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CASE REPORT

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Mutations or exclusion: an unusual case in paternity testing

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Abstract In an immigration case with the scope of family reunification, the DNA extracted from the saliva samples of the male child, the alleged mother and the putative father was typed with 22 autosomal short tandem repeat (STR) systems. In seven STR systems, the alleged mother could be excluded from maternity, and the case then had to be regarded as a deficiency case. Taking this fact into consideration, only two exclusions were found for the putative father, and the question arose whether there was an exclusion of the putative father or the existence of two mutations. Autosomal STR typing could not clarify the case, but the application of eight Y-chromosomal markers showed that the alleged father could be excluded from paternity.

Keywords Paternity case · Autosomal STRs · Mutation · Exclusion · Y-chromosomal STRs

Introduction

Short tandem repeat (STR) systems have been established in forensic stain analysis and in cases of parentage testing due to the high weights of evidence, and STR systems have replaced the conventional blood markers. The multitude of well-examined STR systems enables the experts to clarify any questions concerning paternity cases with a usually very high degree of certainty. The International Society for Forensic Genetics (ISFG) recommendations for paternity testing (Morling et al. 2003) prescribe some fundamental requirements concerning the organisation of the laboratory. The German recommendations for parentage testing in the year 2002 (Adamek et al. 2002) also include more practical aspects, e.g. the treatment of the samples, the number of STR markers to be used and the biostatistics. In general, at least 12 autosomal STR markers located on at least ten different chromosomes have to be examined, which are in normal paternity cases sufficient to clarify the case and to reach a paternity probability of more than 99.9% or to find more than three exclusions. In some cases, it is necessary to examine more than 12 autosomal markers, e.g. in deficiency cases where the mother of the child is absent or where only one or two exclusions are found. In the case of one or two exclusions, the existence of mutations has to be considered (Brinkmann et al. 1995, 1998, 2001; Thangaraj et al. 2004) or that a close relative of the examined man is the biological father. In the past, a multitude of different Y-chromosomal markers were evaluated (Kayser et al. 1997; Schneider et al. 1999; Carracedo et al. 2001; Gill et al. 2001; Barbarii et al. 2003) and gained an important role in paternity testing with male children (Kishida et al. 1996; Santos et al. 1993; Jobling et al. 1997; Kayser et al. 1998; Rolf et al. 2001). Y-chromosomal markers are useful in paternity analysis with a male offspring where the alleged father is deceased and male relatives are available. Any male relative in patrilineage with the deceased alleged father will show the identical Y-chromosomal haplotype, and comparison with that of the male child may help to clarify the case. In the case of an identical haplotype, it cannot be excluded that the deceased alleged father and the child belong to the same paternal lineage. Different haplotypes exclude the deceased alleged father from the paternity to the child. Here, we report a paternity case where, within the scope of family reunification, the DNA

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Table 1 Examination of 13 autosomal STR systems from 11 different chromosomes (first approach)

DNA system	Child	Alleged mother	Putative father
D3S1358	16, 17	15, 17	15, 17
VWA	18, 18	17, 18	18, 18
FGA	20, 21	**22, 24**	20, 25
TH01	9.3, 9.3	6, 9.3	6, 9.3
D5S818	12, 12	**11, 13**	10, 12
D7S820	9, 11	**8, 8**	**12, 13**
D8S1179	10, 14	10, 14	13, 14
ACTBP2	18, 26.2	18, 19	18, 19
D21S11	28, 30	**26, 31**	30, 30.2
D18S51	14, 14	14, 16	12, 14
D10S2325	9, 10	10, 12	7, 9
D12S391	18, 22	18, 18	16, 18
D8S320	23, 25	**25.2, 30**	23, 27

Exclusions for the alleged mother (five) and the putative father (one) are indicated in bold

extracted from saliva samples of the male child, the alleged mother of the child and the putative father were examined with 22 autosomal STR and 8 Y-chromosomal STR systems. Autosomal typing gave inconclusive results, whereas Y-chromosomal typing clarified this unusual paternity case.

Materials and methods

DNA extraction

DNA was extracted from saliva samples taken from the child, the mother and the putative father using the Chelex 100 method (Walsh et al. 1991). All persons were Caucasians and of Turkish origin.

Polymerase chain reaction amplification

Polymerase chain reaction (PCR) amplification was performed according to the protocols and the manufacturers' instructions for MPX-2 (Junge et al. 2003; SERAC, Bad Homburg, Germany) and SE-Filer (Heinrich et al. 2004) and Profiler (ABI, Weiterstadt, Germany). Primer sequences and amplification conditions for the additional STR systems were as follows: D8S320, Junge et al. (2001); D12S391, Junge and Madea (1998); D10S2325 and D1S1656, Wiegand et al. (1999); and Penta D and E, Barbarii et al. (2004). Primer sequences for the STR systems D5S818 and D7S820 were as described in the *STR workshop Handbuch* (Promega GmbH, Mannheim, Germany, 2004).

Table 2 Examination of nine additional autosomal STR systems

DNA system	Child	Alleged mother	Putative father
TPOX	8, 9	8, 9	8, 8
CSF1PO	10, 11	10, 12	10, 12
D13S317	11, 12	8, 11	12, 12
D16S539	11, 12	11, 11	11, 11
D2S1338	20, 25	**24, 24**	18, 25
D19S433	13, 14	13, 15	14, 15.2
Penta D	9, 12	12, 14	**13, 14**
Penta E	11, 13	11, 14	7, 13
D1S1656	12, 12	**15, 16**	12, 15.3

In this second approach, results for ten overlapping STR systems from Table 1 were verified. Exclusions for the alleged mother (two) and the putative father (one) are indicated in bold

Table 3 Examination of eight Y-chromosomal markers

DNA system	Child	Putative father
DYS393	12	12
DYS19	14	**16**
DYS389 I	13	13
DYS389 II	29	**30**
DYS392	13	**11**
DYS390	25	25
DYS385	11, 14	**11, 13**
DYS391	11	11

Bold numbers indicate exclusion for the putative father (four)

The Y-chromosomal markers were amplified using two multiplex kits (*genRES DYSplex-1* and *genRES DYSplex-2*, SERAC, Bad Homburg, Germany) comprising the minimal haplotype DYS19-DYS389I/II-DYS390-DYS391-DYS392-DYS393-DYS385a, b and the sex-specific amelogenin system. PCR amplification was performed according to the protocols and the manufacturers' instructions—*genRes DYSplex-1* (DYS391, DYS390, DYS389I/II, and DYS385a, b amelogenin) and *genRes DYSplex-2* (DYS392, DYS393, DYS389I/II, and DYS19).

Polymerase chain reactions were carried out in a Biometra Trioblock thermal cycler (Germany).

Electrophoresis

All STR systems—with the exception of D10S2325—were electrophoresed and analysed on an ABI Prism 310 Genetic Analyzer as described by Junge et al. (2003). Computer-aided genotyping was done using the software Genotyper (Version 2.5 or 3.7 NT). Electrophoresis of the system

Table 4 The hypothesis of relationship between the woman and the child is preferred compared to the non-relatedness

Hypothesis	Likelihood	A posteriori probability (%)
PV is uncle, PM is three degrees related	6.294×10^{91}	78.86
PV is uncle, PM is unrelated	1.684×10^{91}	21.11
PV is unrelated, PM is three degrees related	2.644×10^{94}	0.03
PV is unrelated, PM is unrelated	3.957×10^{95}	0.0005

D10S2325 was carried out as described by Wiegand et al. (1999).

Statistics

The likelihood quotients were calculated by standard techniques (Elston and Steward 1971) for likelihood calculation in pedigrees and implemented in the programme VAT (Baur, Fimmers, Spitz, Version 1.22).

Results and discussion

Quality assurance

The taking of the saliva samples of the mother and the putative father was carried out at our institute in Bonn, whereas the taking of the saliva sample of the child was taken abroad (Turkey). The proofs of identity were obviously complete and correct.

In general, internal controls are necessary to guarantee the correctness of the results, and various controls were also used in this case for the extraction, amplification and interpretation of the results.

The extraction and the amplification of the saliva samples were repeated on different days. Extraction blanks, negative and positive PCR controls were investigated to exclude the occurrence of contaminations. The STR systems TH01 and ACTBP2 were amplified with the MPX2 multiplex kit and additionally in singleplex amplifications. Results of ten overlapping STR systems were verified by the laboratory in Münster. The interpretation of the results was carried out both manually and through the aid of a computer and was cross-checked to confirm the results.

Autosomal STR typing

In a first approach, 13 autosomal STR systems were examined (Table 1). Surprisingly, five exclusions were found for the alleged mother, and the motherhood was regarded according to Hummel's verbal predicate as "obviously impossible". Therefore, the paternity case was treated as a deficiency case without the mother. Taking this fact into account, only one exclusion was found for the putative father in the STR system D7S820. The genotype of the child was 9, 11, and the genotype of the putative father was 12, 13. It could not be excluded that a one-step mutation had occurred. The question arose whether there is an exclusion or a one-step mutation. The probability of paternity was 94.6%, including the hypothesis of a one-step mutation. The typing of nine additional STR systems resulted in two additional exclusions for the alleged mother and another exclusion in the Penta D system for the putative father (Table 2). Similar to the D7S820 locus, the genotypes of the child (9, 12) and the putative father (13, 14) allows for the assumption of the existence of an additional mutation. The results of the autosomal typing were inconclusive due to the occurrence of only two exclusions, and the probability of paternity, considering that the exclusions were two one-step mutations, was 30% ("merely formal hint for non-paternity", according to Hummel's verbal predicate).

Y-chromosomal STR typing

The sex of the child was male, and typing of eight Y-chromosomal STR (Table 3) was applied and gave additional information; in four systems, the putative father could be excluded from paternity.

Statistics

The results suggest that the putative father may be related to the child. The Y-chromosomal results require that at least one female person is between this person and the child. Therefore, in the first step, we tested the hypothesis that the former putative father is the uncle of the child, i.e. that a sister of the putative father is the mother of the child, against the hypothesis that he is unrelated to the child. This resulted in a likelihood ratio of 15,906:1 in favour of the man being an uncle of the child. With the assumption of equal apriori probabilities, this gives an a posteriori probability of 99.994% for a sister of the putative father to be the mother of the child. An additional idea is that the assumed mother is also related to the child via the true father of the child. Some explorative analyses regarding this point revealed the greatest evidence for a second degree relationship between the putative mother and the true father of the child. Table 4 gives a summary of the comparison of the four possible hypotheses if one is additionally interested in a relationship between the putative mother and the child.

Conclusions

This remarkable case demonstrates impressively the usefulness of Y-chromosomal markers not only in forensics but also in paternity testing. Typing of 22 autosomal STR systems gave inconclusive results, and without the Y-chromosomal markers, the clarification of the case would have been regarded as impossible using only autosomal STR systems. Application of Y-chromosomal systems gave at least a clear statement of the non-paternity of the alleged father.

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