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Short Communication

A case study on limitations of commercial autosomal STR kits for determination of half-sibling by DNA analysis

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Abstract: A case study was conducted to evaluate the significance of adding lineage markers for a complex kinship analysis. Additional genetic information may increase likelihood ratio values for true relationships in a pedigree, while reducing the chance of identifying false relationships. It is very difficult to establish a relationship between siblings by the statistical evaluation with autosomal STR markers. The clear discrimination of true versus false relationships is important for complex kinship cases, such as sibling testing, deficient paternity testing, missing person's identification as well as immigration testing with the help of forensic DNA technology. This case study represents a false parent-child relation or full sibling, one individual is male and other individual is female, where matches were found at least one allele at all 23 tested autosomal STR loci with Combined Likelihood is 75,446,930.822 for Parent-Child and 284,041.488 for Half-Sibling indicates that 266 times more likely parent-child than half-sibling. In order to reach an accurate conclusion, we further included one sister of the putative brother and mother of the putative sister and these four individuals DNA samples were analyzed with X-chromosome STR kits, showing mismatches between claimed siblings in eight loci out of twelve loci and confirm that they are not parent-child. Finally we confirm that they are half-sibling and they are come from same father but different mother. This case emphasizes the necessity of lineage markers like X chromosome or Y chromosome DNA data for interpreting critical sibling cases where one of the siblings is female and their father is dead.

Keywords: autosome; x-chromosome; STR marker; linkage group; paternity; sibling

1. Introduction

Autosomal STR kits are the widely used genetic markers in most of the forensic DNA laboratories in the world. Over the past three decades, a set of core loci has been established by the human identity testing community (Butler, 2006; Butler, 2007). Invariably, a panel of 10-15 STR loci is analyzed in most laboratories, which are available as commercial kits from many different sources. In spite of that, if these additional loci are from the same chromosome, then appropriate linkage analysis data will be required to show that they are independent of each other. Use of non-autosomal STRs, such as Y- or X-chromosome STRs, sometimes helps resolving this kind of situations like complex kinship analysis, deficient paternity testing and missing person's identification. Y-chromosome STR can only help when the disputed child is male. However, its use is limited when a close male relative of the alleged father is involved. On the other hand, X-chromosome STRs can credibly establish the paternity when the disputed child is female as well as the mother–son or sister-sister relationship.

Male individuals inherit their one X-chromosome from their mother while female individuals receive one X-chromosome from the mother and the other one X-chromosome from the father. So, female individuals fathered by same man share their parental X-chromosome. Increasing the number of STR loci resulted in increased discrimination of true and unrelated pairs, although the results were more dramatic for parent-offspring and full

siblings than for half siblings. X chromosome analysis also facilitates the analysis of questioned sibling. A sibling analysis is the comparison of the genotypes of two persons and the calculation of a probability that these two persons are related. The probabilities for different degrees of relationship (parents-child, full sibs, half sibs and first cousin) will be calculated and ranked by their probabilities (Genoproof user manual, Qualitype AG Moritzburger Weg 67, Germany).

2. Materials and Methods

2.1. Sample collection and DNA extraction

Blood Samples from the questioned siblings and the mother was collected in EDTA tubes with written informed consent. Genomic DNA was extracted using the Chelex-100 method (Walsh *et al.,* 1991). Extracted DNA was quantified by NanoDrop-1000 (Nano Drop Technologies, Inc, Washington DE 19810, USA).

2.2. PCR amplification

Approximately 1–2 ng of template DNA was used for each PCR amplification process. PCR amplification was carried out by using Veriti® Thermal Cycler (Applied Biosystems, USA). Temperature programs were employed according to the PCR kits manufacturer's instructions. A total of 23 autosomal STRs were amplified by using AmpFlSTR® Identifiler® Plus (Applied Biosystems, USA), Investigator® ESS Plex (Qiagen, Germany), PowerPlex® 17 System and next generation PowerPlex® Fusion System (Promega Corporation, USA) following the protocol provided by the manufacturer. We also amplified Insertion/Deletion of chromosome at 30 InDel markers with Investigator® DIPplex Kit (Qiagen, Germany). Finally, the X-chromosome analysis was carried out by using Investigator® Argus X-12 PCR kit (Qiagen, Germany) at 12 loci. These STRs loci have been evaluated for forensic purpose by many different groups (Szibor *et al.,* 2000; Moreno *et al.,* 2006; Turrina *et al.,* 2007 & Gomes *et al.,* 2009).

2.3. DNA analysis and STR typing

The PCR amplified products were separated and typed by capillary electrophoresis on ABI 3500 Genetic Analyzer (Applied Biosystems, USA) using POP-4 polymer and Data Collection Software v1.0. Peak sizing and genotype assignments were done by GeneMapper ID-X v1.2.

2.4. Statistical calculation

The Sibling Analysis was calculated by using GenoProof software ver3.0 (Qualitype AG, Germany) and CODIS 7.0 SP3 (FBI, USA) respectively. This calculation used the proper allele frequency of Bangladeshi mainstream population, as published earlier from this laboratory (Ferdous *et al.,* 2006; Ferdous *et al.,* 2009).

3. Results and Discussion

In this study, initially, we genotyped for two questioned fatherless siblings by using most popular autosomal STR kit named AmpFlSTR® Identifiler® Plus and found matches at least one allele at all 15 loci. For further confirmation, we increased STR markers up to 23 loci and surprisingly found matches at least one allele at all 23 tested loci with Combined Likelihood is 75,446,930.822 for parent-child and 284,041.488 for half-sibling and indicates that 266 times more likely parent-child than half-sibling. Moreover we analyzed these two DNA samples with insertion/deletion kits and found matches at all 30 In/Del markers (Table 1). Then we collected the blood samples from the mother of the female and the sister of the male individual and genotyped these samples with both autosome and X-chromosome STRs kits to establish a family relationship. Maternity was proved for the questioned female sibling. Primarily, we calculate the sibling relationship with combined likelihood of the questioned male individual and his sister with GenoProof software ver3.0 and confirm that they are full-sibling. Then we checked the X-chromosome genotype of two sisters and surprisingly we found that they were shared at all 12 loci with four linkage groups and confirm that they are come from same father. Finally, we construct the relationship between two questioned siblings with the help of the mother and the sister's DNA profile.

The apparent coincidental nature of the data presented in Table 1 led us to employ X-STRs only to resolve the situation for two reasons. First, one of the siblings was a girl, and Y-chromosome STR cannot help the situation. Second, we were not able to rule out the possible involvement of a close male relative of the alleged father due to lack of further information and the questionable ethics of raising this issue. DNA markers on the X-chromosome have been shown to be a powerful tool for solving complex relationship cases (Szibor *et al.,* 2003; Szibor *et al.,* 2005). The main application of X- chromosomal markers is in deficient paternity cases especially the investigations of multiple females with the hypothesis that they share the same father. As shown in this work, X chromosome markers were immediately able to include the siblings in fatherless condition.

Table 1. DNA profiles obtained from tested siblings with autosomal STRs (23 Loci), insertion/deletion (30 InDel) and x-chromosome STRs (12 Loci).

(A) Autosomal STRs

Loci	AmpFlSTR® Identifiler®Plus		Investigator® ESS Plex		PowerPlex®17 System		PowerPlex® Fusion System	
	Indv-1	Indv-2	Indv-1	Indv-2	Indv-1	Indv-2	Indv-1	Indv-2
D8S1179	10-13	12-13	10-13	12-13	10-13	12-13	10-13	12-13
D21S11	32.2-32.2	28-32.2	32.2-32.2	28-32.2	32.2-32.2	28-32.2	32.2-32.2	28-32.2
D7S820	10-12	11-12	-	-	-	-	10-12	11-12
CSF1PO	10-11	11-12	-	-	-	-	10-11	11-12
D3S1358	16-18	16-18	16-18	16-18	16-18	16-18	16-18	16-18
TH01	8-9	6-9	8-9	6-9	8-9	6-9	8-9	6-9
D13S317	11-12	8-12	-	-	-	-	11-12	8-12
D16S539	9-11	9-9	9-11	9-9	9-11	9-9	9-11	9-9
D2S1338	19-20	18-19	19-20	18-19	19-20	18-19	19-20	18-19
D19S433	12-13	13-16	12-13	13-16	12-13	13-16	12-13	13-16
vWA	14-17	14-14	14-17	14-14	14-17	14-14	14-17	14-14
TPOX	8-8	8-8	-	-	-	-	8-8	8-8
D18S51	14-16	14-15	14-16	14-15	14-16	14-15	14-16	14-15
D5S818	11-13	11-13	-	-	-	-	11-13	11-13
FGA	21.2-25	21-21.2	21.2-25	21-21.2	21.2-25	21-21.2	21.2-25	21-21.2
D1S1656	-	-	13-15.3	8-13	13-15.3	8-13	13-15.3	8-13
D10S1248	-	-	14-15	15-17	14-15	15-17	14-15	15-17
D22S1045	-	-	16-16	15-16	16-16	15-16	16-16	15-16
D12S391	-	-	18-24	20-24	18-24	20-24	18-24	20-24
D2S441	-	-	10-11	10-11	10-11	10-11	10-11	10-11
SE33	-	-	-	-	12-22.2	12-35	-	-
Penta-E	-	-	-	-	-	-	11-16	11-14
Penta-D	-	-	-	-	-	-	12-12	9-12

(B) Insertion/Deletion

Loci	Investigator® DIPplex Kit	
	Indv-1	Indv-2
HLD77	D77-	D77-
HLD45	D45-/D45+	D45-
HLD131	D131-/D131+	D131-/D131+
HLD70	D70+	D70+
HLD111	D111+	D111-/D111+
HLD6	D6+	D6-/D6+
HLD58	D58-	D58-/D58+
HLD56	D56+	D56+
HLD118	D118+	D118-/D118+
HLD92	D92-/D92+	D92-
HLD93	D93-/D93+	D93-/D93+
HLD99	D99-/D99+	D99-/D99+
HLD88	D88-	D88-
HLD101	D101-/D101+	D101-
HLD67	D67+	D67+
HLD83	D83-/D83+	D83-
HLD114	D114-	D114-
HLD48	D48-	D48-/D48+
HLD124	D124+	D124+
HLD122	D122-	D122-/D122+
HLD125	D125-/D125+	D125-/D125+
HLD64	D64+	D64+
HLD81	D81+	D81+
HLD136	D136-/D136+	D136-/D136+
HLD133	D133-	D133-
HLD97	D97-/D97+	D97-
HLD40	D40-	D40-/D40+
HLD128	D128-/D128+	D128-/D128+
HLD39	D39-	D39-/D39+
HLD84	D84-	D84-/D84+

(C) X-Chromosome STRs

	Loci	Investigator® Argus X-12 Kit			
		Indv-1	Indv-2	Indv-3	Indv-4
LG1	DXS10148	20	25.1-26.1	23.1-25.1	19-26.1
	DXS8378	11	10-10	10-12	10-11
	DXS10135	26	21.1-22.1	22.1-28	21.2-25
LG2	DXS10074	16	7-16	16-17	7-16
	DXS10079	18	19-21	18-21	18-19
	DXS7132	15	13-14	13-13	14-15
LG3	DXS10101	28	28.2-30.2	29.2-30.2	28-28.2
	DXS10103	18	18-19	18-19	18-19
	HPRTB	15	12-13	13-13	12-15
LG4	DXS10148	16	14-16	15-16	14-16
	DXS8378	35	35-36	35-36	35-35
	DXS10135	28	27-29	27-30	28-29

Indv-1=Step-brother; Indv-2=Step-sister; Indv-3=Step-sister's mother ; Indv-4=Step-brother's sister

Like this case, a false mother was included with 46 autosomal STR markers. This case was solved with the help of X-STR and SNP markers and found mismatch at 13 X-STR loci out of 20 loci and 5 SNP loci out of 40SNP markers and finally the putative mother was excluded (Li *et al.,* 2015). It is obviously true that autosomal STR kits has high power of discrimination capacity than lineage marker kits in forensic DNA platform, but sometimes it fails to give conclusive results. Numerous laboratories reported that autosomal STR kits had a chance to false inclusion in paternity testing and sibling testing (Babol-Pokora *et al.,* 2006; Akhteruzzaman *et al.,* 2012). The overall results clearly indicate that the questioned sibling is neither parent-child nor full- sibling. This results give us the conclusion that they were half-sibling. The apparent inconclusive nature of the overall result demonstrates the necessity of X chromosome analysis to give a appropriate conclusion. As one of the half siblings was female, the Y-chromosome STR cannot help in this situation.

4. Conclusions

In this study, we employed four common forensic DNA kits with standard 23 autosomal STRs loci and also 30 InDel markers kit and found surprising complete matches at all genetic loci. For this kind of situation to resolve inconclusive paternity cases or sibling cases, we need to add more autosomal STR markers as well as lineage markers as supplementary analysis for draw a conclusive result.

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