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CASE REPORT

Maternity exclusion with a very high autosomal STRs kinship index

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Abstract This paper reports a maternity testing case to assess the biological relationship between a woman and the boy she was adopting. For all 46 tested autosomal STR loci, the adopting woman and the boy shared at least one allele at each locus, which supported that the woman could be the biological mother of the boy. The pairwise kinship indices (KIs) were calculated for various identity-by-descent distributions. Motherson was the most likely relationship with a very high KI (i.e., 6.91E+08) based on 35 independent autosomal STR loci, but KIs of other pairwise relationships (e.g., aunt–nephew, full sib, etc.) were also high. Further testing of X-STRs and mtDNA excluded the maternity relationship between woman and boy, in which 13 out of 20 X-STR loci were inconsistent and 18 nucleotide mismatches were observed at hypervariable regions I and II of the mtDNA. However, a more distant relationship (e.g., aunt–nephew) cannot be excluded. This case reinforces that possible false identifications can occur in kinship analysis cases yielding very high KIs.

Keywords Short tandem repeat (STR) · X chromosome · Mitochondrial DNA · Kinship analysis

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Introduction

Kinship analysis is an important application of DNA-based forensic analysis. Genotyping results are calculated by a kinship index (KI; e.g., paternity index for paternity testing) given alternative hypotheses to determine supported relationships between or among individuals [1–3]. Most kinship analysis cases are determined by autosomal short tandem repeat (STR) markers. Y chromosome STR or mitochondrial DNA (mtDNA) can be used to further confirm or exclude paternal or maternal lineages, respectively. X chromosome STRs are also very useful, particularly in deficiency paternity cases involving females [4].

In this paper, a rare kinship testing case is reported, in which the biological relationship between an adopting family (including father and mother) and a boy to be adopted was tested to determine if the boy was the biological son of the adopting father or mother. The test was a legal requirement for adoption by the City of Beijing. The alleged father was excluded as the biological father of the boy with 24 inconsistent loci out of the 46 tested autosomal STRs. The

Table 1 Autosomal STR profiles (46 loci) of the woman and the boy

Loci	Woman	Boy	Loci	Woman	Boy
D19S433	14,15.2	13.2,15.2	D1S1677	14,15	10,15
D5S818	11,13	11,13	D11S4463	13,14	14,15
D21S11	30,31	29,31	D1S1627	13	13
D18S51	13,16	16,19	D3S4529	15,16	15,15
D6S1043	12	12,14	**D2S441**	11.3,12	11.3,14
D3S1358	16,17	16,16	D6S1017	10,12	8,10
D13S317	10,11	11,12	D4S2408	8,10	8,9
D7S820	11,	11,	D17S1301	12,13	9,13
D16S539	9,11	11,12	D1GATA113	7,11	7,11
CSF1PO	11,12	12	D18S853	12,14	11,14
Penta D	9,11	11	D20S482	12,13	13
vWA	17,19	17,18	D14S1434	14	14
D8S1179	15,15	10,15	D9S1122	12,13	12,13
TPOX	8,8	8,11	D2S1776	12,13	11,13
Penta E	14,17	12,17	D10S1435	13,14	13,14
TH01	9	9	D5S2500	17,17	17,18
D12S391	18,22	18,22	**D18S1364**	16,20	16,20
D2S1338	19,23	19,23	**D13S325**	20,21	19,20
FGA	23,24	24,25	D2S1772	21,28	24,28
D6S474	14,18	17,18	D11S2368	20,22	18,20
D12ATA63	12	12,17	D22-GATA198	14,17	17,18
D22S1045	15,17	15,16	D8S1132	19	19,22
D10S1248	13,13	12,13	D7S3048	20,23	23,27

Loci in bold were ignored in kinship analysis due to close linkage to other loci

alleged mother and the boy shared at least one allele at all 46 tested autosomal STR loci which yielded a very high KI index based on 35 independent autosomal STR loci between them. Further testing of X-STR loci and mtDNA excluded the maternal relationship between the woman and the boy. The genotypes were tested and confirmed in two separate laboratories (i.e., Beijing and Shanghai) to ensure that no genotyping errors were made during the testing processes. All tested individuals were Chinese Han.

Material and methods

DNA extraction

Genomic DNA was extracted using the Chelex-100 and proteinase K protocol from blood samples of the child and the adopting woman [5]. The quantity of recovered DNA was determined by a spectrophotometric method.

STR typing

Autosomal STRs typing

Nineteen autosomal STR loci plus amelogenin were amplified using the AmpFlSTR® Sinofiler™ kit (Life Technologies, Carlsbad, CA) and PowerPlex®16 System (Promega, USA) following the manufacturers' recommendations. An additional 27 autosomal STR loci plus amelogenin were analyzed using two domestic kits AGCU 21+1 (http://www.agcu.cn/) and STRtyper-10G (http://www.zhcodon.com/). Locus D19S433 was included in Sinofiler™ kit and the domestic kit 21+1. Amplification reactions were carried out using the GeneAmp PCR system 9700 (Life Technologies). The amplified products were analyzed using 3130*xl* Genetic Analyzer (Life Technologies). Genotyping data were determined by GeneMapper v3.2.1 software.

X-STRs typing

Eight X chromosome STR loci plus amelogenin were co-amplified using the Mentype® Argus X-8 Kit (Biotype® AG, Germany) following the instructions of the manual [6]. An additional 16 X chromosome STR loci were co-amplified using an inhouse kit (made by Shanghai Key Laboratory of Forensic Sciences; Institute of Forensic Sciences, Ministry of Justice, People's Republic of China) with 12.5 μL reaction volume, including 10× PCR buffer 1.25 μL, 2.5 mM dNTP, 18.75 mM $MgCl_2$, 1 unit of Golden Taq polymerase, primer mix 1 μL, ddH_2O 4 μL, and the

Table 2 Likelihood of observing the genotype profiles of the woman and the boy given most common pairwise relationship hypotheses based on 35 independent autosomal loci

Pairwise kinship	IBD_0	IBD_1	IBD_2	Likelihood	KI (vs unrelated)
Monozygotic twins	0	0	1	0	0
Same father incest relationship[a]	0	0.75	0.25	1.92E–77	4.15E+07
Parent–child	0	1	0	3.20E–76	6.91E+08
Full sib	0.25	0.5	0.25	1.01E–78	2.19E+06
Half sib Grandparent–grandchild Aunt–nephew	0.5	0.5	0	1.28E–79	2.76E+05
First cousin	0.75	0.25	0	6.19E–82	1.34E+03
Unrelated	1	0	0	4.63E–85	1

[a]The alleged mother is the biological mother of the boy and the biological father of the boy is the biological father of the alleged mother

Table 3 X-STR profiles (totally 20 loci) analyzed with Mentype® Argus X-8 kit (the first 8 loci) and an inhouse kit (the last 16 loci)

Loci	Woman	Boy
HPRTB[a]	**12,14**	**13**
DXS8378[a]	11,11	11
DXS7423[a]	**15,15**	**14**
DXS7132[a]	**15,16**	**13**
DXS10134	37,38	38
DXS10074	**17,17**	**15**
DXS10101	**30,31**	**30.2**
DXS10135	**23,26**	**33**
GATA165B12	**10,12**	**9**
DXS101	24,26	24
GATA172D05	**8,11**	**10**
HPRTB[a]	**12,14**	**13**
DXS981	**13.3,15**	**14**
DXS8378[a]	11,11	11
DXS6795	16,17	16
GATA31E08	**9,11**	**12**
DXS6809	**33,35**	**34**
DXS6803	11,12	11
DXS9902	**10,11**	**9**
DXS6807	11,14	14
DXS7423[a]	**15,15**	**14**
DXS7133	**9,9**	**10**
DXS6810	18,19	18
DXS7132[a]	**15,16**	**13**

Loci in bold are the inconsistent loci between the adopting mother and the boy

[a]Denotes identical loci included in both kits (i.e., HPRTB, DXS8378, DXS7423 and DXS7132)

template DNA 5 μL. Thermal cycling conditions were: 15 min at 95°C follows by 30 cycles at 30 s at 95°C, 90 s at 57°C, 60 s at 72°C, and a final extension at 60°C for 60 min. Both kits include the loci of HPRTB, DXS8378, DXS7423, and DXS7132. Altogether 20 X chromosome STR loci plus amelogenin were tested.

Sequencing of mtDNA regions HV-I and HV-II

Hypervariable regions I and II (HV-I and HV-II) of the mtDNA control region were amplified by using primers pairs L16047/H16464 and L29/H408, respectively. The purified PCR products were then sequenced on an ABI 3130*xl* Genetic Analyzer.

Results

A total of 46 autosomal STR loci were tested (Table 1). Among these 46 loci, 11 pairs of loci (i.e., D11S2368 and TH01, D12S391 and vWA, D13S325 and D13S317, D18S51 and D18S1364, Penta D and D21S11, D22-GATA198B05 and D22S1045, D2S1772 and D2S441, TPOX and D2S1338, D5S818 and CSF1PO, D6S1043 and D6S474, D8S1132 and D8S1179) were physically close in proximity (i.e., <40 Mb) and might not be inherited independently. The locus of each pair with a lower discriminating power in Chinese Han population (i.e., TH01, vWA, D13S325, D18S1364, D21S11, D22S1045, D2S441, TPOX, CSF1PO, D6S474, and D8S1179) was removed from kinship analysis. The likelihoods of the genotype profiles giving various identity-by-descent (IBD) distributions were calculated using MPKin [7]. Various relationships of the mother and boy were assessed from the least related pairwise kinship (i.e., unrelated, $IBD_0=1$, $IBD_1=0$, $IBD_2=0$) to the most related pairwise kinship (i.e., monozygotic twins, $IBD_0=0$, $IBD_1=0$, $IBD_2=1$) as well as a "same father incest relationship" in which the alleged mother is assessed as the biological mother of the boy and they share the same biological father. Table 2 lists the likelihoods and KIs (compared to the unrelated hypothesis) of some common pairwise relationships. The monozygotic twin relationship is not possible. Mother–son was the most likely relationship based on 35 independent autosomal loci with very high KI in all IBD combinations (although other relationships in which high KI values were obtained cannot be excluded). The KI would be higher if the potentially linked 11 loci were considered; the woman and the boy share at least one allele at all these loci.

The adopting family questioned the testing results and insisted on a nonbiological relationship. Further, X-STR (Table 3) and mtDNA (Table 4) testing was performed. There were 13 inconsistent loci out of 20 X-STR loci

Table 4 Sequence results of HV-I and HV-II of mitochondrial DNA

	mtDNA HV-I (16097-16422nt)										
Boy	16138 C	16140 C	16181 G	16183 C	16189 C	16217 C	16223 C	16274 A	16290 C	16319 G	16362 T
Woman	16138 A	16140 T	16181 A	16183 A	16189 T	16217 T	16223 T	16274 G	16290 T	16319 A	16362 C
	mtDNA HV-II (66-407nt)										
Boy	150 T	152 T	235 A	263 G	309.1 C/309.2 C/309.3 T			384 A	399 T		
Woman	150 C	152 C	235 G	263 A	309.1 T			384 C	399 A		

observed between the adopting woman and the boy, including large step differences (e.g., 23 or 26→33 at the DXS10135 locus) and integer allele to fractional allele differences (i.e., 30 or 31→30.2 at the DXS10101 locus). In the mtDNA HV-I and HV-II regions, 11 and 7 nucleotides mismatches were found, respectively. The overall data did not support a mother–son relationship and the woman was excluded as the biological mother. However, other close relationships cannot be excluded. Paternal aunt–nephew relationship or paternal grandmother–grandchild relationship is possible (the woman was 47 years old and the boy was 2 years old) and that relationship does not require the same mtDNA haplotype or at least one allele shared at all X-STR loci.

Discussion

The adopting family was contacted to provide more reference samples in their family to test the possible aunt–nephew relationship. However, they were reluctant to provide any further information or samples and withdrew the adoption application.

This is an extreme case, in which at least one allele was shared at 46 autosomal STR loci. A very high KI (i.e., at least 6.91E+08 for maternity relationship based on 35 independent autosomal STRs) was obtained. The alleged mother would have been falsely included, while the X-STR and mtDNA results showed the alleged mother was not the true parent. It demonstrated that the autosomal STR markers may not be able to provide sufficient information in cases where the true parent may be a close relative of the alleged parent and supplementary typing more genetic markers (e.g. X-STR, Y-STR, mtDNA, SNP) is necessary [8, 9]. The duo case showed that X-STR and mtDNA markers were more informative than the autosomal STR markers.

As indicated in Ge et al. [10, 11], the chance of unrelated or distant relationships being identified as closely related is extremely low but in some cases may still be considered. This real case showed that a risk of a false conclusion with high value of KI can occur. The chance to have at least one allele shared at all 46 loci (assuming independence among all loci) or 35 independent loci for aunt–nephew relationship is 4.5×10^{-8} or 1.9×10^{-6}. Supposing almost a million paternity or maternity testing cases have been done in China in the past more than 10 years, the chance to observe such extreme case is not as low as it seems to be. Given the total number of kinship analysis cases worldwide, the limited number of genetic markers typically used, and limited information in a pairwise comparison, scenarios as described herein will be observed.

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