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DNA Typing Not Only Has Reactive Forensic Application But Also Plays A Proactive Investigation Role

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Abstract

Nowadays DNA typing is an indispensable tool in criminal investigations. However, most of its forensic applications are of the nature of reactive response. We report a particular maternity testing case, in which DNA typing played a proactive investigation role, leading to resolution of the case. Four commercial STR kits and mitochondrial DNA sequence in D-loop were analyzed. Of the 52 STRs tested, 6 mismatched loci (i.e., 88.5% of match percentage, 46 out of 52) were identified between the alleged mother and the baby, however, both having identical mitochondrial DNA sequence. With the two pieces of information, we judged that the biological mother should be a full sister of the alleged mother, and then actively told the police department. Detectives were thus asked to check it up, and the whole story came out as expected. Significance of this case is that DNA typing can also exhibit a proactive investigation function. DNA specialists and detectives could have different perceptions on typing results, so institutionalization of a communication line between the both might be important for solving crime cases.

Keywords: forensic science, DNA typing, short tandem repeats (STRs), proactive investigation, maternity testing

Introduction

The use of DNA typing for human identification appeared in late 1985. Since then, forensic DNA profiling and criminal investigations are now inseparable. Modern DNA profiles analyze short tandem repeats (STRs), using dye probes that detect differences in the number of STRs between individuals. DNA typing was employed predominately by a one-to-one comparison, an evidence profile compared with that from a reference sample. With the benefits of various commercial STR kits, they have been successfully utilized in criminal cases [1], disaster victim identification [2], paternity testing [3], and so on. Amongst the forensic uses, DNA typing always played a reactive role, though an indispensable tool, in proving (or disproving) criminal's association. For example, DNA analysts passively made a conclusion on whether biological evidence was associated with a specific source. Contrary to the reactive role, a proactive role of DNA typing in criminal investigations means that DNA technicians actively specify the direction and/or the method of coping with a crime case. However, the proactive role in practice was seldom seen.

We hereby report a special case consisting of two related suspect mothers (full sisters), in which DNA typing exhibited a proactive function for revealing the truth. Because of an illegal activity, the real mother escaped from a hospital after childbirth, leaving a note with her elder sister's name on it. Six months later, the elder sister (alleged mother, AM) was arrested. She helped to counterfeit the mother's identity and made false statements of abandoning her baby intentionally. Although the criminal investigators checked several

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records including the hospitalization document and the tape of surveillance camera at the hospital, no any suspicious sign was found. According to regulations, a maternity testing should be conducted to make the investigation complete. Unexpectedly, 6 exclusionary and 46 inclusionary loci were identified from the 52 STRs analyzed, implicating that the real mother should be a maternally close relative of the AM. The criminal investigators were thus asked to confirm whether the AM has a full sister, and finally the truth came out.

Materials and Methods

DNA extraction

Genomic DNA was extracted from the oral swabs of the subjects in this case using the QIAamp DNA mini kit (Qiagene, Valencia, CA, USA) under conditions described by the manufacturer.

STRs DNA typing

We typed STR DNA using three autosomal STRs and one X-chromosomal commercial kits. In addition to the gender marker of Amelogenin, the three autosomal DNA kits, AmpFlSTR® Identifiler (Applied Biosystems, Foster City, CA), Investigator HDplex (Qiagen, GmbH, Hilden, Germany), and Genephile G-Plex (GenePhile Bioscience Co., Ltd., Taipei, Taiwan) co-amplify 15, 13, and 15 STR loci, respectively. The X-chromosomal Genephile X-Plex kit (GenePhile Bioscience Co., Ltd., Taipei, Taiwan) includes a multiplex 13 STR loci and also the Amelogenin marker. The STR profiles were identified with an Applied Biosystems 3130 XL Genetic Analyzer according to the protocol of the manufacturer (Applied Biosystems).

PCR amplification of mitochondrial DNA

Two hypervariable fragment lengths of HV1 and HV2 in mitochondrial DNA were PCR amplified. The following primer sets [4, 5], L15997F (5'-CACCATTAGCACCCAAAGCT-3')/H 16401R (5'-TGATTTCACGGAGGATGGTG-3'), and L00029F(5'-GGTCTATCACCCTATTAACCAC-3')/ H00408R(5'-CTGTTAAAAGTGCATACCGCCA-3'), were used for amplifying the HV1 and HV2, respectively.

PCR amplification was performed in 25μL of reaction mixture in a 9700 thermal cycler (Applied Biosystems) with the following conditions: denatured at 95°C for 10 min, followed by 32 cycles of 95°C for 10 sec, 60°C for 30 sec and 72°C for 30 sec. The PCR product was checked on a 3 % agarose gel.

Sequencing of mitochondrial DNA

Prior to the sequencing reaction, the PCR products were purified using a QIAquick® PCR Purification Kit (QIAGEN Inc, Valencia, CA, USA). Cycle sequencing was conducted in a Perkin Elmer 9700 thermal cycler. Automated DNA sequencing was carried out on an ABI 3130 sequencer.

Results and Discussion

Initially, the AmpFlSTR® Identifiler™ kit was carried out on oral swab samples from the alleged mother (AM) and the baby. Unusually, except for one mismatch at D2S1338, the other 14 loci were found matched between the two (Table 1). This peculiar outcome might result from a maternal one-step mutation, and could not exclude her as the biological mother of the baby. In literatures [6, 7], a wrong paternity exclusion case with two mismatches was reported, in which the alleged father is indeed the real father. Moreover, Gunn et al. (1997) and Sun et al. (2012) [8, 9] suggested that exclusionary events at three or more independent STR loci should be essential for indication of non-paternity. Accordingly, we performed other three kits, i.e., Investigator ®HDplex, GenePhile G-Plex, and GenePhile X-Plex. Table 1 shows the 6 exclusionary loci at D2S1338 (ID), Penta E (GP), D3S1744 (GP/HD), D6S474 (GP/HD), D20S470 (GP), and DXS8378 (GX), which obviously ruled out the AM as the biological mother of the baby. It is noteworthy that the other 46 STRs out of the 52 loci tested, i.e., with a percentage of 88.5%, meet the Mendelian inheritance for the AM and the baby, implicating that the biological mother and the AM could have a close kinship relationship. Table 2 presents the same mitochondrial DNA sequence between the AM and the baby, confirming the above inference.

Table 1: Complete STR-typing results of the counterfeiting case. Four commercial STR kits were employed. A total of 71 shared STR alleles between the alleged mother and the biological mother were found. The underlined boldface numbers represent the exclusionary STR loci..

No.	STR Locus	Commercial Kit[a]	Baby	Alleged mother	Biological mother	Shared allele in the sisters
1	D8S1179	ID	13/14	13/14	13/14	13/14
2	D21S11	ID	28/29	29/31.2	29/31	29
3	D7S820	ID	8/11	11/11	8/11	11
4	CSF1PO	ID	10/12	10/11	10/11	10/11
5	D3S1358	ID	15/16	16/17	16/18	16
6	TH01	ID	7/9	9/9	8/9	9
7	D13S317	ID	9/12	8/12	8/12	8/12
8	D16S539	ID	9/11	9/11	9/14	9
9	vWA	ID	14/18	14/17	14/20	14
10	TPOX	ID	8/11	8/11	8/11	8/11
11	D18S51	ID , HD	14/16	14/19	14/19	14/19
12	D5S818	ID	9/12	12/13	12/12	12
13	FGA	ID	22/24.2	22/23	22/23	22/23
14	D2S1338	ID	21/24	**18/23**	18/21	18
15	D19S433	ID	14/14	14/14.2	14/14.2	14/14.2
16	Penta E	GP	5.2/22	**10/16**	10/22	10
17	Penta D	GP	9/9	9/14	9/14	9/14
18	D12S391	HD	19/22	22/22	18/22	22
19	D2S1360	HD	21/22	21/22	22/22	22
20	D3S1744	HD, GP	18/18	**14/17**	17/18	17
21	D4S2366	HD, GP	9/11	9/11	9/11	9/11
22	D5S2500	HD	13/17	14/17	14/17	14/17
23[b]	D6S474	HD	15/15	**14/14**	14/15	14
24[b]	D6S474	GP	15/15	11/15	11.1/15	15
25	D7S1517	HD	23/25	23/25	21/23	23

26	D8S1132	HD	17/18	18/19	18/19	18/19
27	D10S2325	HD, GP	12/13	10/12	10/12	10/12
28	D12S391	HD	19/22	22/22	18/22	22
29	D21S2055	HD	25/34	25/33	25/33	25/33
30	SE33	HD	15/26.2	15/30.2	15/16.2	15
31	D21S1437	GP	11/15	15/17	11/15	15
32	D22S683	GP	12/20.2	12/19.2	12/20.2	12
33	D8S1110	GP	26/28	27/28	23/26	0
34	D12S1090	GP	11/13	9/13	11/12	0
35	D17S1294	GP	12/16	14/16	12/13	0
36	D16S608	GP	7/9	7/10	7/13	7
37	D20S470	GP	15/17	**10/16**	17/17	0
38	D18S536	GP	11/12	12/12	12/12	12/12
39	D13S765	GP	7/8	9/8	8/8	8
40	DXS8378	GX	11/11	**9/10**	10/11	10
41	DXS9898	GX	12/12	12/12	12/12	12/12
42	DXS8377	GX	49/53	49/54	49/53	49
43	HPRTB	GX	12/14	12/14	12/14	12/14
44	GATA172D05	GX	6/8	8/11	8/11	8/11
45	DXS7423	GX	14/15	15/16	14/15	15
46	DXS6809	GX	29/31	31/32	31/32	31/32
47	DXS7132DX	GX	13/15	13/15	13/15	13/15
48	S101	GX	26/26	22/26	22/26	22/26
49	DXS6789	GX	17/19	19/20	19/20	19/20
50	DXS9902	GX	10/12	10/10	10/11	10
51	DXS6807	GX	14/15	11/14	11/14	11/14
52	DXS7424	GX	14/15	14/17	14/17	14/17

[a] Abbreviations: ID, AmpFlSTR® IdentifilerTM ; HD, Investigator ®HDplex; GP, GenePhile G-Plex, and GX,GenePhile X-Plex.

[b] A discrepancy at D6S474 locus (No. 23 and 24) between the HD and GP kit. This locus could be wrongly designated by either the HD or the GP manufacturer.

Table 2: Results of maternal testing by mitochondrial DNA sequencing. Only polymorphic sites in the fragment lengths of HV1 and HV2 are shown. Note that the alleged mother, biological mother, and the baby have the same mitochondrial DNA sequence.

Nucleotide position	Variable region	Anderson sequence	Baby	Alleged mother	Biological mother
160223	HV1	C	T	T	T
160234		C	T	T	T
160316		A	G	G	G
160632		T	C	C	C
73	HV2	A	G	G	G
150		C	T	T	T
153		A	G	G	G
185		G	A	A	A
189		A	G	G	G
263		A	G	G	G
309.1		--	C	C	C
315.1		--	C	C	C

"--": no information in Anderson sequence [22].

In the medical sense, a first-degree relative is defined as a family member sharing about half of genes in common with his (her) blood relative [10, 11] which includes the individual's parents, full siblings, or children. Therefore, the true source may be from a relative of the suspect, if the STR profiles are close with a significant number of alleles in common. In this case, two pieces of information, as high as 88.5% of genetic concordance in the AM-child and identical mitochondrial DNA sequence between the two, lead to an inference that the biological mother and the AM should be the first-order female siblings. With the two pieces of clues, we asked the detective to confirm if the AM has a full sister. As expected, the real mother was caught, with a great resemblance in appearance to the AM, and she finally confessed. Afterward, complete DNA typing for the real mother was done for confirmation. Table 1 also reveals that the two sisters have 68.3% of their STR profiles in common (71 out of 104 alleles tested in total).

DNA typing has been applied to many situations, namely, mass disaster identification (victims' identity confirmation) [2], criminal investigations (whether the suspect is innocent/if evidence related to a specific suspect) [12], parentage testing (who is the father/mother) [3], immigration testing (whether two people are related) [13], missing persons investigation (whose remain compared to biological relatives) [14], armed forces identification (the identity of the killed soldiers) [15], purloined animal identification (whether the wildlife conservation species) [16], exonerate the innocent (preventing false charges and wrongfully convicted) [17], and others (e.g., plant species identification) [18]. Of all the many application scenarios, one feature in common is that DNA typing usually plays a reactive investigation role in solving cases. DNA analysts were passively asked to answer the information the investigator would like to know, and probably without knowing that the laboratory can actually provide more. This could be partially caused by an excess of workload. In Taiwan, for example, during the past five years, the number of forensic DNA tests performed by police across the nation per year was over 150,000, according to an in-house data of the Criminal Investigation Bureau. Additionally, police personnel qualified to do this specialized work are severely in short supply, and the available scientists are too busy to have time paying more attention for interpreting the DNA results in most of the cases. Apart from a high cost of DNA testing, the two challenges, the lack of experts

and the abundance of cases, could also be encountered in other countries. Notwithstanding these difficulties, it is vital to establish a communication channel between the DNA technicians and detectives for interpreting a typing result. In this case, but for an enthusiastic analyst explaining the meaning of the profiling result to investigators, the case could remain unsolved still.

In fact, DNA typing has other instances of proactive role in criminal investigations. For example, sexual offenders in certain populations can be traced using Y-haplotype screening of patrilineages followed by autosomal STR typing [19]. Another one would be that DNA database can be used to extend the investigative lead potential of DNA typing in familial searching for cold cases [20]. Moreover, perhaps in the future, a suspect's age can be determined from a spit of saliva sample [21], dramatically reducing the potential pool of suspects in a crime. Nevertheless, these knowledge and concepts should actively be specified in detail to the investigators so as to effectively help solve more cases and uncover other crimes committed by the same offenders.

Conclusions

In the past 30 years, forensic DNA technologies have had a breathtaking progress, and in the future, advanced scope of their applications in criminal investigations will continuously be expanded. The perception gap between DNA specialists and investigators on the professional knowledge could be widened. The establishment of institutionalized communication channels between the two would be important to shorten the gap.

Significance of this case is that DNA typing could also exhibit a proactive function in solving criminal and/or difficult cases, and that the crime technician should actively inform the detective of the relevant information interpreted from typing results. DNA typing is not only used for identification purposes. It can go beyond the traditional response uses in criminal investigations.

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