CASE REPORT

Forensic casework of personal identification using a mixture of body fluids from more than one person by Y-STRs analysis

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Abstract : We applied Y-STRs (DYS385/DYS19/YCAII) to an adhesive plaster left at a crime scene. This plaster may have included body fluids from more than one person. Firstly, we performed preliminary examinations, ABO-blood type examinations, and commonly used DNA examinations (D1S80, HLADQα, TH01, and PM) on these specimens. As a result of these examinations, we could evidence that suspect A did not contacted with the plaster, but could not confirm the presence of perspiration from suspect B. As the next step, we applied Y-STR examination to the plaster. Using this examination, we detected alleles that coincided to those of suspect B. We also concluded that the fluid from an unidentified person was vaginal fluid based on crime scene investigation. Y-STRs examination data obtained from 124 persons in Tokushima prefecture showed that 1.613% of individuals demonstrated haplotypes 10-18/ 15/19-23, which was detected from the plaster and from suspect B. Therefore, we considered that there was a high probability that the persiration detected in the plaster was that of suspect B. Based on these studies, we concluded that Y-STR examination of trace evidence was very useful to screen suspects using materials that contained body fluid from more than one person. J. Med. Invest. 51 : 238-242, August, 2004

Keywords **:** *forensic casework, mixed body fluid, Y-STRs*

INTRODUCTION

Y-chromosome short tandem repeat (STR) polymorphisms are male-specific and inherited from father to son, and are potential tools for personal identification in sexual assaults and paternity tests between father and sons (1-4). We had studied the distribution of haplotypes on three Y-chromosome STRs including DYS 385, DYS19 and YCAII in a Japanese population from Tokushima prefecture.

In this paper, we report the identification of a suspect by DNA analysis from doubtful material containing mixed body fluids from more than one person. In this case, the suspect was not identified by conventional autosomal DNA typing, such as D1S80, HLADQA1, TH01, and PM system. Y-STR examination established the personal identification of the suspect.

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CASE REPORT

Two suspects, A and B, conspired to break into the victim's house. They suddenly covered the mouth of the victim while she slept, blindfolded her and threatened her to further suppress her resistance. Suspect A tried to forcibly rape the victim. These men were suspected of two other rape incidents, in addition to this one, and there were few items left at the crime scenes. In this case, an adhesive plaster was considered to

have been left behind by suspect B.

MATERIALS AND METHODS

1) Samples

The evidentiary materials were an adhesive plaster though to have been left behind by the suspect. Blood samples were collected from suspects A and B, and from one of the victim.

2) Preliminary examination for body fluids and ABO-Blood type examination

We tested the plaster for the presence of various body fluids using the following methods. Leucomalachite Green method was used as a screening test for blood-stain (5). For the detection of urea, *p*-dimethyl amino cinnam aldehyde (DAC) reagent and urease-bromo thymol blue (BTB) methods were applied (6, 7). ABO Blood type antigens were detected by the absorption-inhibition method (8) and modified absorption-elution method (9) for body fluids, and by the slide method for each blood group (10).

3) DNA-extract method and conventional autosomal DNA typing

DNA was extracted from each material by the modified phenol-chloroform method (11). D1S80 typing was carried out as described in a previous report (12) using D1S80 Primer Mix (LifeCodes, USA). HLADQ A typing and PM system were carried out according to the manufacturer's instructions using an AmpliType PM+DQA1 PCR Amplification and Typing kit (Perkin-Elmer, USA). TH01 typing was carried out according to the manufacturer's instructions using a GenePrint STR System-TH 01(Promega, USA).

4) Y-STRs examination

The primers (DYS385, DYS19 and YCA II) used for PCR amplification were designed as shown in Table 1 (http://ystr.charite.de./). The PCR reaction samples contained : 2 ng DNA template, 1.6 x PCR buffer II (Applied Biosystems), dNTP's200μM, BSA 1.6 μg/ml, AmpliTaq Gold (Applied Biosystems) 2U, $MgCl_2$ 1.5 mM. The total reaction volume was 20 μl. The PCR cycle conditions were as follows:10min at 94°C(soak) 30cycles:1min at 94°C(denaturation), 2 min at 54°C (primer annealing), 2 min at 68°C(primer extension) 60 min at 68°C(extension). PCR products were separated by electrophoresis on4%acrylamide denaturing gel. Band patterns were visualized by silver staining (Fig.1).

Table 1. Primer sequences for PCR amplification of loci in the Y-STR

		Sequence (f : forward, r : reverse)
DYS 385	f :	5'-GTGACAGAGCTAGACACCATGC-3'
	r :	5'-CCAATTACATAGTCCTCCTTTC-3'
DYS 19	f :	5'-CTACTGAGTTTCTGTTATAGT-3'
	r :	5'-ATGGCATGTAGTGAGGACA-3'
YCA II	f :	5'-TATATTAAATAGAAGTAGTGA-3'
	r :	5'-TATCGATGTAATGTTATATTA-3'

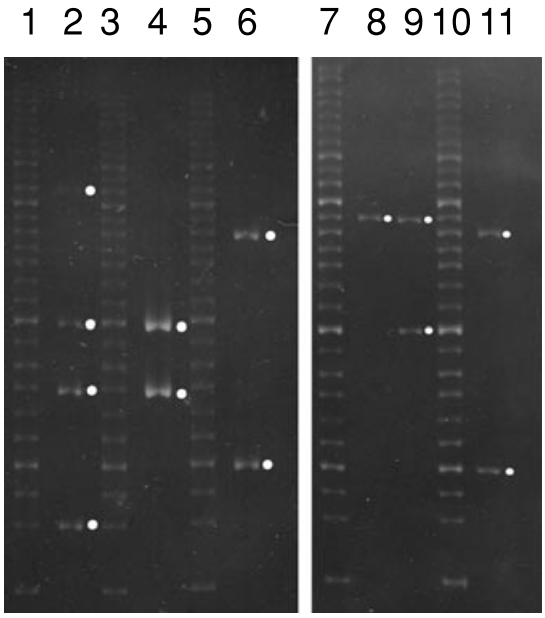

Fig 1. D1S80 typing results obtained from adhesive plaster, the suspect A, suspect B and the Victim
Lane 2 is the adhesive plaster. Lane 8 is suspect A. Lane 4 is suspect B. Lane9 is victim. Lane6and lane11are positive controls. Lane1, lane 3, lane 5, lane 7 and lane 10 are allelic ladder. White dot : main band.

RESULTS AND DISCUSSION

Result of preliminary examination for body fluids and ABO-Blood type examination, and conventional autosomal DNA typing Y-STRs are shown in Table 2.

1) Preliminary examination for body fluids

The plaster showed a weakly positive-reaction by DAC reagent and urease-BTB method. Generally it is known that sweat contains a slight amount of urea (13). Therefore, it was considered that the sweat, containing urea, soaked into the bandage.

Moreover, the Leucomalachite Green method did not show any reaction, indicating that blood had not adhered to the plaster.

Table 2. Summary for the results of preliminary examination, ABO blood and DNA typing

Materials		Adhesive plaster	Suspect A	Suspect B	The Victim
Blood	Leuco*	-			
Sweat and/or urine	DAC	+/-			
	Urease-BTB	+/-			
Blood type	ABO	B	A	B	O
DNA type					
D1S80		16, 21, 24, 32	30-30	21-24	24-30
HLADQA1		1.3, 3, 4.2/4.3, (1.2)	3-4.1	1.3-3	1.3-3
TH01		6, 8, 9	7-9	6-9	9-9
PM	LDLR	BB	BB	BB	BB
	GYPA	AB	AB	BB	BB
	HBGG	BB	BB	BB	BB
	D7S8	AB	AB	BB	AA
	GC	A,B,C	AC	BC	BC
Y-STRs	DYS 385	10-18	12-15	10-18	-
	DYS 19	15	13	15	-
	YCA II	19-23	19-19	19-23	-

- : negative-reaction or no detection. +/- : weak positive-reaction. * : Leucomalachite Green

2) ABO-Blood type examinations

Blood type B was detected from the plaster. The blood type of suspect A was type A, while that of suspect B was type B. The victim's ABO-blood type was type O.

Thus, it was considered that sweat from suspect B had soaked into the bandage. However, the possibility that other person's fluids (B or O type) existed together was left.

3) Conventional autosomal DNA type examination

Electrophoresis showed four bands of D1S80 allele from the plaster (Fig.1). And those bands included the alleles of suspect B, but did not contain the alleles of suspect A nor the victim.

Allele 16 and allele 32 were observed besides alleles of suspect B. Therefore, it was considered that someone's body fluid excluding suspect B and victim soaked into the bandage.

TH01 from the plaster showed 3 bands. And those three bands contained alleles from suspect B and a victim, but there were no alleles from suspect A.

Regarding the HLADQA1type, many alleles from two or more persons were observed from the plaster.

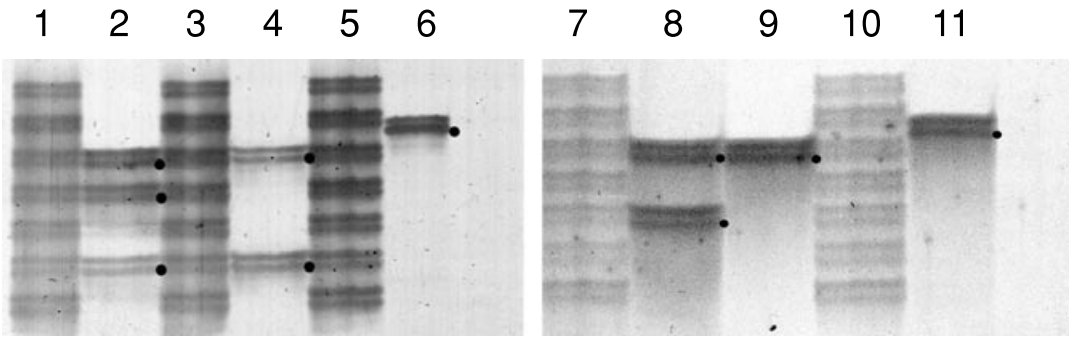

Fig 2. TH01 typing results obtained from the adhesive plaster, suspect A, suspect B and the Victim
Lane 2 is the adhesive plaster. Lane 8 is suspect A. Lane 4 is suspect B. Lane 9 is the victim. Lane 6 and lane 11 are positive controls. Lane 1, lane 3, lane 5, lane 7 and lane 10 are allelic ladders. Black dot : main band.

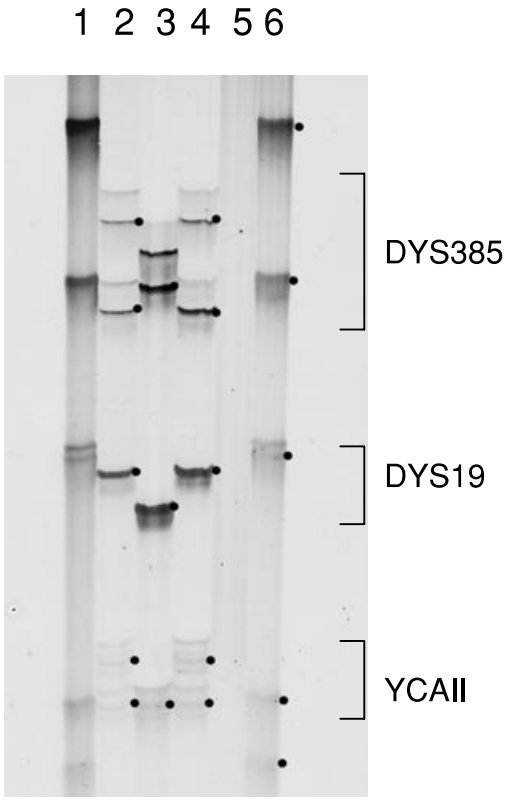

Fig 3. Y-STR (DYS385/DYS19/YCAII) typing results obtained from adhesive plaster, suspect A, suspect B and the Victim Lane 2 is the adhesive plaster [10-18/15/19-23]. Lane 3 is suspect A [12-15/13/19-19]. Lane 4 is suspect B [10-18/15/19-23]. Lane 5 is the victim. Lane 1 and lane 6 are molecular weight standards. Black dot : main band.

The bands contained the alleles of suspect B and a victim, but not those of suspect A. In GC of the PM system, the alleles from the plaster showed many spots from two or more persons. In other types of PM, there was no inconsistency in the alleles from suspects A and B or victim.

One overall judgment, it was considered that plaster contained the sweat from suspect B, and body fluid from someone other than the victim tested. However, the existence of the suspect's sweat had not been definitively proven as also shown by the research results of Fujita *et al*. (14) Furthermore, we found that similar amounts of DNAs were intermixed based on the reports of Budowle *et al*.(15), de Pancorbo *et al*. (16) and Susan C. *et al* . (17).

4) Y-STRs examination

In Fig.3, it can be seen that the alleles from the plaster were identical to those of suspect B, but different from those of suspect A. There were no male alleles other than those of suspect B included among alleles obtained from the bandage. In Y-STR, if homogeneous DNA from two or more persons were present in fairly equivalent amounts and are not the same types, these alleles must surely be detectable (18,19).

As the result of DNA typing, we found that the sweat of suspect B and fluid other than that of the victim tested had adhered to the plaster. And we concluded that the other fluid was vaginal fluid. Vaginal fluid may sometimes contain sufficient urea for cells in the contained fluid, to sink into the plaster. Proof of the fluid's presence usually is obtained by microscopic test and serology test. However, when the sample involves an extremely small quantity, testing becomes extremely difficult quantitatively. In this case investigating the unknown sample using Y-STR yielded significant forensic findings.

Finally, we found that the material had a mixture of male sweat and vaginal fluid adhering to it, and it was considered that Y-STR examination was useful for the personal identification of mixed body fluids from persons of different gender.

5) Individual identification

We studied the frequency of three Y-STRs in a Japanese population from Tokushima prefecture (data not shown). Aler *et al*. (20), Tracqui *et al*. (21) and Honda *et al*. (22) reported that the potential for personal identification using Y-STRs is very high. We were also able to establish highly personal identification in 124 Japanese, as 80 haplotypes was observed, yielding a HD (Haplotype diversity) value of 0.97. According to our study described above, the frequency was 1.613% (2/124 in examined cases). Suspect B was specified with high likelihood as one of the criminals.

In summary, combination of DNA analysis, autosomal and Y-chromosomal DNA polymorphism, demonstrated that the body fluids that had soaked into the bandage were from suspect B and another unidentified female.

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