

MUTATIONS OR EXCLUSION: AN UNCOMMON PARENTAGE ASSESSMENT CASE.

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Abstract: A property partitioning case referred by the honorable District court of Nepal was received in this laboratory for the purpose of paternity establishment based on DNA analysis. The blood samples of the male child and the alleged father was collected on FTA mini card. The purified and dried FTA punch cards were subjected to PCR amplification using the 15 Autosomal STR markers containing AmpF/STR® Identifiler® PCR Amplification Kit. The DNA profiles of both samples (alleged father and child) showed two inconsistencies at D21S11 and D18S51 loci between them. This raised the question whether there was an exclusion of the alleged father or the existence of two mutations. The mismatches were reproduced and confirmed using AmpF/STR® Yfiler™ PCR Amplification Kit which contains 16 Y-STR markers. A total of thirteen Y-STR markers inconsistencies were found between the alleged father and the child. This result was sufficient to exclude the alleged father from the paternity to child.

Keywords: *Paternity case; Autosomal STRs; Mutation; exclusion; Y-STRs.*

INTRODUCTION

Short tandem repeat (STR) systems have replaced the traditional blood markers in cases of parentage testing and facilitate the experts to elucidate paternity issues with a high degree of confidence. The International Society for Forensic Genetics (ISFG) recommended the number of STR markers to be used in parentage testing (Morling *et al.* 2003). Generally, minimum of 12 autosomal STR markers situated on at least ten different chromosomes have to be examined, which is enough to reach a paternity probability of more than 99.9% in normal paternity cases or to find more than three exclusions (Junge *et al.* 2006). Paternity and kinship testing is now routinely performed by using a panel of 15 STRs to obtain exclusion or strong probability of paternity. However, in few cases, this number can be inadequate to solve the case particularly when the mother is unavailable for the test or when the biological father is a close relative of the legal one or where only one or two exclusions are found (Carboni *et al.* 2011). The existence of mutations has to be considered in the case of one or two exclusions (Brinkmann *et al.* 2001, Thangaraj *et al.* 2004). In these cases the test become a tangled and requires analysis of additional genetic markers to clarify the real scenario (Junge *et al.* 2006). Number of different Y- chromosome markers were evaluated in the past (Kayser *et al.* 1997, Gill *et al.* 2001) and gained a significant role in paternity testing with male children (Rolf *et al.* 2001). Comparison of Y-haplotype may help to clarify the case. In the case of an identical Y-haplotype, it cannot be excluded that the

alleged father and the child belong to the same paternal lineage. However, different haplotypes exclude the alleged father from the paternity to the child. Here, a paternity case was reported, where samples of the male child and the putative father were examined with 15 autosomal STR and 16 Y-chromosomal STR systems.

MATERIALS AND METHODOLOGY

Sample collection:

The blood samples of child and alleged father (of Tamang surname) were collected on FTA Mini card.

DNA extraction:

DNA was purified from 1.2 mm punch of bloodstained FTA papers using FTA purification reagent and TE⁻¹ buffer (10mM Tris-HCl, 0.1 mM EDTA, PH 8.0) with the manufacturer's (Whatman) recommendations.

PCR amplification:

- i. Autosomal STR: The purified and dried FTA punch cards were subjected to PCR amplification using the AmpF/STR® Identifiler® PCR Amplification Kit (Applied BioSystems, USA). The kit contains 15 Autosomal STR markers namely D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA and one amelogenin marker for gender determination. The standard method was adopted for PCR amplification (Jha *et al.* 2010).
- ii. Y - STR: The Y-chromosomal markers were amplified using the AmpF/STR® Yfiler™ PCR Amplification Kit (Applied BioSystems, USA),

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following the manufacturer user's manual. Briefly, Y filer enables the simultaneous amplification of 16 loci on the Y-chromosome, namely DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438 and DYS448.

Electrophoresis and Genotype determination:

All PCR products were electrophoresed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) and details of alleles were determined using GeneMapper ID v3.2 software (Applied Biosystems) following manufacture's recommendations.

Quality assurance:

The extraction, amplification and genotype of the samples were cross-checked to verify the results on different days. Negative PCR controls were investigated to exclude the occurrence of contaminations.

RESULT AND DISCUSSION

The initial analysis of the DNA from the suspected father and the child with 15 autosomal STR loci generated by using AmpF/STR® Identifiler kit showed two inconsistencies at loci D21S11 and D18S51 between child and alleged father (Table 1). The alleles for the D21S11 locus in the child and the suspected father were 30/32.2 and 28.2/29 respectively. Similarly, the composition of the alleles of locus D18S51 in the child and alleged father were 13/14 and 19/20, respectively. This raised the question whether it is a case of exclusion or mutation. Generally, if the tested man does not possess the alleles that have been inherited from the biological father, it can conclude that he cannot be the biological father. However, mutations between the father and child could lead to a false exclusion at any given loci (Chakraborty *et al.* 1996, Leopoldino *et al.* 2003), it is standard practice to require exclusion at three or more loci before a test is declared negative (William Goodwin *et al.* 2007). There are examples of double or even triple mutations in investigated cases (Li *et al.* 2011, Mansuet-Lupo *et al.* 2009, Mertens *et al.* 2009). Thus, it could not be excluded the possibility of one-step and multi-step mutation in locus D21S11 and locus D18S51 respectively. The main alternative for laboratories finding such results is addition of extra STRs to improve the probability or, alternatively, to give unambiguous exclusion (Carboni *et al.* 2011, Negi *et al.* 2006). Since the sex of the child was male, we decided to study Y-STR markers. The Y chromosome is haploid and paternally inherited. It is passed from father to son relatively unchanged, except by the gradual accumulation of mutations (Gill *et al.* 2001). Typing of sixteen Y-STR loci reveals thirteen locus inconsistencies between the alleged father and the child (Table 2). In Y-STR, exclusion should be based on differences observed for DNA specimens at a minimum of three loci (Lawrence Kobilinsky *et al.* 2005). Thus obtained result was plenty to exclude the alleged father from paternity. Based on expert opinion, the honorable judge decided for alleged father as non- paternity.

Table 1: Examination of 15 Autosomal STR markers.

Markers	Alleged Father	Child
D8S1179	15,16	14,15
D21S11	28.2,29	30,32.2
D7S820	8,10	8,11
CSF1PO	11,13	11,12
D3S1358	15,16	16,17
TH01	7,9	6,7
D13S317	11,11	11,12
D16S539	11,11	9,11
D2S1338	19,23	19,20
D19S433	14,15.2	13,14
vWA	14,17	14,17
TPOX	9,11	8,9
D18S51	19,20	13,14
D5S818	10,11	10,12
FGA	21,22	19,21

Note: Bold numbers indicate inconsistency for the putative father (two)

Table 2: Examination of 16 Y- STR markers.

Markers	Alleged Father	Child
DYS456	15	16
DYS389I	12	14
DYS390	23	25
DYS389II	29	31
DYS458	16	16
DYS19	14	15
DYS385	13	11,15
DYS393	13	13
DYS391	10	10
DYS439	11	10
DYS635	23	25
DYS392	12	11
Y_GATA_H4	10	13
DYS437	16	14
DYS438	10	12
DYS448	16	20

Note: Exclusions for the alleged father (13) are indicated in bold.

CONCLUSION

Analysis of 15 autosomal STR markers in parentage issues may not be sufficient for conclusive results in all deficiency cases. Because exclusion of the mother increases the possibility of false paternity inclusions (Lee *et al.* 2000, Sa'nchez *et al.* 2008). Thus, the laboratory expert should include the mother or increase the number of investigated loci or should include other typing like Y-STRs to further ascertain the results (Poetsch *et al.* 2006) where two markers inconsistencies observed between alleged father and child. In conclusion, the single parent/child cases should be analyzed very carefully (Thomson *et al.* 2001, De Ungria *et al.* 2002).

Conflict of interest: None

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