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Restriction Fragment Length Polymorphism Analysis of Forensic Science Casework in the People's Republic of China

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ABSTRACT: Restriction fragment length polymorphism (RFLP) analysis for the purpose of individualization is now being used in casework in the People's Republic of China. This report describes the use of the multilocus minisatellite probe 33.15 to solve three cases, including two homicides and a rape. In the third case, fetal tissue was analyzed to prove that the alleged rapist was, in fact, the father. In each case, analysis of deoxyribonucleic acid (DNA) resulted in a positive match. The probability of chance association of the DNA fingerprint was calculated as 5.6×10^{-5}, which is similar to the figures reported in the literature.

KEYWORDS: criminalistics, serology, deoxyribonucleic acid (DNA), restriction fragment length polymorphism (RFLP), minisatellite DNA probes

The development of a procedure which can be used to individualize biological evidence positively has revolutionized forensic serology [*1*]. The method takes advantage of the many deoxyribonucleic acid (DNA) polymorphisms that are present in the human genome [*2*]. The various procedures being used today have been found to be accurate and reliable when appropriate controls are present and when performed correctly using accepted protocols. Although differences exist in the use of particular restriction enzymes and probes, the restriction fragment length polymorphism (RFLP) analyses that are in common use can be divided between those that use single-locus probes and those that use multilocus probes. The use of hypervariable minisatellite DNA in forensic science analyses has been investigated by Gill et al. [*3,4*]. The restriction fragment length polymorphisms, which may or may not be associated with known genes, appear to be inherited in a Mendelian fashion [*2*]. It has been shown that useful RFLP patterns can be obtained from bloodstains up to four years old [*3*].

To the classical genetic marker systems used in the People's Republic of China, and also used in forensic science laboratories around the world, the Beijing Forensic Science Institute has now added the capability of performing RFLP analysis. This report is the first to describe casework from the People's Republic of China.

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Materials and Methods

Peripheral blood samples (5 mL) were collected in tubes containing ethylenediaminetetraacetate (EDTA) from individuals and stored at 4°C until tested. The samples were washed three times with 10 volumes of sterile water. Leucocytes were pelleted by centrifugation at 5000 rpm for 10 min and resuspended in TNE buffer (consisting of Tris, sodium chloride, and EDTA), pH 7.5. Proteinase K (Beijing Chemical Reagents Co.) and sodium dodecyl sulfate (SDS) were added to give final concentrations of 100 μg/mL and 0.5% v/v, respectively. following incubation at 55°C for 5 h, the mixtures were extracted twice with phenol and a third time with chloroform/isoamyl alcohol (24:1 v/v), as described by Gill et al. [*3*,*4*]. DNA was precipitated with 2.5 volumes of ice cold ethanol and then vacuum dried. Purified DNA was redissolved in TE (10m*M* Tris/1.0m*M* EDTA) buffer, pH 7.5.

Dried bloodstains were soaked overnight in TNE. The extract was removed and added directly to a mixture of proteinase K and SDS and incubated at 55°C for 5 h. The further treatment was the same as that just described for fresh blood samples.

Buffers

The following buffers were used:

(*a*) Denhardt's solution (×50), consisting of Ficoll (1%), polyvinylpyrrolidone (1%), and bovine serum albumin (1%) in distilled water;

(*b*) standard saline citrate (SSC) (×20), consisting of sodium chloride (NaCl) (3*M*) and sodium citrate (0.3*M*) in distilled water, pH 7.0;

(*c*) TNE buffer, consisting of tris (hydroxymethyl) aminomethane (Tris) (10m*M*), NaCl (0.5%), and EDTA (2m*M*), pH 7.5; and

(*d*) hybridization buffer, consisting of ×5 Denhardt's solution, ×5 SSC, SDS (0.5%), EDTA (25m*M*), and phosphate buffer (10m*M*), pH 7.0. The volume of Denhardt's solution equaled 1/10 of the volume of the hybridization buffer.

Multilocus Probe

The Jeffrey's 33.15 multilocus probe was labeled by the specific primer extension procedure [*2*].

DNA Restriction

DNA samples (3 μg, as estimated by the yield gel) were exhaustively digested with *Hinf* I (10 units, New England Biolabs Inc., Beverly, Massachusetts) at 37°C for 6 h. The digested samples were mixed with 5 μL of gel-loading buffer (consisting of 30% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanol) and loaded onto a 0.8% agarose gel.

Electrophoretic Separation

The gel consisted of 0.8% w/v agarose in 40m*M* Tris-acetate, 20m*M* sodium acetate, and 1.0m*M* EDTA buffer, pH 8.0. Prior to polymerization, 0.5 μg/mL ethidium bromide was added to the mixture. The cast gel measured 10.5 by 19.0 by 0.8 cm. The gel was subjected to electrophoresis at 25 V for 24 h or until all DNA fragments smaller than 2.0 kilobases (kb) had migrated off the gel.

Southern Blotting

The gel was placed in DNA denaturing solution [consisting of 0.5*N* sodium hydroxide (NaOH) and 1.5*M* NaCl] and then neutralized in 1.0*M* Tris/1.5*M* NaCl buffer, pH 7.5. The DNA fragments were then transferred to a nitrocellulose membrane (Sino-American Biotechnology Co., Beijing, China) by Southern blotting for approximately 20 h. Southern blotting was performed according to the method of Jeffreys et al. [*1*]. The DNA was fixed to the membrane by heating it at 80°C for 6 h. The membrane was then incubated at 42°C for 6 h in hybridization buffer. The filter was then hybridized to a phosphorus-32 (^{32}P)-labeled single-stranded minisatellite probe at 42°C for 16 h. Following hybridization, the filter was washed for 10 min with ×0.5 SSC/0.5% SDS maintained at 42°C. Washing was performed two additional times with fresh buffer. Autoradiography was carried out for 3 days at −70°C with an intensifying screen.

Results

Case 1

A man was killed as a result of head trauma (axe wounds) in Beijing in February 1989. A suspect, who was a security guard in an elementary school, was apprehended soon after. ABO blood grouping and esterase D (EsD) polymorphic enzyme analysis was performed on the bloodstains removed from the suspect's clothing and on the blood of the deceased. The victim was ABO type A, and EsD type 2-1. The suspect was ABO type A and EsD type 1-1. The bloodstains on the suspect's clothing were typed as ABO type A and EsD type 2-1.

As a result of the inability to determine conclusively that the stains were derived from the victim, a decision was made to proceed with RFLP analysis. Figure 1 illustrates that,

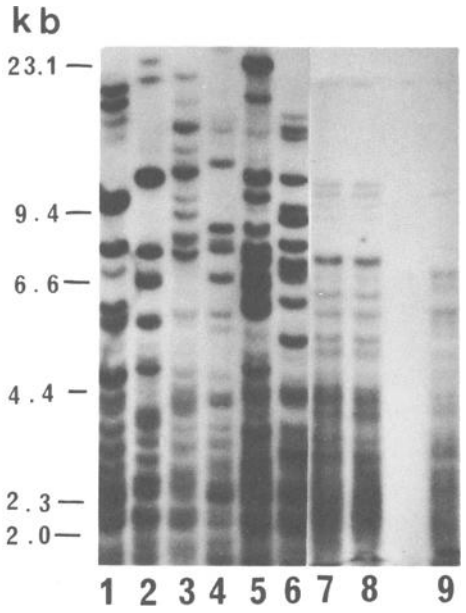

FIG. 1—*Combined photograph of two autoradiographs illustrating the RFLP patterns of DNA taken from six unrelated individuals (Lanes 1 through 6), DNA extracted from the deceased (Lane 7), DNA from bloodstains on suspect's clothing (Lane 8), and DNA extracted from the suspect's blood (Lane 9).*

for each sample, more than 10 resolvable bands can be observed on the autoradiograph. Lanes 1 through 6 represent DNA isolated from unrelated individuals; Lane 7 represents DNA from the victim; Lane 8 represents DNA isolated from the bloodstain on the suspect's clothing; and Lane 9 represents DNA isolated from the suspect's blood. A clear match can be observed in the banding patterns of Lanes 7 and 8. No other DNA samples match.

Case 2

A 31-year-old individual, suspecting that his wife had had sexual relations with another man, killed both his wife and his only child, a 4½-year old, by axing them to death. He was apprehended and jailed. RFLP analysis using the multilocus 33.15 probe revealed that the child was unrelated to him. Figure 2 shows the DNA patterns from five unrelated individuals in Lanes 1 through 4 and 8. DNA from the suspect is shown in Lane 5; DNA from the child is shown in Lane 6; and DNA isolated from the mother is shown in Lane 7. Although the child and mother share many alleles, the father's pattern matches no other pattern on the gel.

Case 3

A 14-year-old female patient attending the Training School for Children with Hereditary Mental Disease complained to her mother about a strange feeling in her abdomen. After the mother discovered that her daughter had become pregnant, she reported the incident to the police. An investigation revealed that the young girl had been sexually abused and raped by a teacher in the school and that the young woman was now 5 months

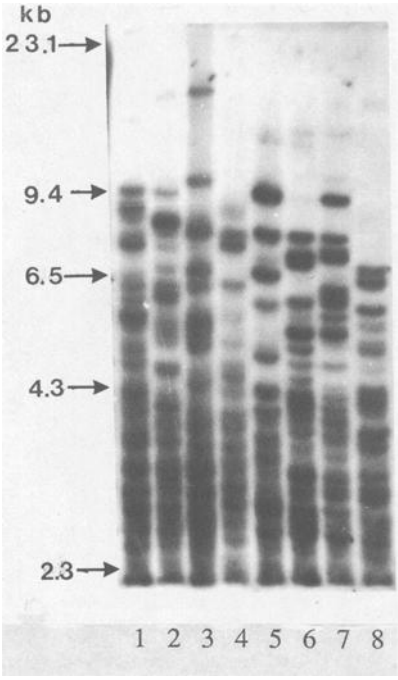

FIG. 2—*Photograph of an autoradiograph illustrating the RFLP patterns of DNA taken from five unrelated individuals (Lanes 1 through 4 and 8), the suspect and legal father (Lane 5), the slain child (Lane 6), and the mother (Lane 7).*

pregnant. The woman underwent an abortion, and paternity testing was performed. The mother was found to be ABO type O, EsD type 2-1. The abortus was blood typed as ABO type B, EsD type 2-1. The teacher was typed as ABO type AB and EsD type 2-2.

RFLP analysis revealed that the teacher had fathered the child (See Fig. 3). Lanes 1 through 4 show DNA RFLP patterns from unrelated individuals. Lane 5 shows DNA isolated from the teacher's blood; Lane 6 shows DNA from the abortus; and Lane 7 shows DNA obtained from the mother.

A database was established, following analyses of 60 individuals residing in Beijing. As a result, the authors have calculated that the probability of chance association of two DNA fingerprint patterns, each obtained from unrelated individuals, is 5.6×10^{-12} (Table 1).

Discussion

In Beijing, the Forensic Science Institute handles the analysis of physical evidence for the police department. As in other forensic science laboratories around the world, the institute analyzes biological evidence by identification, species determination, and individualization. Red cell antigens, such as those used in the ABO and MNS systems, and polymorphic proteins and enzymes are determined using standard serological and electrophoretic methods [5]. When using these methods, exclusion is simple and sometimes very rapid, for example, as in exclusion by ABO blood grouping. Although it is sometimes possible to include a suspect with a statistically high probability (<1 per 1000), this is not always possible. Such had been the situation for the three cases described in this report. In the People's Republic of China, among those of Han nationality (90% of the population) the gene frequency of EsD^1 is 0.6487, and the gene frequency for EsD^2 is 0.3513. The frequencies of the ABO blood grouping system are as follows: Type A = 28%, B = 29%, O = 35%, and AB = 8%. Approximately 94% of Type A individuals

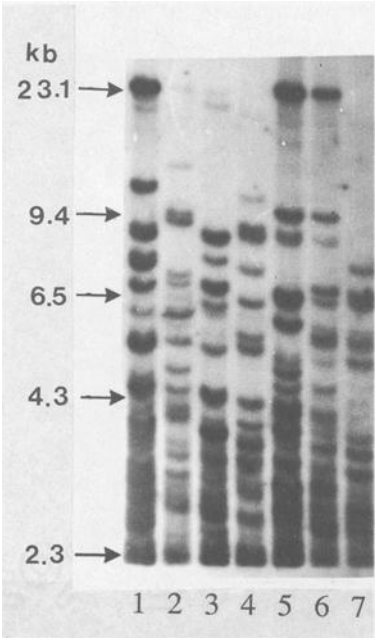

FIG. 3—*Photograph of an autoradiograph illustrating the RFLP patterns of DNA obtained from four unrelated individuals (Lanes 1 through 4), the alleged father (Lane 5), the aborted fetus (Lane 6), and the mother (Lane 7).*

TABLE 1—*Similarities of DNA fingerprints between random pairs of individuals using probe 33.15.*

DNA Fragment Size, kb	No. of Fragments per Individual ± Standard Deviation	Probability that the Fragment in A is Present in B[a]	Maximum Mean Allelic Frequency/Homozygosity
9.4 to 23.1	4.5 ± 0.80	0.18	0.09
6.5 to 9.4	3.2 ± 0.98	0.22	0.11
4.3 to 6.5	3.0 ± 0.89	0.18	0.09
2.0 to 4.3	6.8 ± 0.75	0.30	0.15

[a]A and B represent DNA fingerprints from two unrelated individuals.

are Type A_1. Analysis by these systems had not been sufficiently informative to resolve these cases.

Because of the extraordinary individualizing power of RFLP analysis [*1,6*], the laboratory of the Beijing Forensic Science Institute has begun to perform this procedure in addition to the classical systems described above. It is widely felt that the chances of an accidental genetic match are exceedingly small. As of November 1989, a database consisting of 60 individuals residing in Beijing has been established. Using the method of Jeffreys [*1*], we have calculated that the probability of chance association of a DNA fingerprint pattern equals $0.18^{4.5} \times 0.22^{3.2} \times 0.18^{3.0} \times 0.30^{6.8} = 5.6 \times 10^{-12}$. The Beijing Forensic Science Institute laboratory will soon be investigating the use of single-locus probes for RFLP analysis. Single-locus probes can sometimes allow the investigator to detect alleles present in mixed samples, and even more important, incomplete digestion with restriction enzymes can easily be determined. The laboratory will also soon be developing the polymerase chain reaction technology for amplification and subsequent analysis of the HLA-DQ-alpha region of DNA by the dot blot hybridization technique.

Acknowledgments

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