Molecular Biological tests and their application in parentage cases

Summary of Ph.D. Thesis

Klara Csete

University of Szeged Albert Szent-Györgyi
Medical and Pharmaceutical Centre
Department of Forensic Medicine

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INTRODUCTION

Due to the great development of molecular biology, DNA examinations have spread in all the fields of biology and medical sciences, including forensic sciences as well. They tend to gain ground all over the world in civil and criminal court cases together with blood-group determination tests.
In the parentage cases paternity of the alleged male cannot be either proven or excluded in many instances with classical serological methods and it makes the extension of the testing methods necessary. It is provided by DNA tests. We have applied the DNA examinations in status cases in Hungary first.

OBJECTIVES

In the practice of the determination of paternity only those methods can be applied which are based on known allele frequencies and genotype occurrence with a well established and proven information on the inheritance of the traits. For their applicability in forensic sciences the validation of the DNA systems is required that can be carried out with population genetic tests. Objectives of my thesis are as follows:

1, The examination of distribution of genotypes in 5 somatic chromosomal STR systems (HumVWA, HumTH01, HumF13B, HumFES/FPS, HumFGA) and 2 Y chromosomal STR systems (DYS19, DSY390) in the population of Szeged and its environs;
2, the calculation of the allele frequency values of the individual STR systems;
3, the probability of the exclusion of paternity;
4, as well as calculation of the combined probability of exclusion of the 7 DNA STR systems;
5, by using the probabilities of exclusion I plan to compile a combined protocol of optimal blood group and DNA systems which yield a combined exclusion probability near 100 %;
6, to challenge the practical applicability of DNA examination in the parentage cases, I compare the results of blood group systems that were combined with DNA analysis with the results of blood group systems alone in cases from the year 1997.

BRIEF DESCRIPTION OF THE EXAMINED DNA STR SYSTEMS

HumVWA: The intron 40 of the von Willebrand Factor gene chromosome 12 in the 12p12-pter region. The fragment size is about 150 bp long and TCTA/TCTG bases are repeated (Mercier et al., 1991, Kimpton et al., 1992).

HumTHO1: Intron 1 of Tyrosine hydroxylase gene is located on the 11p15-15.5 chromosome region, the fragment size is about 160 bp long in which 4 pairs of bases (AATG) are repeated (Edwards et al., 1992).

HumF13B: It is the 3'prime end region of XIII b coagulation factor gene chromosome 1. It is about 200 bp long fragment inside which there are pairs of bases (ATTT) repeated. Its polymorphisms were first described by Nishimura and Murray (1992).

HumFES/FPS: It is intron 5 of c-fes/fps protooncogene gene located on the 15q25-qter chromosome region. It is about 250 bp long fragment in which 4 pairs of bases (ATTT) are repeated. It was first described by Polymeropoulos et al. (1991a).

HumFGA: It is intron 3 of the alpha fibrinogen gene. Its chromosomal localisation is 4q28. It is about a 280 bp long fragment with the repeat of TCTT inside (Barber et al, 1996).

DYS19 is about 200 bp long section located on the short arm of the Y chromosome inside which 10-19 repeats of the CTAT/C sequences can be seen (Roewer at al, 1992; Kayser at al, 1997).

DYS390 is about 220 bp long fragment located on the long arm of Y chromosome inside which 18-27 repeats of the CTG/AT sequences can be seen. (Kayser et al. 1997).

MATERIALS AND METHODS

Materials

The DNA was extracted from blood samples applied on a piece of linen and EDTA blood samples that originated from the Albert Szent-Györgyi Medical University of Blood Transfusion Station and the Department of Forensic Medicine's routine parentage tests.There were 489 unrelated adult individuals tested in the case of HumVWA and HumTHO1 systems, 465 in HumF13B system, 360 in HumFES/FPS and HumFGA systems, 308 in DYS19 and 268 in DYS390 systems.

Methods

The DNA extraction from EDTA blood samples was carried out with the NaCl extraction method of Miller et al. (1988).
The DNA extraction from bloodstains was done with Chelex method described by Walsh et al. (1991).
The estimation and quality testing of the DNA isolated from blood is done with agarose gel electrophoresis followed by ethidium-bromide staining. For the estimation of DNA concentration a 50, 100, 200, 400 and 600 ng/ul solution of the DNA originating from the K562 cell line (Promega , Madison) is added into adjacent lanes of the same gel.
After the PCR (Polymerase Chain Reaction) reaction the detection of genotypes are visualized in native discontinuous buffer (Allen et al.,1989) by polyacrylamide gel electrophoresis (PAGE) followed by silver nitrate staining (Budowle et al.,1991). The DNA samples can be typed by comparison with an sequented allelic ladder constructed by known alleles from Department of Forensic Medicine Münster and standardised in our laboratory.

RESULTS AND DISCUSSION

Evaluation of the results of the population genetic studies

The blood samples of unrelated adults from the Blood Transfusion Station of Albert Szent-Györgyi Medical University and from parentage cases of the Forensic Medicine Institute of Albert Szent-Györgyi Medical University have been tested 7 DNA-STR systems. The allele frequency values and distribution of the genotypes were calculated. In the knowledge of the allele frequency values of expected heterozygosity (H), discrimination index, gene and haplotype variance (h) and data characteristic of polymorphism of the systems (PIC) can be determined in order to characterise the individual STR systems.
Among the results gained on the basis of genotype occurrence in the 5 tested somatic DNA systems there were typed 26 genotypes in the HumVWA system, 23 genotypes in the HumTH01 system . In the HumF13B system 15 genotypes and in the HumFES/FPS STR system 22 genotypes were defined. HumFGA is the most polyphormic system in which we found 46 different genotypes.

On the basis of the distribution of the genotypes occurrence we calculated the allele frequency values for each STR system. In HumVWA system 9 alleles were typified and we typed in HumTH01 system 7 alleles.In HumF13B system 6 alleles were typed, and 10 alleles in the HumFES/FPS STR system. In HumFGA system we typed 14 alleles. Among the STR traits localised on Y chromosome 6 alleles were typed in DYS19 system and 6 alleles in the DYS390 system. On the basis of the allele frequencies I performed an χ^2 test to see the level of significance difference between the calculated genotype and the found genotype .The frequency values do not differ from Hardy-Weinberg equilibrium.
The value of the heterozygosity, the variance of genes and the degree of polymorphism in the systems of higher allele number, i.e. in the hypervariable systems (HumVWA, HumFGA), are higher. At the same time the statistic values are lower in systems of lower allele number or in systems where one allele can occur with greater probability than any other allele (HumTH01, HumF13B, HumFES/FPS).
It can be seen in the comparison of allele frequency values of European, Hungarian, Gypsy and Asian populations that in the somatic STR groups there is no significant difference between our data and the European population data.
The tests on genetic distance show that the allele frequency values of the somatic STR systems in European population groups do not differ significantly which is probably the result of the migrations in the course of history.
However, the characteristics of Y chromosome that is inherited on the father's line show a close connection with Asian (Mongolian) population will be tested in our laboratory.

Applicability of the DNA tests in parentage cases

In the second part of my paper I analyse the applicability of the DNA STR systems in parentage cases.
Our Institution has been conducting blood group tests since 1979. In the beginning we analysed 14 and since 1984 17 blood group systems. In 30-40% of the analysed cases the probability value of the parentage with blood group tests was between 30-99% and in 2-6% of the cases it was excluded in one system which require more tests in order to prove its paternity or its exclusion without doubt.
It makes necessary to perform further tests that can either increase the probability value or result in the exclusion of the paternity. In our institute we started to utilise the possibilities provided by DNA STR systems in paternity cases in 1996. At first three somatic STR systems and since 1997 five somatic and two Y-STR systems were tested.
Among the somatic STR systems the exclusion probability of the HumFGA is the highest and that of the HumTH01 is the lowest. It is alone greater than that of any blood group system. In most of the blood groups only 2-3 alleles are combined in the population and it does not make greater discrimination possible.
In 1996-98 we performed DNA-tests together with blood group tests in nearly 150 family in paternity cases. Blood group tests indicated a probability rate below 95% in 27 cases out of 119. In 30 cases paternity was probable and in 43 cases probability was above 99%. Exclusion was found in 19 cases, 7 of which were from one system and 12 were from more systems. Blood group tests with paternity probability below 95% indicated probability above 95% in only 6 cases, whereas in 18 cases it was above 99% and in 3 cases it was excluded with DNA examinations. Blood tests with probability results between 95%-99%, except 2 cases,indicated probability above 99% with DNA tests.
Where paternity is not excluded with either a blood group test or a DNA test, we calculate a combined probability. The combined probability values calculated from blood group tests and DNA tests done in 1997. In 6 cases where probability of paternity was below 95% the DNA

tests indicated probability between 99% and 99.75%, whereas in 18 cases it was above 99.75%. In 6 out of 30 cases where paternity was "probable" according to the blood group tests, the DNA tests indicated that paternity was "probable to a great extent" and in the remaining 24 cases it was "practically proven". In 3 cases where blood group tests showed paternal probability values between 99% and 99.75%, the probability value was not changed, whereas in 21 cases it rose above 99.75%.
Despite this fact, it is not recommended to exclude blood group tests because with the joint application of blood group and DNA test it is possible to examine the different protein and DNA systems that are located on several different chromosomes. With their help any incorrect opinion can be eliminated that might be caused by probable mutations. At the same time the 16 blood group characteristics become unnecessary. Only those should be used that has a relatively high exclusion probability and the costs of which are not high. I recommend a series of tests of 9 blood group characteristics and 7 DNA systems with the help of which parental exclusion can be defined with 99,9 % probability. Data from other authors and our own data also indicate that the classical exclusion rules (Bernstein I and Bernstein II) can no longer be applied in their original form. I recommend the alteration of the exclusion rules in a way that they should only be acceptable if the characteristics are located on more chromosomes than one , because of probable mutations.

LIST OF PUBLICATION RELATED TO THE SUBJECT OF THE THESIS

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