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Identification of Human Cadaver Remains by DNA Fragment Analysis in Disputed Paternity

A. Apostolov, E. Angelova

Department of Forensic Medicine and Deontology, Medical University, Faculty of Medicine, Sofia, Bulgaria

This study presents the results of expertises carried out using the DNA fragment analysis, associated with disputed parental origin and identification of the individual, performed in the laboratory for DNA analysis at the Department of Forensic Medicine and Deontology (DFMD) of the Medical Faculty (MF) at the Medical University (MU) – Sofia for a period of ten years /2001 - 2010/.

For the 2001 – 2010 period we worked on 538 cases of investigations to establish the parental origin, paternity and maternity, and in 102 cases (19.17%) the paternity of the compared men was excluded. These negative results from the applied DNA fragment analysis are based on the established presence in the child's genotype of alleles that cannot be inherited from the alleged father.

In parallel and independent of studies of disputed parental origin we carried out successful DNA identification profiling of cadavers, changed beyond recognition, and cadaver parts, which allowed the person's identification and led to the successful completion of the ongoing police investigations.

The data, presented by us, allow to lay the foundations for a broader and more comprehensive research, concerning the issues of parental origin and identification of the individual.

Key words: forensic science, DNA typing, paternity tests, identification, X and Y chromosomes

Introduction

In some criminal investigations, conducted by the Ministry of Interior, as well as in judicial investigations of the prosecutors and the court, it is necessary to establish the identity of cadavers, changed beyond recognition, or cadaver remains. In these cases, carrying out identification expertises by the method of DNA analysis, in particular, DNA fragment analysis, is indispensable and gives very good results **[6]**.

In considering civil cases many different issues arise, one of which is to establish the parental origin of children, in paternity or maternity in cases of disputed parental origin. In these cases, DNA analysis aims at comparing the genetic profiles of the mother, the child and the alleged father and to answer some basic questions: Can a child's genotype be deduced from the characteristics of the genotypes of the respondents, is the man the biological father of the child and if so, what is the probability of his paternity in relation to that child **[11]**. The methodology of AmpFLP analysis allows to give a definitive answer to these questions and two options are possible, namely, first, either a definitive exclusion of paternity or, second, its confirmation with a probability of over 99.9999%. The same principle can be used to exclude or to prove the probability of a woman being the biological mother of the child.

Research related to establishing paternity is based on three basic methods of research and statistical analysis of the results including: a/a group of families, randomly selected or selected according to ethnic, social and geographic criteria; b/a group of children selected on the same principle **[8,9]**; c/ individuals tested in relation to civil cases filed in court **[4,10]**.

In the preliminary proceedings, conducted on the Criminal Procedure Code, with investigations related to the identification of unknown persons, mutilated corpses, or parts of them, DNA expertises for the identification of bodies or to prove the origin of parts of a deceased person are ordered. This is possible using a comparative analysis of the genetic profiles of possible relatives of the wanted persons, who are reported missing or unaccounted for, which aims to find out a probable relationship between the compared persons. DNA identification can also be performed using other family relations, as carriers of the unchanged Y-chromosome in the male line, for example, brothers, uncles, grandfathers and great grandfathers **[7]**.

In our study we present data from expertises, related to disputed parental origin and the identification of individuals, carried out in the laboratory for DNA analysis in the Department of Forensic Medicine and Deontology of the Medical Faculty at the Medical University of Sofia for a ten-year period /2001 - 2010/.

Material and Methods

For the presented period of time 415 women, 557 children and 543 alleged fathers were tested for disputed parental origin, paternity. For the same period 9 tests for disputed parental origin, maternity, were made and 7 human cadavers and cadaver remains were tested for identification of parental origin.

1. DNA extraction. Extracting DNA from buccal mucosa smears of the compared persons was carried out under a FBI report provided by LIFE TECHNOLOGIES (Debra Nickson, technical services; 29.01.97). Stain Extraction Buffer with 0.01 M Tris, 0.01 EDTA, 0.1 M NaCl, 0.039 M DTT, 2% SDS characteristics is used and Protinase K (20mg / ml) is added later. An organic phenol extraction (phenol: chloroform: isoamyl alcohol = 25:24:1) was carried out after an 18-hour incubation at 56°C. DNA precipitation was performed with absolute alcohol, cooled to - 20°C. The extracted DNA was dissolved in TE-4 Buffer to a volume of 50 microliter, stored at - 20°C.

The blood samples, taken from compared persons, were processed for DNA extraction as described by Promega Corporation **[5]**: consecutive pouring the material over with erythrocyte lysis buffer (155 mM NH4Cl, 10mM KHCO3, 0,1 mM EDTA) and nucleo lysis buffer (75mM NaCL, 25mM EDTA), treatment with 2% SDS and Proteinase K (20mg/ml) and subsequent organic phenol extraction and precipitation with absolute alcohol, cooled to – 20°C. The extracted DNA was dissolved in TE-4 Buffer to a volume of 50 microliter, stored at -20°C.

2. PCR (polymerase chain reaction) involves three basic steps: thermal denaturation, annealing and extension. The polymerase chain reaction for the samples was carried out, using Peltier Termal Cycler 200 (MJ Research USA) in a 25-nl volume, containing: 1 X Buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.17 mg / ml BSA, Cy 5 ,Primer A and Primer B 0.4 pmol / nl for the studied STR markers for the material from the buccal mucosae and the blood samples from the compared individuals (Pharmacia LKB), 1U-Taq DNA polymerase, Recombinant- GibcoBRL-licensed by Life Technologies, Inc. under US patent N 5,338,671, ddH2O and template (extracted DNA).

3. Fragmental analysis was performed using automatic sequencer ALFexpressTM DNA Sequencer (Amersham Pharmacia Biotech) by ultrathin (0.5 mm thickness of the gel on the short thermocassette) vertical, denaturing polyacrylamide high voltage electrophoresis 6% PAAG 0.65 X TBE, 7M Urea or ReproGel™ (Ge Healthcare Bio-Sciences AB) 1 X TBE, 1500V, 60 mA, 25W, 50°C, laser detection of fragments and computer analysis by Fragment managerTM V1.2 Software (Amersham Pharmacia Biotech) **[3]**.

Analysis control was accomplished by: internal standards - AMEL 106 BP and H16401-L16110 347 BP, external standard Sizer 50-500 (Amersham Pharmacia Biotech) and sequenced allelic ladders for relevant STR markers, as it is at Ronny Decorte and kindly disposed by National Laboratory of molecular pathology at University Hospital of Obstetrics and Gynaecology, Sofia, Bulgaria **[12]**.

We did bio statistical analysis of results according to the frequencies of matching alleles of the compared persons using the respective genetic markers. The identified allele frequencies and the forensic statistical parameters for the set of tested basic STR's markers are published by St. Hristov et al. **[13]**.

Results and Discussion

During the 2001– 2010 period we developed 538 cases of parental origin, for paternity and maternity, as 102 cases were connected with exclusion of paternity (19.17%) based on the presence in the child's genotype of alleles that cannot be inherited from the alleged father.

Table 1. Distribution of the number of persons undergoing DNA identification tests and percentage of exclusions of paternity for the 2001-2005 period.

Year / tests	2001	2002	2003	2004	2005	Total number
Tested women	34	43	44	52	49	222
Tested children	49	52	51	64	59	275
Tested men	49	50	56	61	58	274
Percentage %	12.50	14.00	13.73	22.95	15.52	15,74 %
Tests for maternity	-	2	-	1	1	4
Identification of diseased persons	1	1	-	-	-	2

Table 2. Distribution of the number of persons undergoing DNA identification tests and percentage of exclusions of paternity for the 2006-2010 period.

Year / tests	2006	2007	2008	2009	2010	Total number
Tested women	37	48	47	38	23	193
Tested children	50	66	68	51	47	282
Tested Men	50	62	64	47	46	269
Percentage %	34.04	19.35	16.92	31.37	13.33	22.59 %
Tests for maternity	-	1	1	3	-	5
Identification of diseased persons	-	1	1	2	1	5

In 7 out of 9 comparative studies on suspicions of a baby mix-up, which occurred in maternity wards, exclusions of maternity were not established. In two of the legal cases maternity was excluded /2009/ but they were not cases of suspected baby mix-up (Table № 1 и Table № 2).

The analysis of investigation results over the 2001-2005 period showed that a total of 268 expertises about disputed parental origin were made. In 41 of these cases the paternity of the compared man was excluded. The estimated average rate of paternity exclusions for the 2001-2005 period was 15.74%. For the 2006- 2010 period a total of 270 expertises about disputed parental origin were carried out. In 61 of these cases the paternity of the compared man was excluded. The estimated average rate of paternity exclusions for the surveyed period (2006-2010) was 22.59%.

According to the data from the studies conducted by us using the DNA fragment analysis, the average rate of paternity exclusions during the 2001-2010 period was 19.17% (102/538).

During the target period we carried out seven successful DNA identifications of cadavers and cadaver parts, changed beyond recognition, using comparative analysis with their relatives, which allowed their identification and led to the successful completion of the ongoing police investigations.

Conclusion

The application of the method of DNA profiling, and in particular, the DNA fragment analysis in establishing the parental origin of the children led to the opportunity to definitively establish paternity in order to achieve social and legal responsibility for raising children. The obtained results also provide an opportunity for discussion of some moral and ethical issues as an important addition to the psychological portrait of Bulgarian population in its broad multiethnic complex.

The estimated average rate of 19.17% for paternity exclusions for the studied time period (2001-2010) provides an opportunity for future research encompassing comparison with results from other similar national studies and, in addition, with European and worldwide studies.

For the ten-year period we carried out successful profiling and identification of individuals that led to an increasing number of identified individuals on the basis of non-identified cadavers. The ability to identify individuals using DNA analysis of cadavers, changed beyond recognition, and cadaver parts allowed the identification of individuals, victims of natural disasters, murders, suicides, accidents, car accidents and more.

The data, presented by us, allow for laying the foundations for a broader research concerning the issues of parental origin and identification of the individual. To make the main conclusions on the issue, it is necessary to study many other diverse factors such as age, socio-economic status of respondents, socio-political climate, the behavior of individuals in different ethnic communities, etc.

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