CASE REPORT

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DNA Testing of Klinefelter's Syndrome in a Criminal Case Using XY Chromosomal STR Multiplex-PCR*

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ABSTRACT: We report genetic typing of Klinefelter's syndrome applied to casework in forensic DNA testing. In this case, by using extracted DNA from body samples (muscle and bones), we could identify two distinct X alleles in two out of three X-STR loci (HPRTB and ARA), in addition to Y alleles (DYS390, DYS393). The extra X was found to have originated from father, and the victim turned out to have 47XXY Klinefelter's syndrome. The victim was a 30-year-old male, born from relatively elderly parents as a second child. His father was a severe alcoholic and had been malnourished for more than 20 years at the moment of his birth. He exhibited slight mental retardation as a child, and belonged to a criminal group as an adult. The method presented here was useful to accurately diagnose sex chromosomal abnormality instead of conventional chromosomal analysis and Xg blood group typing. A subtype of this syndrome, 48 XXXY or mosaic, for example, could be identified if the intensity of the overlapped X bands were calculated.

KEYWORDS: forensic science, DNA typing, Klinefelter's syndrome, multiplex polymerase chain reaction, short tandem repeat, X and Y chromosomes

In the field of forensic identification, DNA testing using STR (short tandem repeats) polymorphism is performed worldwide. In particular, STR markers in Y chromosome are useful in rape investigations because they can be detected selectively from the male DNA, ignoring the female victim's DNA, in mixed stains (1). In addition, it is also convenient for the paternity testing of male offspring, because since Y chromosome is inherited from father to son directly we are able to omit the examination of maternal DNA types. In a previous study, we have already demonstrated the superiority of Y-STR polymorphism in the practical DNA testing required officially by a court (2).

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In this report, we describe our findings and the methods used to diagnose sex chromosomal abnormality in criminal DNA identification.

Case Report

In 1999, a murder was reported in Osaka, Japan. A teen-age gang killed the victim by multiple brute force. The victim was dismembered using an electric saw and the body parts were disposed of along a river in a mountainous forest. Wild animals consumed most of the evidence but a police investigation discovered four fragments of the skull and the lower parts of the tibia and fibula. In addition, the well preserved amputated left hand of the victim (Fig. 1) was found in the house where the murder took place, and a small piece of dried flesh, measuring 0.6 cm by 0.4 cm, was picked up in the room where the body was dismembered. A simian line in the hand, sometimes accompanied by chromosomal disorder, was evident (3,4).

These pieces of evidence were identified as parts of a human body by morphological examination. However, in order to establish the identity of the victim, and confirm that they all originated from the same person, DNA testing was performed.

Methods

DNA Typing

DNA was extracted from all the samples using standard procedures described elsewhere (5). Extracted DNA was quantified by measuring its optical density at 260 nm: a value of 1.0 corresponds to approximately 50 μg/mL DNA (Beckman-DU®70 Spectrophotometer, Fullerton, CA). One to 10 ng of DNA was used in all PCR reactions.

Sex Identification

First, we identified the sex of the biological samples by X-Y Amelogenin PCR, and found them all to be male because both X and Y fragments were detected (6). However, we found that the intensity of the X-band was stronger than the Y-band. Further analysis of images with NIH Image 1.55 software showed the X band was about 2-fold the intensity of its companion Y band (Fig. 2). Similar results were obtained when using fluorescently labeled amelogenin primers and analysis with Genescan software (7).

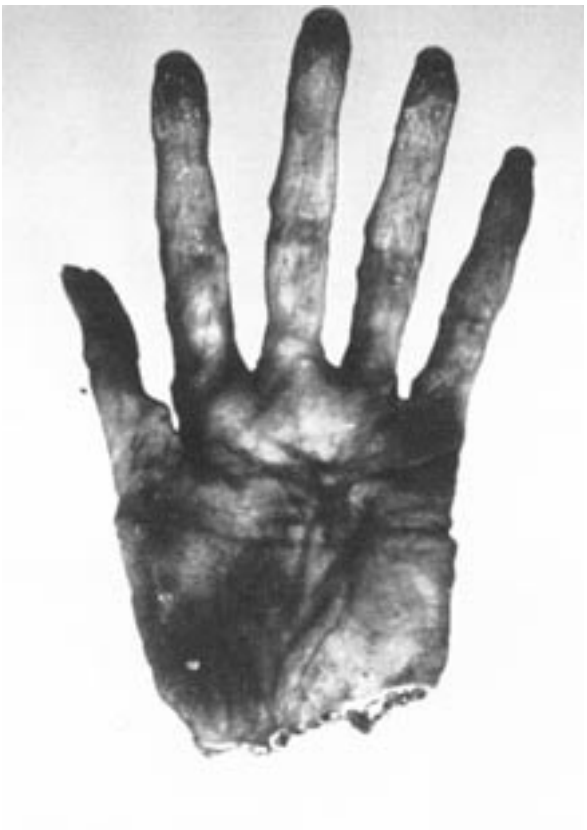

FIG. 1—*Amputated hand. It was preserved by wrapping tightly with several rolls of bandages after dipping into honey.*

XY STR Typing

Next, we tested X and Y-STR systems and found complex results. We applied X and Y-STR markers simultaneously by multiplex-PCR using fluorescently labeled primers. Electrophoresis and analysis of DNA fragments were performed using an ABI PRISM™ 377 DNA sequencer (PE, Applied Biosystems, Foster City, CA) and Genescan® Analysis 2.1.1 and Genotyper® 2.5 softwares (PE Biosystems). The detail procedures have already been described in our previous report (8). As a result, we identified that the alleles of these samples were all identical, thus the samples were all derived from the same person. However, astonishingly, we found that the DNA types of all samples had two distinct Xs (HPRTB, ARA), in addition to Y fragments (DYS 390, 393). Hence, we suspected that the victim had a sex chromosomal aberration, 47 XXY Klinefelter's syndrome.

Results and Discussion

It has been reported that Klinefelter's syndrome is not a rare abnormality, and its frequency in the population is estimated to be one per 1000 male offspring, (0.1%) (9). Perhaps due to social maladjustment, the occurrence of Klinefelter's in people involved in criminal groups has been noted to be high (10). Therefore, the chance of finding Klinefelter's in this DNA test was not so rare.

The genesis of this syndrome is not yet known, but one of the risk factors of this abnormality is delivery from an elderly mother (11,12). In the presented case, the victim was born to a 38-year-old mother, as a second child. His elder sister of two years older is normal, and has a healthy child. The victim himself was infertile with a tendency towards unusual sexual behavior.

After further investigation it was found that the father who had been a long-distance truck driver and had an alcohol dependency was highly likely to have been the source of the medical impairments, rather than the mother. After marriage the couple remained childless for nine years. The victim was raised at home until he was 13 years old, however, at puberty, mental retardation and maladjustment at school became marked, and he was sent to a school for the handicapped. After 18 years of age, he worked at nightclubs and gradually become involved in criminal groups. Finally, he was targeted and killed by a gang before being dismembered and dumped in a remote mountain area.

To identify the victim, and to confirm this abnormality, blood from the presumed father and mother was collected and X-Y STR typing was performed. Comparing the bands of the parents and son, we found that one X was inherited from mother normally, however, the additional X originated from the father's chromosome. Y chromosome (DYS390 and DYS393) had been inherited from the father normally (Fig. 3). We also performed other somatic STR typing and found complete matching between the samples (Table 1). Therefore, we concluded that the victim had a chromosomal aberration of Klinefelter's syndrome and was the biological son of parents who were tested. Moreover, further investigation of Y-STR haplotypes of Klinefelter persons, including the present one, showed that most of them are extremely rare in the normal population (13).

We demonstrated that sex chromosomal aberration could be diagnosed and the origin of the inheritance was as easily determined by multiplex PCR of X and Y STR markers, as by recognizing heterozygous alleles from parents. In this case, his additional X chromosome proved to originate from the father's sperm, which suggests that incomplete meiosis of the sperm was the cause of the abnormality. Severe alcoholism and poor health, and administration of penicillin were strongly suspected as the causes of the abnormal meiosis of sperm, and might also explain the nine years infertility in his father.

TABLE 1—*DNA typing of dismembered hand and possible parents.*

	X-STR			Y-STR		Somatic STR		
	HPRTB	STRX1	ARA	DYS390	DYS393	THO1	FES/FPS	TPOX
Father	16	13	28	22	13	7,9	11,13	8,11
Hand*	13,16	13,13	23,28	22	13	6,7	11,12	8,8
Mother	13,13	13,15	23,24	…	…	6,7	12,12	8,11

* Other samples (muscle and bone fragments) have the same DNA types as the hand.
Please note that somatic DNA types give no clue on sex chromosomal abnormality.

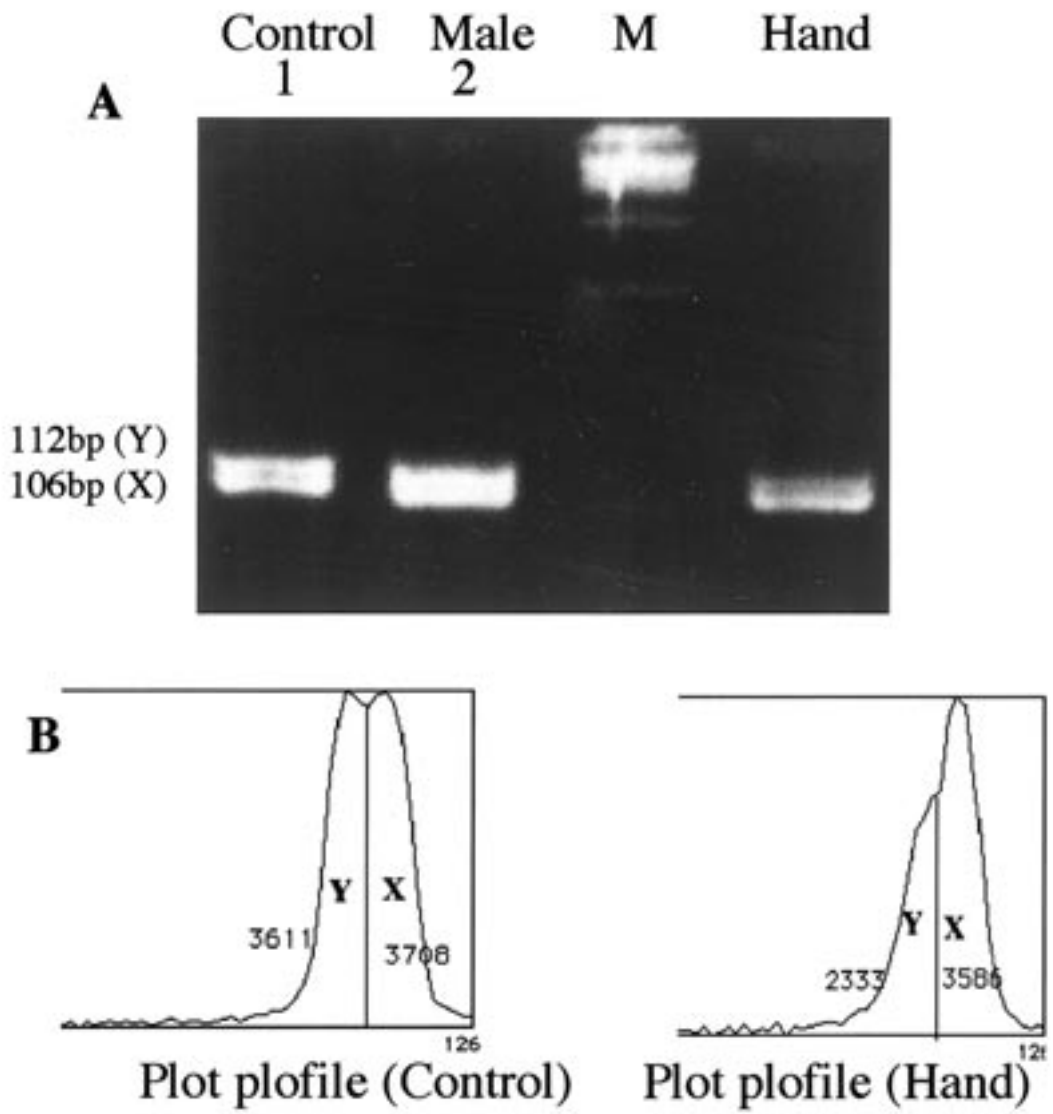

FIG. 2—(A) *X-Y Amelogenin PCR products run in 4% metaphor gel. The intensity of the X band in the hand sample is stronger than the Y band.* (B) *Gel image analysis of the bands shows that the intensity of the X band in the hand is nearly 2 times stronger than the Y band. M = 100 bp marker.*

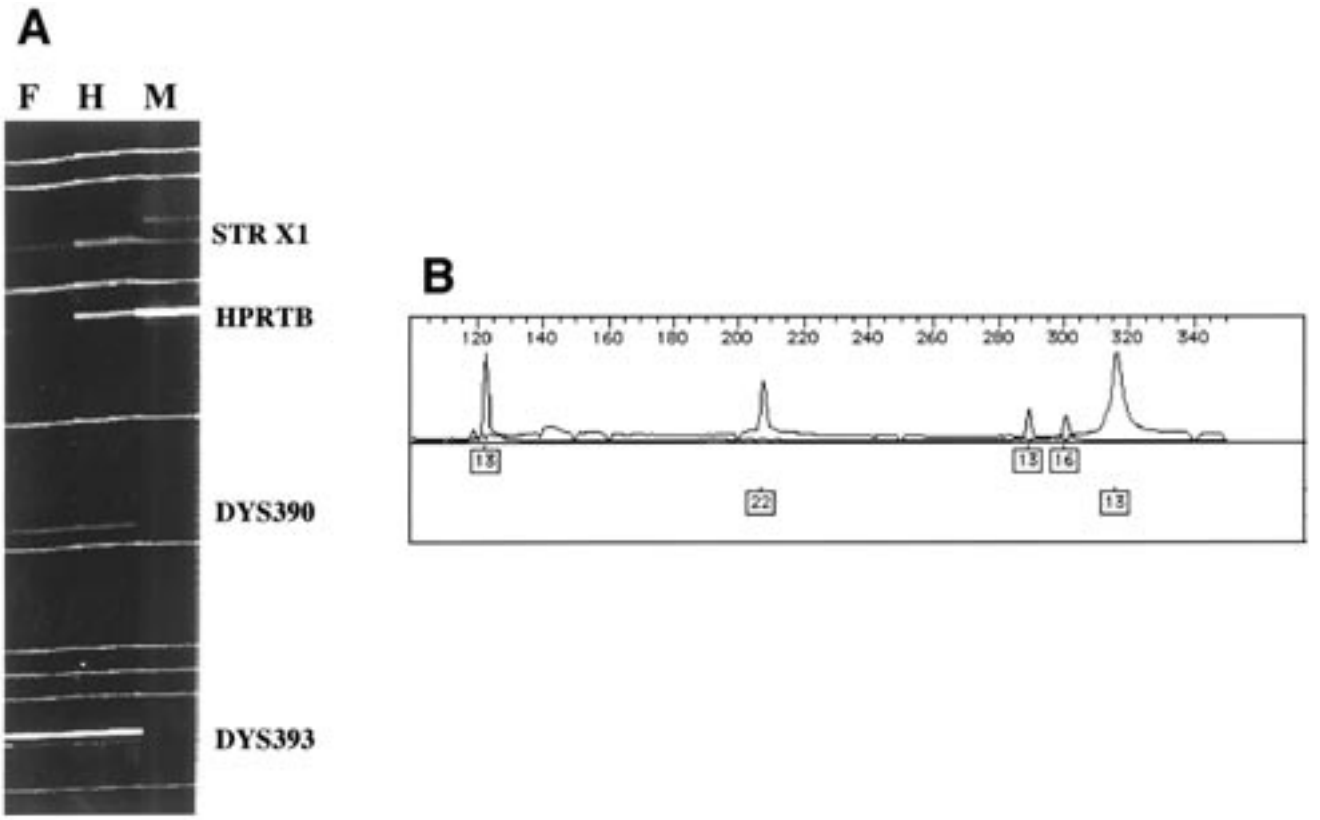

FIG. 3—(A) *Gel image of two X-STR (STRX1, HPRTB) and two Y-STR (DYS390, DYS393) multiplex-PCR products of samples from the father, hand, and mother.* (B) *Electropherogram of hand sample. It showed 2 X alleles at HPRTB locus. For typing details, please refer to Table 1. F = father, H = hand, M = mother.*

The method, presented here, was useful to diagnose sex chromosomal abnormality accurately, and we could omit conventional chromosomal analysis and Xg blood group typing (14). A subtype of this syndrome, 48 XXXY or mosaic, for example, could be identified if the intensity of the overlapped X bands were calculated. In addition, any kinds of biological samples can be used in this method. We conclude that multiplex XY STR detection is as effective in clinical diagnosis as in forensic identification.

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