



Letter to the Editor

The Ashikaga case of Japan—Y-STR testing used as the exculpatory evidence to free a convicted felon after 17.5 years in prison

Dear Editor,

In 1990, a 4-year-old girl was murdered and abandoned at the bank of a river (known as the Ashikaga case in Japan). The short-sleeved shirt of the victim found at the river side was an important piece of evidence. It was confirmed by microscopic examination that a lot of sperm stuck to the shirt.

Two similar cases occurred in a residential area in 1979 and 1984. This suggested that the Ashikaga case was suspected as a set of multiple murders of young girls. Numerous homicide detectives were involved in the search of the criminal, however; no suspect was identified for a period of more than six months.

Eventually, “Mr. S”, the bus driver for kindergarten students, was identified as a suspect. One and a half years later, the National Research Institute of Police Science (NRIPS) stated that the D1S80 [1,2] type and ABO blood type of the sperm on the shirt were identical to those of Mr. S. As a result, he was arrested and was forced to confess to the crime. According to the NRIPS original report, both the sperm on the shirt and Mr. S had 16-26 (D1S80) and B (ABO blood typing). This was the first case that DNA testing was used for a criminal arrest in Japan.

However, a serious problem concerning the D1S80 typing method was reported just after an appraisal of the original DNA test was submitted. It had been observed in other studies that DNA molecular size standard (such as a 123 bp ladder marker) did not simply migrate according to its molecular size in native polyacrylamide gel, so that the amplicon size cannot be determined precisely only by comparing it to a standard marker [3,4]. After presentation of this problem in the court room, the NRIPS changed the original typing and revised the results from 16-26 to 18-30 during trial. Nevertheless NRIPS still insisted that the DNA type of the sperm is the same as that of Mr. S also for the revised DNA type (18-30).

However, a very terrible thing occurred subsequently. Five years after Mr. S was arrested, a similar murder case of a 4-year-old

girl occurred in the neighbouring city of Ashikaga, and her body has never been discovered. Obviously, Mr. S was not involved in this case because he had already been taken into custody.

Nevertheless, the Supreme Court accepted the NRIPS's DNA test at last and rejected the defense's appeal for retrial. In the end, the original life imprisonment sentence was confirmed on July 17, 2000.

Mr. S. has repeatedly demanded DNA re-testing, but his request was denied by the court of law. In December 2008, the first DNA re-test was granted by the Tokyo High Court. The Tokyo High Court nominated two forensic scientists for the re-test: Dr. S (prosecution recommendation) and Dr. H (defense recommendation). Nineteen years had passed since the crime had occurred, the new DNA analysis was considered difficult and challenging. The shirt was divided between the two experts. Dr. H selected the piece of shirt with greenish discoloration parts indicating the presence of semen in which moss and mould could easily grow.

As reference samples, blood and oral swab were taken from Mr. S who was incarcerated at the Chiba prison. Unfortunately, the differential DNA extraction method [5] was not applicable because the crime scene sample was very old. Instead, total DNA extraction from each sample was performed using conventional methods of organic extraction. This technique is suitable for old and contaminated samples [6]. D1S80 amplification was performed as previously described [7].

In addition, Y-chromosomal STR testing was performed instead of autosomal STR to exclude potential cross-contamination by the DNA of the victim and to improve the efficiency of the amplification [8]. The DNA extracts were typed using Y-STR markers with the addition of VCL₄ in the reaction buffer for PCR enhancer. The sizing of PCR products was performed by capillary electrophoresis with an ABI PRISM 3130xl Genetic Analyser. The typing results using PowerPlex™ Y System (Promega) are shown in Table 1. The Y-STR results with seven exclusion constellations between the evidence and Mr. S are fully conclusive and exclude the suspect as the donor of the stain.

The frequency of the perpetrator's haplotype found in the sperm trace was calculated as follows: no observations in 1503 Japanese reference haplotypes from 21 populations (YHRD release

Table 1
Results of Y-STR typing.

Locus	DYS391	DYS389I	DYS439	DYS389II	DYS438	DYS437
Sperm	10	13	11	28	13	14
Mr. S	10	13	10	29	10	14
Match or no match	Match	Match	No match	No match	No match	Match
Locus	DYS19	DYS392	DYS393	DYS390	DYS385	
Sperm	16	13	13	23	10-18	
Mr. S	16	11	18	24	14-21	
Match or no match	Match	No match	No match	No match	No match	

39 from 17.02.12; www.yhrd.org [9]). Using the counting method ($1/(\text{database size} + 1)$) the frequency is 6.6×10^{-4} . In the full East Asian metapopulation including the Japanese ($n = 14,012$, YHRD release 39) the haplotype is observed 2 times resulting in a frequency of 3.8×10^{-5} [confidence interval limits (CI): 4.6×10^{-6} to 1.4×10^{-4}].

On the other hand, the DYS393 allele 18 from Mr. S was extremely rare; it has only been observed two times in YHRD release 39 ($n = 101,055$ haplotypes; [$f = 1.979 \times 10^{-5}$ (95% CI: 2.397×10^{-6} to 7.149×10^{-5})] in 2 of 766 populations, once in Mongolia, and once in South Korea. Even the difference only for this locus (DYS393) represents strong evidence of exclusion.

The Y-STR haplotype of the sperm stain clearly indicates a single male (or male lineage) origin that did not match with Mr. S's haplotype. Similarly, the other expert (Dr. S) also confirmed that the DNA type of the evidence was different from that of Mr. S. It was therefore concluded that Mr. S was not the true perpetrator. On June 4, 2009, the public prosecutor accepted the results of the new DNA test done by Dr. S, so that Mr. S was released from the prison.

In addition, D1S80 re-typing by Dr. H showed that the type of Mr. S. was 18–29. So, the D1S80 type defined by NRIPS of the sperm (18–30) did not match with Mr. S's (18–29). Therefore, the DNA typing results using D1S80 could have been inconclusive in this case since the beginning.

Furthermore, the victim's umbilical cord and an oral swab from her mother were obtained by Dr. H as reference samples. The data indicated that the D1S80 types of victim and mother were the 18–31 and 30–31, respectively, and it was suspected that the 18 and 30 alleles detected in the original D1S80 type done by NRIPS could have been the result of a cross contamination from the victim's and her mother's DNA.

The public prosecutor did not easily accept the results of the new DNA test including D1S80 typing done by Dr. H. At the Utsunomiya District Court (re-trial court), the prosecutor argued that the new DNA test by Dr. H had detected contaminated DNA that was not from the true criminal. For the objection, Dr. H stated that the major DNA types attached to the sample were highly conserved, and that this indicated that the DNA types came from the true criminal.

The Utsunomiya District Court accepted DNA re-test as a crucial evidence, which revoked the results of the old DNA analysis (D1S80 typing) that was performed by the NRIPS. The retrial request was admitted and Mr. S was finally declared innocent on March 26, 2010. Similar exoneration cases have been published recently by the "Innocence Project" [10].

These results also show that Y-STR typing proved to be a very powerful tool for exonerations. The results of this study show that

Y-STR typing is possible, even from a 19-year-old sperm stain. At the same time, it revealed that the previous D1S80 typing by the NRIPS yielded false typing results, and that an innocent man was imprisoned for 17.5 years. The results of DNA re-testing in the Ashikaga case indicate that multiplex STR typing may be a useful tool in the review of other retrial cases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fsigen.2012.07.004>.

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