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CASE REPORT

CRIMINALISTICS

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Y-haplotype Screening of Local Patrilineages Followed by Autosomal STR Typing Can Detect Likely Perpetrators in Some Populations

ABSTRACT: The male-specific, human Y-chromosomal short tandem repeats (Y-STRs) are very useful in forensic analysis and human evolution studies. The authors report two sexual crime cases in which the perpetrators were successfully traced using Y-haplotype screening of local patrilineages followed by autosomal STR typing. First, several main local patrilineages from local cases were investigated using Y-STR haplotyping, aimed to find the pedigrees whose haplotypes were identical or similar to those of the crime scene samples. Then, several key suspects were defined from the screened pedigrees, and autosomal STR typing was performed to identify the perpetrator of the crime. The application of Y-haplotype screening of local patrilineages followed by autosomal STR typing in these two cases demonstrates its usefulness for solving sexually related crimes in certain populations.

KEYWORDS: forensic science, short tandem repeats, Y-chromosome, patrilineage, haplotype, sexual crime

The male-specific, human Y-chromosomal short tandem repeats (Y-STRs) are becoming a useful tool for tracing human evolution through male lineages (1) as well as application in a variety of forensic situations (2) including those involving evidence from sexual assault cases containing a mixture of male and female DNA (3,4). In sexual assault cases, the detection of Y-STR haplotypes can produce additional information for crime investigations (5), for example, their pedigree-specificity may allow determination of the origin of a male lineage. In this article, we report two sexual crime cases in which the perpetrators were successfully traced using Y-haplotype screening of local patrilineages followed by autosomal STR typing.

Materials and Methods

Genomic DNA was extracted from whole blood of suspected males, victims, seminal stains, and the cotton swabs that were used to wipe the breast of a victim using the Chelex-100 method (6). Y-chromosomal and autosomal STR typing were performed using the AmpFlSTR® Yfiler™ PCR Amplification Kit and AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's manual. After PCR amplification, 1 μL of the amplified products was mixed with 10 μL of Hi-Di™ formamide and 0.3 μL of GeneScan-500 LIZ size standard (Applied Biosystems) and then samples were denatured at 95°C for 5 min. The electrophoresis was run on an ABI 3130 Genetic Analyzer using 3130-POP-4 polymer (Applied Biosystems). The raw data were collected using 3130 Genetic Analyzer Data Collection Software version 3.0 and then analyzed with GeneMapper® ID Software v3.2 (all Applied Biosystems).

Cases

Case 1

In April 2004, an 85-year-old woman was found dead at home, which is located in a remote mountain village in Dengzhou, Henan province, China. Forensic autopsy revealed that the woman died of brain injury caused by trauma from a hoe and that she was raped after death. At the scene, a spot mark was found on the bed sheet and identified as seminal stain. ABO genotyping was performed for the seminal stain, and its genotype was *AO*. More than 200 suspects were investigated one after another, and all of them were excluded according to their ABO genotypes. Because this case took place in a relatively remote region, and because few of the locals had contact with or married outsiders, the investigation team decided to trace the patrilineal origin of the seminal stain collected at the death scene to narrow the scope of investigation.

Two male individuals including the oldest male from each pedigree were first selected to determine the representative patrilineal Y-STR haplotype. After comparing the typing results of 17 Y-STR

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loci of eight major pedigrees in the area where the case occurred, it was found that the haplotype from the seminal stain was identical to that in males having "Lee" as surname (Table 1). To save the cost, therefore, the investigation team selected several key suspects who were young and middle-aged males (without consideration of teenager and old-aged males in this stage) from the Lee patrilineage for autosomal STR typing. Among these male suspects, a man named A. Lee was found to have identical genotypes with the seminal stain in seven STR loci (D21S11, D7S820, TH01, D13S317, vWA, D18S51, and FGA). He also shared one allele in all other loci, except for CSF1PO (Table 2). The avuncular index of A. Lee to the suspect's type was estimated by using the I-T-O method (7) to be 9.20×10^2. This result strongly suggested that the rapist and A. Lee were closely related males. Hence, 10 immediate male relatives of A. Lee, including his father, uncles, brothers, sons, and nephews, had to be further investigated. The results showed that the DNA profile of B. Lee, A. Lee's nephew—a 19-year-old high school student—exactly matched all 16 autosomal STRs identified in the rape case (Table 2). The frequency of the DNA profile identified was estimated to be 9.53×10^{-21}. Later, B. Lee confessed his criminal offense.

Case 2

On March 14 of 2006, a 32-year-old woman was killed in her house, which is located in a remote mountain village in Dengzhou, Henan province. Scene examination found that there were multiple drop-like, wipe-like, drag-like bloodstains and bloody hand prints on the ground, and a bloody wooden stick beside the body. The clothing of the decedent was in disarray, and her face was cyanotic. Obvious pinch-marks and bite-marks were observed on her neck and breast, respectively. Postmortem examination revealed that death was because of asphyxia by throttling. Spermatozoa were not found from vaginal swabs examination. Analysis of the cotton swabs used to wipe the victim's breast revealed mixed genotypes, including that of the victim and that of an unknown male (Table 2).

Because the area where this case took place was relatively remote, very few outsiders visited the village. Also, the victim was not married and living alone. Thus, it was highly possible that the victim had been assaulted by a local person. Hence, the investigation team traced the patrilineal origin of the male component in the cotton swabs through Y-STR haplotyping. In total, similar to case 1,

TABLE 1—*The results of Y-chromosomal STR typing for cases 1 and 2.*

	Case 1				Case 2			
	Seminal Stain	Surname Lee	A. Lee	B. Lee	Cotton Swabs	Surname Peng	A Branch of Surname Fang	A. Fang
DYS456	15	15	15	15	13	13	13	13
DYS389I	11	11	11	11	12	12	12	12
DYS390	23	23	23	23	24	24	24	24
DYS389II	27	27	27	27	31	31	31	31
DYS458	17	17	17	17	16	16	16	16
DYS19	14	14	14	14	15	15	15	15
DYS385a/b	9,10	9,10	9,10	9,10	13,22	13,22	13,22	13,22
DYS393	11	11	11	11	12	12	12	12
DYS391	11	11	11	11	11	11	11	11
DYS439	15	15	15	15	11	11	11	11
DYS635	22	22	22	22	21	21	21	21
DYS392	14	14	14	14	13	13	13	13
Y-GATA-H4	12	12	12	12	13	12*	12*	13
DYS437	14	14	14	14	14	14	14	14
DYS438	10	10	10	10	12	12	12	12
DYS448	19	19	19	19	20	20	20	20

*Indicates alleles were different with that of the cotton swabs used to wipe the victim's breast.

TABLE 2—*The results of autochromosomal STR typing for cases 1 and 2.*

	Case 1			Case 2		
	Seminal Stain	A. Lee	B. Lee	Victim	Cotton Swabs	A. Fang
D8S1179	10,15	10,13	10,15	10,15	10,14,15	10,14
D21S11	30,32.2	32,32.2	30,32.2	31	29,31	29
D7S820	12	12	12	11,13	11,12,13	11,12
CSF1PO	9,12	11	9,12	10	10,12,13	12,13
D3S1358	15,17	15,18	15,17	15,16	15,16,17	15,17
TH01	9	9	9	9	9	9
D13S317	8,9	8,9	8,9	11,12	8,11,12	8,12
D16S539	11,12	9,11	11,12	8,9	8,9,12	12
D2S1338	23,27	17,23	23,27	21,23	17,21,23	17,23
D19S433	13,15	13	13,15	14.2,15.2	13,14.2,15.2	13
vWA	17	17	17	17	14,17	14
TPOX	11	9,11	11	9,11	9,11	9,11
D18S51	14,18	14,18	14,18	13,20	12,13,14,20	12,14
D5S818	13	11,13	13	13,14	11,13,14	11
FGA	22,24	22,24	22,24	23	22,23	22
Amelogenin	XY	XY	XY	X	XY	XY

27 main family clans within seven villages located around the crime scene were investigated. A particular family clan, the "Peng," drew our attention. The Y chromosome haplotype in their family pedigree was almost identical with the haplotype (in all 17 loci analyzed) obtained from the cotton swabs, except for one allele difference at the Y-GATA-H4 locus (Table 1). Further investigation revealed that the males in the Peng pedigree were the offsprings of a man who had been adopted by the Peng family from the Fang family. Therefore, males with the surname "Fang" were further investigated as key suspects. The haplotypes of a branch of the Fang pedigree were identical with those of the Peng family, and the haplotypes from another branch were identical with those found on the breast swabs. With this clue, the suspect A. Fang, a 43-year-old man, was identified by autosomal STR typing (Table 2). Presented with the evidence, he confessed his criminal offense.

Discussion

In China, it is common practice for children to take their father's surname. It is also a biological truth that a son inherits the Y chromosome of his father, so it follows that males sharing the same surname eventually share the same haplotype in the nonrecombining segment of the Y chromosome. In forensics, the haplotype of Y chromosome could be used as a primary screen in certain cases, such as sexual assault, in which a male leaves his Y chromosomes at the crime scene.

In these two sexual assault cases reported in this article, offenders were successfully traced using Y-haplotype screening of local patrilineages followed by autosomal STR typing. First, several large main genealogies around the case locale were investigated using Y-STR haplotyping, aimed to find pedigrees whose haplotypes were identical or similar to those of the biological samples collected at the scene. Then, several key suspects who were mainly young and middle-aged males were defined from the screened pedigree, and autosomal STR typing was performed to identify the perpetrator of the crime.

It is worth noting that these two cases had occurred in remote mountain villages where few local people married outsiders and that the population rarely wandered outside the villages. So, it was highly possible that the crimes were committed by the locals. This is the key to track down a criminal successfully using a Y-haplotype-based local patrilineages screening method. This method would probably not have been as effective in a large city where the genealogical investigation would have been more difficult, and the pool of potential perpetrators is open and unknown.

Mixtures of bodily fluids from different individuals in sexual assault cases are a frequent finding in forensic work. In these cases, it is important to isolate sperm DNA from vaginal swabs mixed with semen using a two-step differential extraction procedure (8). This procedure, however, is inapplicable for other body–fluid mixtures (e.g., saliva–skin, skin–sweat) from victims and suspects of different sexes (3,4). Y-STR haplotype analysis is considered to be very useful for such cases, and it allows the assailant's haplotype to be determined simply. In case 2, the cotton swabs that were used to wipe the breast of the victim contained the saliva–skin mixture. Mixed genotypes formed by the victim and an unknown male were detected using autosomal STR typing. This makes it difficult to carry out the criminal investigation. However, the assailant's haplotype can be simply determined by using Y-STR analysis, and this information can be directly used to find the pedigree the criminal belongs to.

The cases described here show that it is extremely useful to trace offenders using Y-haplotype screening of local patrilineages followed by autosomal STR typing during forensic investigation of sexually related crimes, especially to those occurring in remote areas where close family marriage is present and where the population is not very mobile.

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