been estimated to comprise theoretically up to 10¹⁰

specificities. Since each B lymphocyte is endowed with a single antibody specificity, this estimate corresponds to the number of different B cells that can be generated. A major source of antibody diversity in the preimmune repertoire is the stochastic recombination of V, D, and J elements of the immunoglobulin heavy (IgH) chain locus. It takes place in B cell precursors in the bone marrow, and it results in the generation of genes encoding immunoglobulin heavy chains. Apart from random selection of V, D, and J elements, diversity is increased by the random addition of non-germline encoded nucleotides (N sequences) and the addition of nucleotides palindromic to the termini of rearranging gene segments (P nucleotides) resulting in extremely diverse stretches of approximately 45 bp. These stretches should be specific markers to aid forensic differentiation between monocytic twins because, due to their extreme diversity, they are highly unlikely to be shared by two individuals in the subset of B cells that forms the population of memory cells. Upon stimulation by an antigen, specific B cells are activated. In the germinal centers (in secondary lymphoid organs like spleen, lymph nodes, Peyer's Patches) they proliferate and differentiate into antibody secreting plasma cells and memory cells. At the same time, further diversity is generated by the introduction of point mutations into Ig genes (somatic hypermutation). In contrast to naïve, antigen inexperienced B cells, memory cells are clonal. They are long-lived cells and can provide immunity to the specific antigen for decades. Even if two individuals will have undergone an immune response to the same antigen, the pools of memory cells generated are likely to differ due to the variability in naïve B cells that are recruited into the response. This means that from a statistical standpoint, it is unlikely that monocytic twins will share a majority of identical VDJ stretches in memory B cells. VDJ genes from B lymphocytes in a blood stain can be isolated by PCR and sequenced. Sequences that are derived from memory B cells can be identified by the presence of somatic hypermutations. The PCR primers used are specific for sequences downstream of J and inside of V elements, respectively. This will result in amplification of VDJ regions of all B cells in a given sample. 25% of all amplicates are expected to represent VDJ sequences from memory cells. The forensic question to be asked in a case involving monocytic twins would be: In whom of both twins are memory B cells (i.e. specific VDJ combinations) present that are identical to the ones found in a given stain? Technically, native blood samples of both twins would be taken, and memory B cells would be isolated by fluorescence-activated cell sorting. Then, possible VDJ sequences matching the stain-derived ones would be detected by PCR using primers specific for the sequences in question. If a specific VDJ sequence of one of the twins' blood cells matches a VDJ sequence in a stain, a possible match is established. Due to the high variance in specific VDJ sequences, false positives are unlikely. On the other hand, false negatives are possible, as the lack of a specific VDJ sequence in one individual might not exclude this individual: Depending on the size of the memory B cell clone no cell of a given type might be found in an actual sample of native blood. The data needed to calculate the probabilities for the exclusion of the matched twin and for the inclusion of the other twin are not yet fully available. In particular, comprehensive statistical data concerning the diversity of the available B cell repertoire and on the size of memory clones in humans have still to be established. In any case, an important piece of circumstantial evidence might be obtained by our investigation method. Apart from the potential use for forensic purposes, the method described here can also be applied to distinguish individuals within a population of animals, e.g. sheep or cattle. This might be inbred lines, twins, or clones.

Correspondence:

Dipl.-Biol. Dr. rer. medic. Mark Benecke,

Internat. Forensic Research & Consult.,

Postf. 250411, 50520 Köln, Germany,

e-mail: forensic@benecke.com, Fax +49-221-660-2644,

http://www.benecke.com/

Adaptation of different PCR systems to the identification of criminal postmortem human remains

A.G. Smolyanitsky, A.I. Smolyanitskaya, V.L. Popov and G.I. Zaslavsky

Forensic Bureau of Leningrad District, Saint Petersburg, Russia

Identification of the bone remains using DNA-typing is highly complicated task. In such cases some commonly used system like VNTR and others are not always applicable. As usual DNA isolates from the old bone samples, which were hold for a long time in ground, in water, or destroyed due to fire, are contaminated with some impurities inhibiting polymerase reaction. As a result even after additional purification stages the DNA-typing remains to be impossible. Our experience shows that with the samples from humid places the sex determination using extremely sensitive system based on the amplification of the segment of the amelogenin gene was the only possible test. Nonetheless we found that some amplification systems can be applicable in most of practical situations. We compared several commercial AMP FSP systems: HLA DQ A1, LDLR, GYPA, HBG, D7S8, GC (all manufactured by Perkin-Elmer Corporation, USA) and AMP FLP systems: D1S80, ApoB, Ig-JH, Col2A1 and D17S30 (manufactured by Helix, Ltd., Russia). We found that even in highly complicated cases when the bones are strongly destroyed the above systems can be optimized and adapted to reach the positive DNA-typing. For instance, decalcification of samples as well as an increase in the amount of the bone material (from 200 mg to 5 g) leads to positive results. Also important is the type of bones. Better results were obtained with large tubular bones, teeth and cranial bones. The most successful adaptation was observed with the first six systems. Thus even in most complicated cases the problem of positive identification of criminal postmortem human remains can be successfully solved using suitably optimized procedure for the DNA sample isolation as well as relevant adaptation of the PCR system.

Address for correspondence:

Dr. Andrew Smolyanitsky, Forensic Bureau of Leningrad District, Shkapinstr. 36/40, Saint Petersburg, 198092, Russia, Tel.: +7 (812) 251 4000, Tel./Fax: +7 (812) 251 1600, e-mail: v_sidorov@mail.ru

Family Relationship proven by Chromosomal and DNA Examination

Georgette Bujdoso^{1,2}, Peter Sotonyi^{1,2}, Andras Laszik¹, Max P. Baur³

¹*Institute of Forensic Medicine, Semmelweis University, Budapest, Hungary*

²*Research Unit sponsored by Hungarian Academy of Sciences*

³*Institute of Medical Biometry and Epidemiology, University of Bonn, Germany*

The authors present some interesting cases from the huge archive of their past 20-year practice in the field of family member identification. These people were mostly adults who could find their family roots after being in doubt for decades.

One of the most interesting cases will be presented, where the "last" hungarian Prisoner of War (POW) was identified with his relatives after more than half a century. In this case not only the traditional methods (blood groups, anthropology) but also the recent cyto- and molecular genetical methods (chromosomes, DNA) were used.

Incestuous Offspring Detection Inference by VNTR Homocigosity Increment

D. Corach, G. Penacino, M. Marino and A. Sala

Serv. de Huellas Dig. Genéticas y Cát. Genética y B.M.,
Fac. Farm. y Bioq., Univ. Bs. As., Argentina

Almost universally, sexual relations between parent and child and between sister and brother are forbidden. This kind of sexual intercourse is defined as incest. In it, the people involved are usually legally prohibited from marrying because of their close matrimonial or blood relationship. In addition, when children are involved violence and sexual abuse are usually committed, at least in most modern civilizations. Recent studies have indicated that incest, although rarely discussed, is a continuing problem in modern society. Sexual molestation by family members is a recurring form of child abuse. Prohibition of incest as a social behaviour may rely on a genetic base. It is known that inbreeding fosters the concentration of no desirable recessive genes. Reduction of heterozygosity levels in polymorphic genomic sequences are also characteristic in incestuous offspring.

In the forensic field, the statutory crime of incest consists of either cohabitation or sexual intercourse between closely related persons, such as between parent and child or between siblings. As a special case of paternity tests, the unique unbiased approach for incestuous offspring identification is the DNA typing. In addition this technique also may provide information that may allow inferring the incestuous origin of an offspring even in the absence of the biological mother. In some cases incest detection may arise during a regular paternity test. The aim of this contribution is to present the comparative analysis of incest cases, involving fathers – daughters, investigated by means of diverse VNTR platforms, ranging from manual minisatellites (YNH-24, MS-1, LH-1, PH-30, TBQ-7, EFD-52 and CEB-42) to microsatellites in manual platform (HUMTHO-1, HUMFABP, TPOX, CSF1PO, VWA, F13AO1, FES/FPS, D16S539, D7S820, D13S317 and D6S366) or by means of automated STR typing (using the commercial kits: Profiler Plus, Cofiler and Powerplex 16). The frequency distribution of homozygous markers is completely skewed when compared with non-incestuous offspring. Although, some overlap may be detected when incestuous-non incestuous distribution of homozygous are compared, if the homozygosity percent is over 40% the offspring can be confidentially be considered as incestuous. As expected minisatellite results denote a lesser degree of homozygosity in incest cases, due to the higher allele number and a more homogeneous distribution of the allele frequency.

This contribution may facilitate the interpretation of cases in which the daughter-wife, sister-mother is unavailable, as well as when an unrelated suspect is accused to be a biological father, but the offspring depicts a homozygosity percentage over 40%.

Determination of blood volume of blood stains on clothes. A case report.

A. Pifarré, E. Ramírez, D. Solá, R.M. Fernández, J.L. Valverde.

Instituto Nacional de Toxicología, Dept. de Barcelona,
Sección de Biología. Barcelona, Spain

Death due to hypovolemic shock may occur when lost blood volume reaches about one third of the whole volume contained in an adult human body. Bloodstained clothes and other objects found in a car were sent to our laboratory. The aim was to investigate the entity and the amount of blood present on clothes and objects in order to determine if the amount of blood present on them was enough to cause death.

DNA extractions have been performed from each sample. AmpliFSTR Profiler Plus and Cofiler kits (Applied Biosystems) have been used for PCR amplifications to determine the entity of blood stains.

Dry weight of blood measurement has been performed using Strassmann and Ziemke method, by which identical surfaces have been cut, both completely stained with blood or totally clean. Weight means for both stained and clean cuttings have been calculated for each clothing. Dry weight of blood contained in spots on clothes per surface unit has been obtained by calculating differences between both values. Total spotted surface has been calculated using tracing paper. Stains have been traced and the resulting piece of paper weighed. Applying weight/surface ratio, surface of each stain has been obtained. Harmstem ratio (1 L of blood yields 211 g of dry weight) has been applied and volumes obtained for each clothe summed. Results are shown on table 1.

DNA profiles obtained from blood stains were all identical, supporting the hypothesis that blood stains were from the same person. Other DNA-based identification procedures have been performed by other experts.

Table 1.

Sample	Weight/surface ratio g/cm ²	Spots surface cm ²	Total dry weight g	Blood volume L
Blanket 1	0.043440	2344.600	101.84942	0.48269
Blanket 2	0.045150	4518.330	204.00	0.97142
Shirt 1	0.002100	1619.066	3.474	0.01646
Shirt 2	0.000044	242.330	0.0106626	Negligible
T-shirt**	0.008359	8254.220	68.997	0.32700

Total volume calculated: **1.79757 L**

No corpse was found, but suspects (one woman and two men) were convicted.

Address for correspondence:

Dr. Alex Pifarré, Instituto Nacional de Toxicología,
Departamento de Barcelona, Sección de Biología,
C/Mercè 1, 08002 Barcelona.

Barcelona, Spain, Tel. 00 34 93 3174061; Fax: 00 34 93 3182530;
e-mail: biolog@bcn.inaltox.es

Development of two new Multiplex Systems (M1: D3S1358, D8S1179, D7S820, D16S539, Penta E and M2: D5S818, D13S317, D10S516, Penta D) for routine and forensic casework

C. Proff, C. Schmitt and M. Staak

Institut für Rechtsmedizin, Universität Köln, Germany

In recent years, multiplex PCR-based DNA profiling has become a routine method at forensic laboratories across the world. A number of different Multiplex Systems has been developed in order to improve sensitivity and throughput of DNA analysis, thus substantially reducing the costs per profiling task. Regarding these objectives, commercial Multiplex Systems offering high discriminating power as well as extraordinary sensitivity in only one PCR are of particular interest. Separation, detection and analysis performed according to these systems are based upon the ABI PRISM™ technology, with three different labelling dyes. ALFexpress™ owners cannot use this technology (or even parts of it) because of the limitation to Cy5-labelling of primers.

When the PowerPlex16™ primer sequences had been published, institute staff chose to develop two new Multiplex Systems comprising nine STRs based upon some of these PowerPlex16™ primers, combined with established STRs and the highly polymorphic D10S516. The implementation of the two pentanucleotide repeats Penta D and E for the ALFexpress™ was of specific interest.

Before combining the different Cy5-labelled STRs within two Multiplex Systems the loci were established in singleplex reactions in order to produce allelic ladders containing the most commonly observed alleles.

Investigations regarding the two multiplexes M1 and M2 included PCR conditions (FailSafe™ PCR Premix selection kit was tested), examination of 180 unrelated caucasian samples and their allele proportions, statistical evaluation, sensitivity tests, forensic stains, and finally DNA mixtures. Problems arising in the course of investigations concerned external standards, different signal intensity and evaluation software.

The two designed multiplexes proved to be extremely useful in population genetic studies as well as forensic applications and offer a reasonably priced alternative to commonly available testing kits.

*Dipl.-Biol. Carsten Proff
Institut für Rechtsmedizin der Universität Köln
Melatengürtel 60-62, D-50823 Köln, Germany
Phone: +49 (0)221 4784295
Fax: +49 (0)221 4783496
e-mail: cproff@smail.uni-koeln.de*

DNA Typing from biological stains: a casework experience

N. Cerri, M. Franchi, S. Mascadri and F. De Ferrari

Institute of Forensic Medicine University of Brescia, Italy

In a crime scene many different exhibits can be found and a forensic lab can be asked to examine the biological stains identified upon them. The aim of this study is optimize PCR DNA typing of non haematic fluid to a concrete application in forensic casework.

DNA from different stains (semen, post-coital champion, saliva, urine, nasal mucus, stool, beads of perspiration, tears) was extracted by two different methods: Chelex and the standard procedure with pCIA after the digestion with proteinase K.

DNA was amplified and analyzed at first with manual procedure; Dot-Blot Analysis (Dqalpha and Polymarker Kit) and silver staining on a denaturing electrophoresis (Microsatellite DNA polymorphism and Amelogenin by using Gene Print STR by Promega) and sequently by automatic procedure. PCR was performed using the AmpFISTR Profiler Plus and SGM Amplification Kit, according to the manufacture's recommendation and detection was obtained by capillary electrophoresis in an Applied Biosystems 310 instrument. The size fragment and genotype were determined with the genescan and genotyper software.

*Prof. Dr. Med Francesco De Ferrari, Institute of Forensic Medicine,
University of Brescia, c/o Spedali Civili,
Piazzale Ospedale 1, 25100 Brescia, Italy.
Tel. ++39/030/3995838; Fax. ++39/030/3995839,
e-mail: deferrari@master.cci.unibs.it*

DNA Typing from Epiglottic Cartilage of Exhumed Bodies

S. Gino, C. Robino, E Bonanno and C. Torre

Laboratory of Criminalistic Sciences, Department of Anatomy, Pharmacology and Legal Medicine, University of Turin, Italy

DNA typing is the method of choice to identify decomposed human remains, when routine techniques such as fingerprint analysis and X-ray comparisons fail to establish identity. In paternity testing as well it is sometimes necessary to study DNA polymorphisms on remains of humans who have been dead for long time periods.

In both cases postmortem degradation of DNA raises a serious obstacle to the success of the analysis. Cadaver blood and soft tissues were seen to be unsuitable for DNA typing after relatively short postmortem intervals, because of rapid DNA damage.

In decomposed bodies, bones and teeth are the most reliable sources of nuclear DNA amenable for PCR typing of STR loci. However, DNA extraction from this kind of specimens requires long and painstaking laboratory work and DNA final yields are often extremely small.

With all this in mind, we have evaluated elastic cartilage of the epiglottis as an alternative source of nuclear DNA in decomposed human remains. Cartilage is a specialized type of connective tissue formed by cells, the chondrocytes, lying in an amorphous matrix rich in collagen and elastic fibers. It can be surmised that this matrix may act as a physical barrier protecting DNA from chemical breakdown. Moreover, cartilage is lacking in blood vessels: for this reason chondrocytes may be less prone to degradative factors such as microorganisms. Unlike costal cartilages, epiglottis does not undergo extended calcification as a consequence of ageing and therefore it is suitable for DNA isolation by means of straightforward extraction procedures, without previous decalcification.

In a preliminary study, samples of epiglottic cartilage were collected from three bodies exhumed after 1–2 years.

In all the samples staining with hematoxylin-eosine demonstrated the presence of nucleated chondrocytes, though signs of karyolysis and karyorrhexis were evident.

DNA was isolated by standard organic phenol/chloroform extraction, quantified using the Quantiblot Human DNA Quantitation Kit (Applied Biosystem) and subjected to amplification of six STR loci plus Amelogenin with the AmpFISTR Cofiler PCR Amplification Kit (Applied Biosystem).

In one case it was possible to compare the results of DNA typing with the STR profile obtained from a blood sample collected at the time of postmortem examination: complete concordance of genotypes was observed at all loci.

Dr. Sarah Gino, Dipartimento di Anatomia, Farmacologia, Medicina Legale, Lab. di Scienze Criminalistiche, Università degli Studi di Torino, C.so M. D'Azeglio 52, 10126 Torino, Italy, Tel. 00 39 (0)11 6707731; Fax: 00 39 (0)11 6707732; e-mail: sarah.gino@unito.it

Paternity determination in criminal case by DNA typing in South Ukraine

Yu.M. Sivolap, A.F. Brik, G.F. Krivda

Molecular-Genetics Expertise Center of Odessa Region Forensic Service Odessa, Ukraine

First the DNA-analysis usage in Odessa region Forensic Service (South Ukraine) was on 1992 after PCR-technique installation for DNA polymorphism investigation. It was the paternity determination by typing DNA isolated from blood. Since 1995 suspect genotypes identification by comparison of DNA-profiles from biological traces was begun. In the first stage (1995–1997) 6 minisatellite loci – D17S5, IgH, D1S80, ApoB, p33.6, RB1 were used. Since 1998 DNA-typing was realized with microsatellite loci: HUMTPOX, HUMCSF1PO, HUMF13A01, HUMF13B, HUMHPRTB, HUMFES/FPS, HUMTH01, HUMLPL, HUMvWA, HUMCD4, HUMCYAR04, D6S336, D19S253, HUMvWFII, HUMSE33 and Amel for sex determination.

Principle and technique of paternity determination used for relatives ascertaining and for personal identification are in following example:

- unknown man corpse identification in sharp corruption stage. For DNA isolation the hair, cartilage and collar-bone fragment were used. 11 SSR-loci (HUMF13A01, HUMF13B, HUMTPOX, HUMFES/FPS, HUMTH01, HUMSE33, D19S253, HUMCD4, HUMLPL, HUMHPRTB, HUMCSF1PO) were analyzed. DNA profiles of prospective victim's mother, daughter and biological remains were compared. Molecular-genetic analysis showed the woman can be as victim's mother with probability $n = 99,85\%$ (value of the cumulative probability of casual concurrence of genotypes was $P_{cum} = 1,49 \times 10^{-3}$), and girl can be as victim's daughter with probability $n = 99,99\%$ (value of the cumulative probability of casual concurrence of genotypes was $P_{cum} = 2,51 \times 10^{-6}$).
- remains identification. DNA extracted from skull, boiled down and open with varnish, and fired bones tested on 7 microsatellite loci (D19S253, D19S253, HUMCYAR04, HUMCD4, HUMF13A01, HUMF13B, D6S336) and locus Amel. Obtained PCR-profiles were compared with those of prospective victim's mother. The skull was female and can belong to daughter of analyzed woman with probability $n = 99,99\%$ (value of the cumulative probability of casual concurrence of genotypes was $P_{cum} = 1,34 \times 10^{-5}$), and bones remains from fire place were male.
- blood spots identification on asbestos pipe fragments by person disappearance. DNA was analyzed on microsatellite loci: HUMF13A01, HUMF13B, HUMTPOX, D6S336, D19S253, HUMFES/FPS, HUMTH01, HUMSE33 and Amel. Also the prospective parents DNA was investigated. Blood spots on the pipe belong to man who can be these parents son with probability $n = 99,99\%$ (value of the cumulative probability of casual concurrence of genotypes was $P_{cum}^{fatherly} = 8,65 \times 10^{-8}$ and $P_{cum}^{motherly} = 2,16 \times 10^{-5}$).

Further study on suitability of Profiler Plus in personal identification

L. Buscemi, M. Pesaresi, C. Sassaroli, F. Alessandrini and A. Tagliabracci

Istituto di Medicina Legale, Università di Ancona, Italy

Since their discovery, STR loci have become routine markers for forensic identification and parentage testing. Introduction of multiplex PCR, combined with highly sensitive multicolor fluorescent dye-labeling technology in automated detection, has enabled rapid simultaneous amplification of several STR loci. The coamplification in a single reaction tube has the advantages of best optimizing the available amount of DNA, often scarce in forensic casework, to reduce at least the possibility of contamination and laboratory operator time, sometimes valuable in the cause of justice. Several commercial PCR kits are currently available for these purposes; nevertheless, systematic validation studies are a critical precondition before new ones can be introduced into forensic applications.

This work reports a validation study on the AmpFISTR Profiler Plus™ kit (PE/AB) on the ABI PRISM CE 310 Genetic Analyzer (PE/AB). It permits coamplification, in a single PCR reaction, of nine of thirteen STR loci selected in the CODIS STR standardization project, plus the amelogenin locus.

In order to evaluate the performance of this multiplex STR system for forensic applications, sensitivity tests were carried out to establish the optimal input amount of extracted genomic DNA required for interpretable and reliable profiles on the capillary electrophoresis instrument. To satisfy requirements for reproducibility of typing results, DNA was extracted from various biological samples of the same subject. The next stage of the study verified the suitability and efficiency of multiloci analysis in practical forensic applications. The most significant examples of casework reading our laboratory were selected with the aim of: 1) examining the most representative number of different biological samples and stains on various substrates; 2) investigating the robustness of multiplex STR systems on forensic DNA samples, very often exposed to a great variety of environmental factors or chemical contaminants, which may degrade DNA or inhibit PCR amplification.

Prof. Dr. Adriano Tagliabracci, Istituto di Medicina Legale, Policlinico, Torrette, I-60020 Ancona (Italy), tagliabr@popcsi.unian.it

A case of Disputed Parentage with two Apparently False Exclusions of Paternity at STR Loci

C. Di Nunzio¹, F. Corradi², M. Pizzamiglio⁵, R. Landi¹, M. Gramegna³, D. Stangalini³, E. Cosentini⁴, C. Casillo¹ and L. Garofano⁵

¹*Istituto di Medicina Legale, II Università degli Studi di Napoli, Italia*

²*Dipartimento di Statistica, Università degli Studi di Firenze, Italia*

³*CLONIT s.p.a., Milano, Italia*

⁴*Istituto di Immunoematologia, Università degli Studi di Napoli "Federico II", Italia*

⁵*Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italia*

The worldwide data regarding the use of STR loci, currently validated for forensic purposes, and the availability of commercial kits have definitely shown, beyond reasonable doubt, that these loci are quite efficient and have adequate power to solve most paternity cases. In this paper we report on an interesting case in which two apparent exclusions of paternity were obtained at loci D13S317 and HUMCSF1Po from a combined battery of 35 different markers, consisting of 24 DNA systems (20 STRs and 4 VNTRs) and 11 standard biochemical systems. We introduced a simple mutation process with parameter estimates, as previously published, and obtained the overall weight of evidence which strongly supported the hypothesis that the putative father was the biological father, but that two independent mutations had occurred. Based on this case, we agree with most Authors and with the recommendation of the American Association of Blood Banks suggesting that non-paternity is declared on the basis of exclusions at two or more independent loci considered.

DNA Typing on small Latex Gloves' Fingertips

M. Pizzamiglio, F. Biondi, T. Floris, A. De Meo and L. Garofano

Raggruppamento Carabinieri Investigazioni Scientifiche, Reparto di Parma, Italia

Forensic labs are receiving an ever increasing number of latex gloves used by criminals in serious crimes, such as murders and robberies. These gloves are a precious source of information, as the criminal may be identified by highlighting fingerprints and/or typing DNA.

In this paper we report on a real case of a murder and describe the strategy devised by our lab for a thorough genetic analysis of the exhibit, consisting of three small gloves fingertips left at crime scene. We were able to extract, amplify and type conventional STR markers from trace amount of DNA still present in both the inside and outside of the evidence which made it possible to prove the culprits' responsibility and push them to confess the murder.

P175

DNA Typing of Latent Blood Traces from a Case of Murder

M. Pizzamiglio, C. Bellino and L. Garofano

*Raggruppamento Carabinieri Investigazioni Scientifiche,
Reparto di Parma, Italia*

Investigations on evidence from cases of murder often constitute a delicate problem for the forensic geneticists, as very frequently small and/or washed traces of blood material is to be analysed, rendering the identification of the aggressor/victim very difficult. In this work we describe a real case of a murder in which we had to examine latent traces of blood still present in the culprit's cellar where the victim had been hit and cut in pieces even though it had been washed very accurately in order to eliminate any evidence of the homicide. We describe the methods we used to recognize and identify the blood and the strategies we followed to successfully extract, amplify and type STR markers which made it possible to show the culprits' responsibility and solve the case.

P176

The rapid identification of railway disaster victims by DNA analysis

P. Hoff-Olsen, B. Mevåg and K. Ormstad

Institute of Forensic Medicine, University of Oslo, Norway

At 01.12 PM on the 4th of January 2000 a local train and an express train crashed in a wooden area, approximately 150 kilometers north of Oslo. Both trains were expected to move at a speed of 80–90 kms/hr before the incident. The northbound local train was expected to carry 15 passengers and 2 employees. The southbound express train was expected to carry 81 passengers and 2 employees. A diesel-fueled locomotive and several passenger wagons tipped over and were fiercely ignited by the fuel. At 07.00 PM on the day of the accident the fire was extinguished and by midnight the same day 11 casualties were brought out from the scene. At day 2 after the disaster a total of 18 deceased were recovered from the scene. At day 4 the 19th and last victim was brought out.

For the victim identification it was decided to use DNA profiling in addition to traditional means, i.e. forensic pathology and odontology. Furthermore, it was decided only to use PCR-based methods, due to the expected degradation of the material.

A total of 24 bodies and body parts were found at the scene. Blood samples from first degree relatives of 17 of the deceased were harvested as reference material. A surgical pathology tissue block had to be used as reference material for one of the victims. Reference biological material was not available for one of the subjects. For the extraction of DNA from all tissue and blood samples the phenol-chloroform extraction was used, partly as a rapid truncated method. DNA from a single sample of skeletal tissue was extracted by a Silica method. DNA from all the reference samples was extracted by a Chelex-based method. All samples were amplified with the AmpFISTR SGM plus kit®.

By the evening of day 3 after the accident, all samples received in the afternoon of day 2 were successfully analyzed. Some samples arrived at our laboratory by the afternoon of day 5. These were successfully analyzed by the next morning. All 18 victims with available reference samples were identified by DNA analyses. This work represents a further example of DNA profiling as a rapid and safe way of mass disaster victim identification. The choice of DNA extraction methods, and sample harvesting at the scene will be further discussed.

Adress for correspondence:

*Dr. Per Hoff-Olsen, Rettsmedisinsk institutt,
Rikshospitalet, 0027 Oslo, NORWAY.*

Tel. 00 47 23071258 Fax: 00 47 23071260,

e-mail: per.hoff-olsen@labmed.uio.no

P177

Extraction of DNA from a Variety of Substrates in a Single Step

A. Linacre

Forensic Science Unit, University of Strathclyde, Glasgow

A wide range of biological substrates are encountered in forensic science. The substrates, such as blood, saliva, and spermatozoa, may be at trace amounts. It is frequently the smallest bloodstains that are the most informative. Equally a few spermatozoa may be all that are present on a swab. The method used in collecting, preserving, and isolating the DNA from the sample is crucial for the success of gaining a DNA profile.

One of the most common methods used is the chelating resin based procedure using Chelex®. This process, while being safe and inexpensive, is inefficient at isolating DNA from trace substrates. A method is described that isolates DNA from liquid blood, bloodstains, and buccal scrapes using a simple liquid solution. The bloodstain, liquid blood, or swab is placed into the solution (20 µl) which is then processed in a thermalcycler for 20 minutes. The solution (microLysis™) is now available from a commercial source. An aliquot of the microLysis/DNA solution can be used to generate full STR profiles from commercial kits.

The process takes approximately 20 minutes, involves no tube changes in the extraction process, is safe, and is inexpensive.

*Dr. Adrian Linacre, Forensic Science Unit,
Department of Pure & Applied Chemistry,
University of Strathclyde, Glasgow, G1 1XW, UK.*

Tel +44-141-548-4377, Fax +44-141-548-2532,

e-mail: a.m.t.linacre@strath.ac.uk

Importance of canine identification in the Hungarian forensic practice

Z. Pádár¹, B. Egyed¹, K. Kontadakis², S. Füredi¹, J. Woller¹, L. Zöldág² and S. Fekete²

¹DNA Laboratory, Institute for Forensic Sciences, P.O. Box 314/4, H-1903 Budapest, Hungary

²Szent István University Faculty of Veterinary Science, H-1078 Budapest, Hungary

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. Nowadays, 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 rising, 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 (e.g. blood) or in the dog's stomach (e.g. bone, tissue) can prove the animal as perpetrator. Lack of these evidence the availability of certain canine specific polymorphic STR loci is a significant tool to identify the 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 sequenced allelic ladders – what 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.

Correspondence:

Zsolt Pádár

Institute for Forensic Sciences

POB 314/4, H-1903 Budapest, Hungary

Tel. 00 36 1441 1474

Fax. 00 36 1441 1473

e-mail: padarzs@free-mailc3.hu

Validation and practical experiences with the multiplex kits genRES[®] MPX-2 (SERAC) and GenePrint[®] PowerPlex[™] 16 (Promega)

A. Junge, M. Steevens and B. Madea

Institute of Legal Medicine, University of Bonn, Germany

In the past a tool of different multiplex systems were validated and established for forensic identification purposes and are now commercial available. The aim of the establishment was to reduce time, cost and material and to maximize the forensic power of discrimination. At the beginning of the multiplex area the systems consisted of no more than 3 short tandem repeat systems (STR). Meanwhile the number of systems have raised up to 16 STRs + Amelogenin. Before application of multiplex systems in routine casework extensive validation studies have to be performed e.g. sensitivity and mixtures studies, experimental stain case work and different storage conditions in order to get information about reproducibility, robustness and specificity. Validation studies were performed with the commercially available multiplex kits *genRES* MPX-2 and *Geneprint* PowerPlex 16. The *genRES* MPX-2 kit contains 8 STRs (TH01, VWA, FGA, ACTBP2, D21S11, D3S1358, D8S1179, D18S51) which are components of the German DNA data base (BKA Wiesbaden) + the sexspecific Amelogenin whereas the *GenePrint* PowerPlex 16-kit co-amplifies 15 STRs + Amelogenin, including the 13 CODIS tetranucleotide STR loci and 2 pentanucleotide STR loci. All investigations were performed according to the manufacturers protocol and the samples analysed on an ABI Prism 310 Genetic Analyzer using a 50 µm capillary, the polymer POP6 and the module GS STR POP6 with filter set A. Investigations on the intra- and inter reproducibility showed that both systems show a high degree of precision and accuracy. Sensitivity and mixture studies were performed with different amounts of defined template-DNA. In general reproducible results and correct assignment of the alleles were achieved in the range of 200 to 500 pg DNA for both *genRes* MPX-2 as well as *GenePrint* PowerPlex 16 depending on the allele sizes of the locus. The detection limit of the minor component in a mixture was heterogeneous ranging from 1:2 (Penta E; PowerPlex 16), 1:5 (ACTBP2; MPX-2) and 1:20 (VWA, FGA; MPX-2). Simulated casework on fresh and old material (bloodstains, saliva, hair roots) was carried out as well as on real casework samples including e.g. epithel cells, blood mixtures and tissue samples. The results of the validation study and the forensic parameters are presented in summary.

Dr. rer. nat. Anke Junge, Institute of Legal Medicine, University Bonn, Stiftsplatz 12, D-53111 Bonn, Germany, Tel. 0049 (0) 228 738355, FAX 0049 (0) 228 738339, e-mail: a.junge@uni-bonn.de

Cyt-B analysis and dog hair comparison in a serial robbery case

A. Berti, A. Virgili, G. Zignale, C. Franchi, M. Serafini and G. Lago

*Raggruppamento Carabinieri Investigazioni Scientifiche,
via Aurelia 511, 00165 Roma, Italy.*

In October 2000 a serial robber carried out over six robberies in Rome. The victims reported that the robber was always a man with a dog. Moreover he used the dog to frighten the victims.

The police found in all the victim's car many dog hairs which were immediately submitted to our laboratory.

To diagnose hair species and, in particular, to confirm the dog hair nature we extract DNA from the hair root and we perform CytB analysis for species identification. We amplify one region of 290bp of the CytB mt-DNA gene and we sequenced it by Big Dye™ Terminator method. The samples were analyzed on ABI 310 analyser and the sequence was submitted on the BLAST sequences DNA database (<http://ncbi.nlm.nih.gov/cgi-bin/BLAST>). The Database search confirmed that the hair belongs from a dog.

After genetic analysis, in order to diagnose dog race, we observed at microscope the hair and we compared them with different dog races hair reference samples. One of the reference hair sample perfectly matched, by size, color and morphology with the evidence hair. The results were immediately transmitted to investigators and the robber was soon arrested.

Address for correspondence:

*Cap. Dr. G. Lago – Sezione Biologia –
Reparto Carabinieri Investigazioni Scientifiche Rome,
Italy, via Aurelia 511, 00165 Roma, Italy.
Tel. +39 6 66394644 +39 6 66394665 Fax +39 6 66394737
e-mail: gplago@tiscalinet.it*

DNA typing after α -Amylase test

C. Rapone, G.D' Errico, M. Bruno, M. Serafini, A. Berti and G. Lago

*Raggruppamento Carabinieri Investigazioni Scientifiche,
via Aurelia 511, 00165 Roma, Italy*

Saliva traces in forensic cases can be found in many different samples like cigarette-butts, stamps or face masks. In these cases we are able to evaluate saliva presence by α -Amylase test (BNP-Amylase test, Sclavo), an enzymatic method which utilizes blocked p-nitrophenyl-maltoheptaoside (BpNPG7) as substrate. The α -Amylase (1,4- α -D-glucan glucanhydrolase) catalyses the endohydrolysis of α -1,4 glucosidic linkages to produce p-NP-labelled fragments (p-NPGn). Glucoamylase (exo-1,4- α -D-glucosidase) and maltase (α -glucosidase) quickly hydrolyse non-reducing ends of the chains liberating p-nitrophenol. The absorbance of p-nitrophenol (p-NP), stoichiometrically formed, can be measured at 405 nm. The rate of its liberation is directly proportional to the α -Amylase activity in the sample.

This test is rapid, highly sensitive and allows the forensic scientists to exactly find the saliva traces on the substrates. When we have positive response with α -Amylase test, we directly extract the DNA from the substrate with a modified Phenol-Chloroform method. We have done over 200 tests with reference saliva samples on cigarette-butts, stamps or face masks. No differences have been observed between α -Amylase tested vs not tested samples as DNA recovery or successfully typing in ABI Prism 377 sequencer analysis.

Address for correspondence:

*Cap. Dr. G. Lago – Sezione Biologia –
Reparto Carabinieri Investigazioni Scientifiche Rome,
Italy, via Aurelia 511, 00165 Roma, Italy.
Tel. +39 6 66394644 +39 6 66394665 Fax +39 6 66394737
e-mail: gplago@tiscalinet.it*

STR typing from human faeces: a modified DNA extraction method

G. Iacovacci, M. Serafini, A. Berti and G. Lago

Raggruppamento Carabinieri Investigazioni Scientifiche,
via Aurelia 511, 00165 Roma, Italy

Human faeces can be found in forensic cases in a small but significant proportion of crime cases in Italy. This biological sample is a complex mixture of micro-organisms, digested and undigested food residue, mucus, soluble and insoluble products of the gastrointestinal tract and degradative enzymes derived from cells, food and bacteria (Iyengar et al. 1991; Sidransky et al. 1992).

Although an estimated 10^{10} cells per day are lost from gastrointestinal tract (Sidransky et al. 1992) Routine STR analysis from faeces was not possible following extraction by current extraction methodology. It is known that faecal constituents such as bilirubin and bile salts inhibit PCR even when present at low concentrations (Widjoatmodjo et al. 1992). Therefore for PCR analysis, DNA purification is required prior to amplification; alternatively, inhibitors can be reduced by dilution of the extract, but this is accompanied by a loss in sensitivity proportional to the dilution factor.

The success of STR analysis depends on the amount of DNA, PCR inhibition factors and the degradation level. In faeces samples all of these factors are present and the success depends on the extraction methodology and purification strategy used.

We have tested different extraction methodology such as Chelex[®] 100, Phenol-Chloroform and Qiagen[®] extraction. None of these allows us to obtain positive results.

In this report we present a modified methodology using a Phenol-Chloroform extraction method followed by a Microcon[®] 100 and a Sephadex purification step.

These modified method allow us to obtain full STR profiles from the faeces samples.

Address for correspondence:

Cap. Dr. G. Lago – Sezione Biologia –

Reparto Carabinieri Investigazioni Scientifiche Rome,
Italy, via Aurelia 511, 00165 Roma, Italy.

Tel. +39 6 66394644 +39 6 66394665 Fax +39 6 66394737

e-mail: gplago@tiscalinet.it

DNA paternity testing of five-year-old exhumed remains

L. Simjanovska¹, M. Kicurovska², D. Plaseska¹, A. Dimovski¹,
A. Duma³ and G.D. Efremov¹

¹Research Centre for Genetic Engineering and Biotechnology,
Macedonian Academy of Sciences and Arts, Macedonia

²Department of Criminal Technique, Ministry of Interior Affairs,
Macedonia

³Department of Forensic Medicine, Faculty of Medicine, Macedonia

DNA marker analysis is a powerful methodology for paternity testing especially in specific cases when the biological material is present in traces or is decomposed. We describe a case of paternity testing from five-year-old exhumed remains of the putative father. DNA from the putative father was isolated from the remains of bones, while from the presumed child, her mother and alleged relatives (brother and two sisters of the putative father) were isolated from the peripheral blood leukocytes by the phenol/chloroform/isoamylalcohol extraction method. DNA samples from the remains were subjected to multi-step purification protocol in order to obtain good quality DNA for PCR amplification. Six conventional genetic markers were analysed by AmpliType HLA DQA1+PM kit, 9 short tandem repeat (STR) loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820) and Amelogenin locus by ABI Prism 310 DNA analyzer, 6 STR loci (CSF1PO, TPOX, THO1, F13A, FESFPS, LPL) by PAGE and non-radioactive hybridization detection, and 6 VNTR loci (D1S7, D7S21, D7S22, D12S11, D2S44 and D10S28) by Southern blot analyses. The results showed exclusion of the paternity. Different sized alleles of the child and putative father were obtained for 5 STR loci (vWA, FGA, D5S818, D7S820, F13A). The analyses of the HLA-DQA1, HBG and D7S8 loci showed different polymorphisms, as well. The exclusion of paternity was confirmed by the analyses of DNA from the close relatives of the putative father. The analyses from the 6 VNTR, 9 STR and Amelogenin loci showed a biological relationship between the exhumed material, and his brother and sisters but excluded their relationship with the child. These results increased the confidence of the exclusion of paternity.

Genetic Analysis of Fingernails Debris: Application to Forensic Casework

A. Fernández-Rodríguez, M.J. Iturralde, L. Fernández de Simón, J. Capilla and M. Sancho

Sección de Biología, Instituto Nacional de Toxicología, Madrid, Spain

In the course of violent crimes multiple actions of aggression and defense are frequent. In cases where victims struggled or defended themselves, victim fingernails debris has been described as a possible source of DNA from the perpetrator. In this work we present the results of the genetic analysis of 106 samples of fingernails debris from 40 forensic cases.

Most of the cases (33) were homicides, 7 were rapes and in 2 cases the objective was to confirm a natural death by ruling out the possibility of struggle. Fingernails clippings were the most frequent sample (65.09% of 106), while the remnants under the fingernails (without any physical support) were submitted by the forensic doctor in a 18.87%. Fingernails debris was sent on wood or plastic sticks or sterile swabs in a 16.03%. Most of the samples (94.32%) belonged to the victim, except 4.72% which were taken from the suspect and one fingernail found at the crime scene.

DNA was extracted following the phenol-chloroform technique and concentration with the centricon 100 device. Any possible cellular debris from fingernails clippings was eluted in sodium acetate for an hour at 56°C and then extracted. Samples were quantified with the Quantiblot System. DNA typing was performed by the PCR analysis of different multiplex amplification systems including some with manual detection and those coupled to the ABI Prism 377 DNA Sequencer.

DNA was obtained from almost all the samples (95.28%). While in most of the samples the DNA profile matched that from the donor, seven samples (6.6%) yielded a mixture profile coming from, at least, two individuals. In five of these cases the mixture matched the profiles of the victim and the suspect. In the other two cases the suspect was not identified. In six samples (5.66%) a profile matching the victim with some alleles of the offender was found. Inconclusive results were obtained only in one sample (0.94%). Finally one sample (0.94%) yielded a mixture from, at least three individuals, which matched the victim and two offenders.

These results suggest that DNA analysis of fingernails debris could be a useful approach, especially when a struggle between the victim and the offender is suspected. Further investigation is needed to evaluate the influence of the handling and the laboratory procedures on the possibility to obtain DNA from the scratched.

Correspondence:

A. Fernández-Rodríguez,
Sección de Biología Instituto Nacional de Toxicología,
C) Luis Cabrera nº9, 28002 Madrid, Spain.
e-mail: amparo.f@wanadoo.es

DNA analysis of ABO blood group system detected single-base nucleotide substitutions in a paternity case

S. Nakamura, H. Matsushita, T. Nagai, H. Sugie, M. Furukawa and K. Kurihara

Department of Legal Medicine, Kitasato University School of Medicine, Sagami-hara, 228-8555, Japan

Introduction

The ABO blood system is one of the major blood group systems in man and is important in transfusion medicine, forensic serology and anthropological genetics. With the establishment of the molecular genetic basis of the ABO grouping by Yamamoto et al., it became possible to determine the ABO blood groups at the DNA level. The A₁ allele differs from the B allele in seven single-base nucleotide substitutions at positions 297, 526, 657, 703, 796, 803 and 930, in which the four nucleotides at positions 526, 703, 796 and 803 lead to amino acid changes in the final transferase protein. A₂ allele differs from A₁ allele by a single-base substitution (np 467) and a single-base deletion (np 1060). The O allele (O₁) is identical to the A allele except for a single-base deletion at nucleotide position (np) 261. In addition, another O allele (O₂) without the nucleotide 261 deletion characterized by typing as the B allele at position 526 and A allele at positions 703, 796, and 803 has been reported. In the present investigation, DNA analysis of ABO blood group system detected single-base nucleotide substitutions in a paternity case is reported.

Materials and methods

EDTA-treated blood samples were collected from the trio (mother, child and putative father), and genomic DNA was isolated by proteinase K / phenol / chloroform extraction. The ABO phenotype of the trio was determined by agglutination test, and the ABO genotyping was performed at nucleotide positions 261 and 703 by PCR-RFLP described Lee and Chang. The PCR products were sequenced using BigDye Terminator Cycle Sequencing FS Ready Reactoin Kit (PE Biosystems) according to the user's manual provided by the manufacturer.

Results and discussion

The ABO phenotypes of the trio were typed as A with the agglutination test. The zygosity at the ABO locus of the trio was determined by using the method described above. The genotypes of the mother, child and putative father were AB, BO and AO, respectively. In this case there was a discrepancy between the serological determination of the blood group and the PCR ABO genotyping, so that a sequence determination was additionally carried out. The nucleotide sequence of mother had six nucleotide differences, at positions 297, 526, 657, 703, 771 and 829, compared with A₁. The mother and child possessed these differences each other. These nucleotide differences were coincided with that of ABO**R*₁₀₁ allele previously reported by Ogasawara et al. (1996) that detected in an A₂ individual. As the nucleotide (np703) of the **R*₁₀₁ allele is the same as that of B allele, the analysis of at least three nucleotide positions, i.e. nps 261, 796 and 803, is necessary to avoid mistyping of the ABO genotype.

Address for correspondence:

Dr. S. Nakamura, Department of Legal Medicine, Kitasato University School of Medicine, Sagami-hara, 228-8555, Japan,
Tel & FAX: +81 042-778-9026, e-mail: nakasige@med.kitasato-u.ac.jp

A 5 Years Study on DNA Recovered from Fingernail Clippings in Homicide Cases in Milan

A. Piccinini, F. Betti, M. Capra and A. Comino

Istituto di Medicina Legale, Università di Milano, Italy

Debris recovered from under the fingernails represents a well known source of DNA in assault cases.

In 1996 the collection of fingernail clippings on a routine basis started in cases of homicide from stab wounds at the Institute of Legal Medicine of the University of Milan.

The collection of fingernail clippings in other homicide cases has also been performed depending upon the circumstances of each case.

A total of 179 homicides was observed in the period 1996–2000. 60 of them were from stab wounds, 72 from gun shot wounds, 47 from other causes.

In 66 homicide cases fingernail clippings were collected and a Court order for their examination (DNA typing) was issued in 31 of them.

Special precautions were taken in all cases in order to avoid contamination.

Organic phenol-chlorophorm DNA extraction was performed in each case on the debris scraped from under each fingernail.

Both singleplex (silver staining detection) and multiplex PCR (fluorescent detection – ABI 373) were used.

The results obtained were informative (presence of a foreign profile) in 11 of 31 cases; 3 of them were inconclusive; 17 were negative (no foreign profiles detected).

These results demonstrate the usefulness of routine fingernail clipping during the autopsy in homicide cases.

Address for correspondence:

*Dr. Andrea Piccinini, Istituto di Medicina Legale,
Università di Milano, Via L. Mangiagalli 37, I-20133 Milano, Italy,
Tel. 0039 (0)2 2666433; Fax: 0039 (0)2 26680129,
e-mail: andreapiccinini@yahoo.com; romeo@unimi.it*

Validation of multiplex STR systems and population databases for the investigation of immigration cases

H. Sippel, M. Hedman and A. Sajantila

Department of Forensic Medicine, University of Helsinki, Finland

In 2000 our Department was requested by the Finnish Immigration authorities to verify presumed family relationships by DNA-technology.

We have previously reported validation of the AmpFISTR SGM Plus and AmpFISTR Profiler multiplex systems for the resolution of complicated forensic cases. We have now extended this work to consider DNA-testing in family reunion where the claimed relationship of both parents is in doubt and also cases where other relationship, such as sibship must be tested.

Although STRs are commonly studied in forensics, East African population studies are particularly scarce. A Somali population was studied in a total of 171 unrelated individuals to establish a DNA database. Forensic and paternity statistical parameters were analysed. The studied Somali population was compared with our Finnish population database by means of chi-square analysis. Significant statistical differences were observed, so a specific database is required for forensic purposes.

The paternity/maternity indices and the likelihood ratios were calculated using the DNA-view immigration program (C. Brenner, Berkeley, USA) and this calculation was based on a database constructed from the respective ethnic group. This was done in order to test the effect of the use of databases constructed from different populations.

Practically any first-degree biological family relationship can be established with the same technique as that for paternity investigation. Conventional paternity testing usually assumes that the mother is the true biological mother. However, in family reunion testing the aim is to investigate whether the family is a true biological family, so there are several potential constellations, including the maternity and sibship, to test.

The goal of this study was to evaluate the efficiency of the two Multiplex STR PCR Kits SGM Plus and Profiler (Applied Biosystems) in family reunion testing. The SGM Plus Kit is composed of Amelogenin and 10 STR systems: D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01 and FGA. The second Kit designed as Profiler combines 9 STR loci and amelogenin: D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820.

The results demonstrate the higher efficiency of the SGM Plus loci compared to the Profiler loci in paternity testing. The Profiler loci are, however, useful in combination with the SGM Plus loci to solve one parent paternity cases and cases with mutations. On the basis of this study the use of a minimum of fifteen STR loci is recommended for African and Asian applicants of unknown ethnical origin.

Correspondence:

*Helmuth Sippel
Department of Forensic Medicine, University of Helsinki
P.O.Box 40
FIN-00014 University of Helsinki
Tel. +358-9-19127476
Fax +358-9-19127518
e-mail: helmuth.sippel@helsinki.fi*

Two fathers for two twin sisters

S. Lebeau-Le Guiner¹, F. Guidet¹, T. Bompil¹, C. Marka² and O. Pascal¹

¹Laboratoire de Génétique Moléculaire, CHU de Nantes, France

²Laboratoire de Médecine Légale et Toxicologie, Faculté de Médecine de Grenoble, France

When a mother gives birth to twins, it is assumed that whether or not identical, they share the same father.

In our laboratory, we recently had a case involving two little twin sisters A and B and a putative father Mr Y. We started by using conventional serological tests. They showed that the two little girls were not identical twins (systems MNS, Duffy and HLA) but more importantly Mr Y was excluded as the father of girl A and a non conclusive result was obtained for girl B. We decided to solve the matter using STR-DNA analysis. Surprisingly, whilst confirming the exclusion of Mr Y as the father of girl A, our data showed clearly that Mr Y is indeed child B's father. Although documented, this kind of situation is rather rare.

Biological analyses are more and more frequently used to solve paternity disputes. This casework is an illustration that DNA analysis is the method of choice in comparison to other haematological techniques in parentage testings.

P190

A fall in doubt

D. Stiller¹, K. Trübner¹, P. Wiegand², M. Kleiber¹

¹Institut für Rechtsmedizin, Martin-Luther-Universität Halle-Wittenberg, Germany

²Institut für Rechtsmedizin, Universität Ulm, Germany

A 27-year-old prisoner was found dead in the sanitary cell of his cell midst of bloodstained broken pieces of an porcelain urinal. The cause of death was a stab wound of the left side of the neck perforating the common carotid artery and the jugular vein. Furthermore there were additional superficial cuts in the face and the left upper arm. The statement of the fellow prisoners that the man sustained the lethal injury by falling into the urinal was strongly doubted by prosecutor. However, experiments with pig skin showed that broken pieces of an urinal could cause wounds like those of the deceased.

During the trial at the county court suspicions arose that a broken piece of the urinal with a plastic drain pipe was used as a stiletto-shaped stabbing weapon.

Swabs of this part were taken for which epithelial cells and blood cells could be demonstrated microscopically.

DNA typing of the swabs has been carried out using 5 STR systems (TH01, VWA, D8S1132, FGA, CD4) leading to a mixed stain which could be explained by the combined allele pattern of the victim and the suspect.

Considering these results a second trial at the district court was ordered.

During the trial the defense attorney succeeded in questioning the evidence of the DNA opinion as the defendant claimed regular contact with the urinal. Finally the accused was found to be guilty of bodily injury but not of manslaughter.

Adress for correspondence:

Dankwart Stiller, Institut für Rechtsmedizin, Martin-Luther-Universität Halle-Wittenberg, Franzosenweg 1; D-06112 Halle/Saale, Germany
Tel.: 0049 345 557 1768, Fax.: 0049 0345 557 4553;
e-mail: dankwart.stiller@medizin.uni-halle.de

P191

Use of STRs in paternity testing in the Flemish population

A. Vandenberghe^{1,2}, N. Mommers¹, H. Heylen¹, L. Boutrand² and G. Mertens¹

¹Antwerp Blood Transfusion Centre, Edegem, Belgium

²Faculty of Pharmacy, University Claude Bernard Lyon I, France

We have now performed over 1,700 paternity tests in the Flemish population mainly using DNA polymorphisms. Testing started in 1987 and initially used minisatellite and VNTR markers. From 1994 on, we decided to switch to non-isotopic detection with exclusive use of STRs and silver-staining. A large number of tests were performed with 3 triplex sets as described by Alford et al. (Am J Hum Genet 55:190-195, 1994). One of these triplex sets contains an STR in the HPRT locus which is located on the X-chromosome and is of no value when male descent is being tested. From 1997 on, we replaced this triplex (HPRTB, FABP and CD4) by the MultiPlex I kit from Lifecodes (D3S1744, D12S1090 and D18S849). D12S1090 has proven to be especially useful (heterozygosity 93%, number of alleles > 30). We characterised this locus in detail by cloning and sequencing the majority of alleles. These revealed to be very heterogeneous having a complex composite organisation. We also characterised both the other MultiPlex I loci, for which sequence information is available at NCBI (access numbers G07992 and G08246). Discordance was found for locus D18S849 since the published sequence did not correspond to the sequence in the kit although it contained a polymorphic GATA repeat. In 2000, MultiPlex I was no longer available and we replaced this set by the Gamma STR-kit from Promega (D16S539, D7S820, D13S317). In 2001, we further introduced the FFv triplex from Promega (F13A01, FESFPS, vWA), thus avoiding the need to type for HLA B* and HLA DRB1* by PCR SSO (InnoLIPA from Innogenetics), which we had been using as additional markers to solve complex problems.

During our work, STR mutations were found in FESFPS (1x), D12S1090 (3x), TH01 (1x), D13S317 (1x), D3S1744 (2x) and CSFPO (2x). All mutations were characterised (when necessary by cloning and) by sequencing. Paternal to maternal mutation ratio was 2:1 and the ratio repeat gain to repeat loss was 7:3. As expected, the most heterozygous locus showed the largest number of mutations (D12S1090) and in composite loci, mutations occurred in the longest stretch of repeats. An apparent paternal mutation of CD4 was due to drop-out of a paternal allele which showed a rare sequence variant in the penultimate position of the reversed primer.

Address for correspondence:

Pr. A. Vandenberghe, Laboratoire de Génétique Moléculaire Humaine, Faculté de Pharmacie, Université Claude Bernard Lyon I, 8 avenue Rockefeller, F-69008 Lyon, France.
Tel 00 33 (0)4 78 77 72 31; Fax 00 33 (0)4 78 77 75 68;
e-mail: vandenbe@rockefeller.univ-lyon1.fr

Quali-quantitative analysis of DNA recovered from fingerprints

M. Pesaresi, L. Buscemi, F. Alessandrini and A. Tagliabracci,

Istituto di Medicina Legale, Università di Ancona, Italy

Most crimes committed in Italy are against property (theft, robbery) and against the person (bodily harm, sexual violence, murder) and very often the perpetrators are not punished since traces with analysable biological material which could identify the perpetrator cannot be found. Apart from the few cases of premeditation, in which protection system are adopted, in most crimes of this type, criminals leave only their fingerprints on the scene. These may be used to identify the criminal when they can be interpreted and when they have already been registered; all the others escape identification by traditional methods. The literature contains preliminary studies or cases histories on the possibility of recovering DNA from fingerprints left on the skin or in rope, cord, wire, etc. used for strangling, gloves, knives, solid parts of cars and other objects, and on the interference of substances used to highlight fingerprints on later genetic analysis. These works report isolated experiments, dictated by need to resolve definite cases, but systematic studies on recovery techniques, interference by contaminants, the influence of individual and exogenous factors in the number of cells left with the fingerprint, the quantity of DNA which can be extracted from prints prepared with various modes of contact and on various types of substrate (wood, glass, metal, skin, textiles), and the percentages of success in PCR analysis of the genetic impression from nuclear DNA, have not been exhaustively carried out.

This study aims at verifying whether fingerprints may be used as a source of biological material for analysing genetic profiles. A secondary aim is to verify the capacity to interpret mixed genetic profiles by capillary electrophoresis and, indirectly, to reveal possible contamination of biological findings after brief contacts by different subjects.

Operational methods were as follows:

- 1) formation of fingerprints on the following substrates: glass, metal, wood, plastic, textile fibres, skin. Where possible, formation was carried out in three different ways: brief contact by touch, pressure at standard times (from 5 to 30 sec), and tangential contact with rolling friction effect on the skin. Some of the experiments used already manipulated substrates, to verify phenomena of contamination, simulating caseworks;
- 2) removal of fingerprints by swabs impregnated with extraction buffer;
- 3) extraction of DNA;
- 4) quantitative analysis with a probe specific for the human genome using the dot-blot technique;
- 5) PCR amplification of microsatellites of nuclear DNA and allele typing by capillary electrophoresis.

Prof. Dr. Adriano Tagliabracci, Istituto di Medicina Legale, Policlinico, Torrette, I-60020 Ancona (Italy), tagliabr@popcsi.unian.it

Fingerprints from Fingerprints

M.K. Balogh¹, K. Bender², P.M. Schneider², J. Burger¹ and K.W. Alt¹

¹*Institute of Anthropology, Johannes Gutenberg University, Mainz, Germany*

²*Institute of Legal Medicine, Johannes Gutenberg University, Mainz, Germany*

A validation study was conducted to demonstrate that DNA can be successfully extracted from latent fingerprints and analysed using short tandem repeat (STR) profiling and/or mitochondrial DNA (mtDNA) sequencing. Latent fingerprints were deposited from a number of donors on various surfaces (such as paper and clothing). In contrast to the relatively high number of epithelial cells from saliva or from excessively pressured fingerprints during strangulation, the experiments of this study are expected to generate only a very small number of epithelial cells from objects softly touched by hands. Furthermore the microscopic analyses showed that the majority of epithelial cells from fingerprints were nuclei-free corneocytes with a minimal incidence of nucleated cells. To solve these problems various DNA extraction methods were tested to achieve the best performance in terms of sensitivity. In addition, the DNA extracts were amplified using the commercial AmpFISTR® Profiler Plus™ PCR Amplification Kit, surveying nine STR loci simultaneously, consisting of: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, as well as the sex determining marker Amelogenin. Finally a fluorescent-based capillary electrophoresis was carried out using ABI Prism 310 Genetic Analyzer. The results prove that successful DNA extraction, amplification and typing for 9 STR loci plus Amelogenin is possible, but the validation depends on several factors, such as time and intensity of touch or type of skin. Nevertheless, fingerprint material yielded STR profiles. Upon completion of the validation study the two hypervariable segments (HVI and HVII) of the control region of mtDNA were analysed using the capillary electrophoresis sequencing method. Hence there is a possibility that forensically typeable DNA could be extracted from latent fingerprints. Therefore, a fingerprint can be considered a potential source of DNA for forensic identification, even when latent fingerprints can not be visualized with traditional methods.

Marta Kinga Balogh, Institut für Anthropologie, Johannes Gutenberg Universität Mainz, Colonel Kleinmann Weg 2, D-55099 Mainz, Germany, Tel. 00 49 (0)6131 3923575, e-mail: balokooo@mail.uni-mainz.de

Identification of a Carbonized Body found Inside a Car

Anna Barbaro*, Patrizia Cormaci*, Aldo Barbaro**

*Department of Molecular Genetics –
SIMEF – 89128 Reggio Calabria-ITALY

**Department of Haematology –
SIMEF – 89128 Reggio Calabria-ITALY

**Medico-Legal – Director of SIMEF –
SIMEF – 89128 Reggio Calabria-ITALY – <http://www.simef.com>

A prejudged man disappeared in the city of Cosenza (Calabria).

After some days his car was found burned in a brushwood. Inside the car there was a burnt body.

DNA extraction from the unknown body has been performed from a bone (after pulverization), and a tooth using phenol-chloroform procedure and from a partial carbonized brain tissue by Instant Gene Matrix (Biorad) treatment.

For the comparison we extracted DNA from saliva samples belonging to the parents of the disappeared man.

STRs amplification was carried out by GeneAmp 9700 thermal cycler (Perkin Elmer), using Power Plex kit (Promega): that coamplifies the repeat regions of 16 STRs repeat loci.

A segment of the X-Y homologous gene Amelogenin is also amplified. According to the kit protocol, positive and negative controls were enclosed during the amplifications.

Amplified products were analyzed by capillary electrophoresis on two ABI PRISM 310 Genetic Analyzers (Applied Biosystems) employing ABI softwares (DATA Collection, GeneScan Analysis, Genotyper Fragment Analysis).

For fragment length determination of the products, the internal lane DNA standard ILS 600 (Promega) was used for calibration.

Amelogenin test confirmed that the body was from a man while the other loci typing confirmed that the body found inside the car was from the prejudged man.

In fact, paternity test performed with the DNA from the disappeared man family, showed that DNA profile from the unknown body was compatible with DNA profiles from the disappeared man parents.

The cumulative probability of paternity (P50%), calculated by a special software PATER, using allele frequencies of Cosenza, was 0,99999990 (99,999990%). P(Ex) was 0,99999940 (99,999940%).

Are DNA Tests Infallible?

G. Penacino, A. Sala and D. Corach

Serv. de Huellas Dig. Genéticas y Cát. Genética y B.M.,
Fac. Farm. y Bioq., Univ. Bs. As., Argentina

Modern technology strongly influenced most fields of knowledge. Forensic sciences do not escape to this reality. With the advent of the application of molecular biology to human identification by means of DNA typing, conceptual conflicts were introduced. After over fifteen years of worldwide experience, the robustness and reliability of DNA analysis was demonstrated. However in our country, judges, prosecutors and defenders, due in part to different educational background as compared to scientists, may ignore potential restrictions concerning DNA profiling results.

Probably for this reason, from its beginnings, DNA testing was surrounded by an aura of infallibility. This was a misconception fed by the popular belief that genetic information was unique and unrepeatable for each individual, with the only exception of identical twins. In those times only a few variable regions were analysed of the millions that each person possesses. Nowadays, a big number of highly polymorphic genetic markers, included in commercial kits, as well as automated devices for DNA extraction and purification, PCR amplification, electrophoresis and data analysis are available. Nevertheless, errors may occur?

This presentation describes the main causes for errors in DNA testing in forensic laboratories that simultaneously processes hundreds or thousands of samples a month, and makes suggestions for their solutions or their reduction to a minimum.

The errors at the different stages of the testing can be classified into:

- * Collection, transportation and storage of evidences: this stage presents the largest fallacies, as the samples are not always handled by specially trained personnel.
- * Handling within the laboratory: includes the possibility of confusion of samples by the operator as from the reception of the material, and the contamination with exogenous DNA.
- * Results interpretation: some multiplex STRs systems frequently present spurious bands or peaks, that matches with real alleles of the system in analysis. This situation may lead inexperienced operators to interpret a homozygote as a heterozygote or a single donor sample as a mixed profile. Other sources of errors are mutations and the differential amplification of some alleles in detriment of others.

As a conclusion, the present work contains recommendations for the novel forensic labs to prevent each particular problem. In addition, we would like to suggest to Justice authorities operating in our geographical region, that DNA testing should be considered one more piece of evidence within the context of a criminal or forensic investigation, and that the judicial sentences should be based on the evidence as a whole and not just on the genetic studies.

