CASE REPORT

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The Application of Minisatellite Variant Repeat Mapping by PCR (MVR-PCR) in a Paternity Case Showing False Exclusion Due to STR Mutation

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ABSTRACT: A boy and a girl with their mother brought a paternity suit against an alleged but deceased father. We tested six conventional genetic markers, the AmpliType PM+DQA1 and twelve STR loci the children and mother together with the alleged paternal grandparents. We also DNA typed the bloodstain found later in the alleged father's medical record. Only the result at D3S1358 in a nineplex STR system excluded the alleged father from parentage of the boy, whereas all markers were inclusive for the girl. Accordingly, we performed sequence analysis at D3S1358 to confirm the presence of a paternal exclusion or mutation. The sequence analysis indicated that the boy's allele 17 could have originated from either of the alleged father's allele 16 or 18 by a single-step mutation associated with slippage mutation in STR loci. We carried out minisatellite variant repeat mapping by PCR (MVR-PCR) at loci D1S8 (MS32) and D7S21 (MS31A) and mapped allele haplotypes of all individuals except the deceased alleged father. The MVR-PCR analysis showed that the boy has no inconsistency with the relationship between the alleged grandparents, and was very effective at increasing the paternity index (PI) value. We conclude that there is biological relationship between not only the girl but also the boy and the alleged father.

KEYWORDS: forensic science, DNA typing, paternity test, short tandem repeat, MVR-PCR, multiplex PCR

Short tandem repeats (STRs) have been widely used for paternity testing (1–3). Since a single STR locus has limited discriminating power, more than ten loci are usually used to obtain a high probability of paternity. However, since mutation rates of individual STRs in the germline may be of the order of 0.7% as the highest (4), mutation cannot be considered negligible in a system using as many as ten STR loci. It may be expected that false exclusion would happen at the rate of 2% in trio cases if ten STR loci with average mutation rates (0.12%) reported previously (5) are used. An apparent false exclusion at two STR loci in a child has already been reported, but mutation rather than non-paternity was only concluded from statistical estimates (6). Minisatellites (or VNTRs) have been used to confirm such apparent STR mutation cases in paternity tests (7). However, the mutation rates at minisatellites are generally higher than at STRs (8,9). Thus to date, no appropriate method has been established to discriminate STR mutation and true exclusion in paternity testing.

Minisatellite variant repeat mapping by PCR (MVR-PCR) (10) is a powerful approach for analyzing individual variation, and allele-specific MVR-PCR can be achieved by virtue of single nucleotide polymorphisms of DNA flanking both the D1S8 (MS32) (11) and D7S21 (MS31A) (12) loci. We have analyzed MVR allele structures in Japanese (13,14), and reported the potential applications to forensic samples for personal identification (15) and a practical case for maternal identification (16), as well as the potential contribution of MVR-PCR to paternity probabilities in a motherless case (17).

Recently, we carried out a paternity test using blood samples of a boy, a girl, their mother, and the alleged paternal grandparents; we observed a single exclusion between the boy and the alleged grandparents at one STR locus in a nineplex STR system. At that time, paternal blood was not available since the alleged father had died a few years previously. Fortunately, during the course of our investigation, a bloodstain from the alleged father was found in his medical record. From the bloodstain, we could type 18 DNA markers by multiplex PCR and performed sequence analysis at D3S1358 which shows a mismatch between the boy's paternal allele and the alleged father. In order to investigate this family relationship, we applied MVR-PCR mapping at D1S8 (MS32) and D7S21 (MS31A). As a result, we finally conclude that there is no inconsistency with the relationship between the boy and the alleged father.

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Materials and Methods

Samples—Five mL of peripheral blood was collected in EDTA-Na_2 from the boy, the girl, their mother, and the alleged paternal grandparents. A bloodstain from the alleged father was found later in his medical record (Fig. 1).

Conventional Genetic Markers—The phenotypes of ABO and Rh blood-groups, those of phosphopglucomutase-1 (PGM_1), acid phosphatase (AcP) and esterase-D (EsD), and that of haptoglobin (Hp) were determined by hemoagglutination tests using human monoclonal antibodies (Ortho Diagnostic System, NJ), by electrofocusing, and by vertical electrophoresis, respectively.

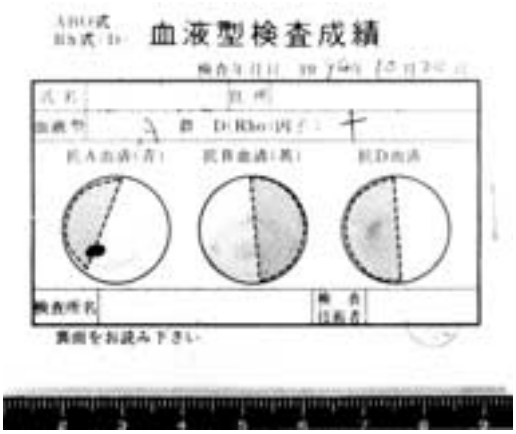

FIG. 1—*Bloodstain on the paper sheet taken for ABO and Rh(D) blood groups typing and found in the alleged father's medical record. Semicircular regions (shaded) were used for DNA extraction.*

DNA Extraction and Quantification of Extracted DNA—DNA was extracted from blood and the bloodstain with the organic extraction method described previously (18). The amount of extracted DNA was determined fluorometrically using a TKO 100 Mini Fluorometer (Hoefer Scientific Instruments, CA) (15).

DNA Amplification and Typing—Six loci (LDLR, GYPA, HBGG, D7S8, GC, and HLA-DQA1) were typed using the AmpliType™ PM + DQA1 kit (Perkin Elmer, NJ). Three STR loci, LPL, F13B, and F13A01 were amplified and typed with the Gene*Print*™ STR Systems (Promega, WI) by silver staining. The PCR amplification and typing at nine STR loci (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, and D7S820) were performed according to the protocol using the AmpFℓSTR Profiler PCR Amplification kit (PE Applied Biosystems, CA) by the Genetic Analyzer 310 (PE Applied Biosystems, CA) and the Genotyper 2.0 (PE Applied Biosystems, CA) software.

Sequence Analysis at D3S1358—Using primers described previously (19), the D3S1358 locus was PCR-amplified and each allele was cloned using the TA-cloning kit (Invitrogen, CA). Plasmid DNA for a dozen clones derived from each of the alleles of the six individuals was isolated with the QIAprep Spin PLASMID KIT (QIAGEN, Germany), and sequenced by the dye primer method

TABLE 1—*Typing results of paternity testing in the present case at six conventional markers and 19 DNA markers.*

Locus	Alleged Grandfather	Alleged Grandmother	Boy	Girl	Mother	Alleged* Father
Conventional genetic markers						
ABO	O	A	A	A	A	A†
Rh	CCDee	CcDEe	CcDEe	ccDEE	ccDEE	…
PGM_1	2+2+	1+1+	2+1+	2+1+	1+1+	…
AcP	BA	B	B	A	BA	…
EsD	2	2–1	2–1	2–1	2–1	…
Hp	2	2	2	2	2	…
DNA markers						
AmpliType PM+DQA1						
DQA1	3, 4.1	1.3, 3	1.1, 4.1	1.2, 1.3	1.1, 1.2	1.3, 4.1
LDLR	AB	AB	BB	BB	AB	BB
GYPA	AB	AB	AA	AA	AB	AA
HBGG	AB	AB	BB	BB	AB	BB
D7S8	AB	AB	AA	AB	AB	AA
GC	BB	BC	BB	BB	BB	BC
Gene Print STR						
LPL	10, 10	10, 12	10, 10	10, 10	10, 10	10, 12
F13B	10, 9	10, 9	10, 9	10, 9	10, 9	9, 9
F13A01	6, 3.2	6, 3.2	3.2, 3.2	6, 3.2	6, 3.2	3.2, 3.2
AmpFℓSTR Profiler						
D3S1358	15, 18	15, 16	15, 17	15, 16	15, 15	16, 18
vWA	16, 17	16, 18	17, 18	17, 18	16, 18	17, 18
FGA	22, 24	18, 22	18, 21	21, 24	21, 23	18, 24
TH01	6, 7	7, 9	7, 10	7, 10	7, 10	7, 7
TPOX	11, 12	7, 9	9, 9	11, 11	9, 11	9, 11
CSF1PO	10, 12	12, 13	12, 13	12, 12	12, 12	12, 13
D5S818	11, 11	10, 11	10, 12	11, 13	12, 13	10, 11
D13S317	11, 11	8, 11	11, 11	11, 11	10, 11	11, 11
D7S820	8, 11	11, 14	10, 11	8, 13	10, 13	8, 11
Amel	X, Y	X	X, Y	X	X	X, Y

… Not tested.
* Results of the alleged father were obtained from a bloodstain in his medical record.
† Result of typing sheet.

with automated DNA sequencer ABI 373XL (PE Applied Biosystems, CA).

Allele Mapping of MVR-PCR at D1S8 (MS32) and D7S21 (MS31A)—Allele specific MVR-PCR was performed as described previously at D1S8 (11) and D7S21 (12,14). Allele-specific MVR-PCR is based on the selective amplification of one allele over the other from total genomic DNA using an allele-specific PCR primer directed to a polymorphic site in DNA flanking the minisatellite (11). In the case of individuals homozygous for each of the flanking polymorphic sites at each locus, MVR-PCR was performed after the alleles were physically separated as described previously (13,20).

Results and Discussion

We typed six conventional genetic markers and 18 DNA markers along with a marker for gender identification summarized in Table 1. The results from the alleged grandparents and the girl did not exclude the deceased alleged father being the true father of the girl. However, with respect to the boy, allele 17 at D3S1358 in the boy was not observed in the alleged grandparents. Thus, allele 17 of the boy was inconsistent with the proposed relationship to the alleged father. We then calculated the paternity index (PI) of each child using typing results from the grandparents and children. According to the AABB Accreditation and Requirement Manual (21), in a case where a VNTR allele is mutated, PI is estimated as μ/ARE, where μ is mutation rate of the locus and *ARE* is average rate of exclusion. Since the mutation rate of D3S1358 in the Japanese population has not been investigated, we used the average mutation rate (1.2×10^{-3}) reported previously (5) as the mutation rate of the locus. *ARE* was calculated from the Japanese allele distribution at D3S1358 described previously (*ARE* = 0.448, Ref 22). The PI of the boy at the locus therefore was estimated as 2.68×10^{-3}, and the accumulated PI of the 24 loci in the boy was estimated as 21.7 while that in the girl is estimated as 604 (Table 2). Compared with the PI from the girl, the PI from the boy was very low although it still showed possible paternity between the boy and the deceased alleged father. These two values gave probabilities of paternity (W) of 95.6% and 99.8%, respectively, assuming a prior probability of 50%.

In order to investigate whether this was a single but genuine exclusion or a consequence of mutation, we asked the court to look for a sample traceable to the alleged father before his death, and a stain on his ABO- and Rh(D)- blood typing sheet was found in his medical record. We typed the DNA from this bloodstain for the 19 markers shown in Table 1, and found no incompatibilities with his parents, the alleged grandparents. Nevertheless, the alleged father remained genetically incompatible with the boy because he had alleles 16 and 18 at locus D3S1358. To shed further light on this, we performed sequence analysis. As shown in Fig. 2, this analysis shows that either of the alleged father's alleles (16 or 18) could be the progenitor of the boy's allele 17, given that mutations in STR loci usually arise by a single-step process (4,5).

Brinkmann et al. investigated mutation rates and structures of mutants at STR loci, and reported that around 90% of mutation involved a single repeat gain or loss (4). Based on AABB formula for estimating PI at RFLP loci (21), Brenner proposed a new formula for calculating PI when an STR allele in a child shows an apparent single repeat mutation: PI = μ/4q, where μ is mutation rate of the locus and *q* is the frequency of the progenitor allele (23). Using this formula, the accumulated PI was estimated 8.35 or 43.4 depending on the frequency of the progenitor allele 16 and allele 18, respectively, and *W* was calculated as 89.3 or 97.8% assuming 50% prior probability. These values are even lower than those estimated by the AABB formula.

We performed allele mapping by MVR-PCR at D1S8 (MS32) and D7S21 (MS31A) in order to clarify whether this case was a

TABLE 2—*The paternity index (PI) of the present case at six conventional markers, 18 DNA markers and 2 MVR loci.*

Locus	PI		Accumulated PI	
	Boy	Girl	Boy	Girl
Conventional genetic markers				
ABO	1.19	1.19	1.19	1.19
Rh	1.15	0.98	1.36	1.16
PGM_1	3.18	3.18	4.34	3.70
AcP	0.95	2.03	4.13	7.52
EsD	1.01	1.01	4.17	7.58
Hp	1.36	1.36	5.65	10.3
DNA markers				
AmpliType PM+DQA1				
DQA1	3.79	1.28	21.4	13.2
LDLR	0.62	0.62	13.2	8.11
GYPA	0.88	0.88	11.6	7.17
HBGG	0.70	0.70	8.19	5.05
D7S8	0.81	1.00	6.62	5.05
GC	1.46	1.46	9.64	7.35
Gene Print STR				
LPL	1.05	1.05	10.2	7.74
F13B	1.08	1.08	10.9	8.34
F13A01	1.52	1.15	16.7	9.58
AmpFℓSTR Profiler				
D3S1358	2.68×10^{-3}*	0.86	0.0447	8.23
vWA	0.95	0.95	0.0426	7.85
FGA	11.36	1.75	0.484	13.7
TH01	2.04	2.04	0.989	28.0
TPOX	3.57	0.71	3.53	19.8
CSF1PO	2.34	1.17	8.25	23.2
D5S818	1.17	2.98	9.64	69.0
D13S317	3.15	3.15	30.4	217
D7S820	0.71	2.78	21.7	604
MVR-PCR				
MS31A	12.40	12.40	269	7480
MS32	26.50	26.50	7130	198000

* This value is estimated by the AABB Accreditation and Requirement Manual (Ref 21, see Results and Discussion).

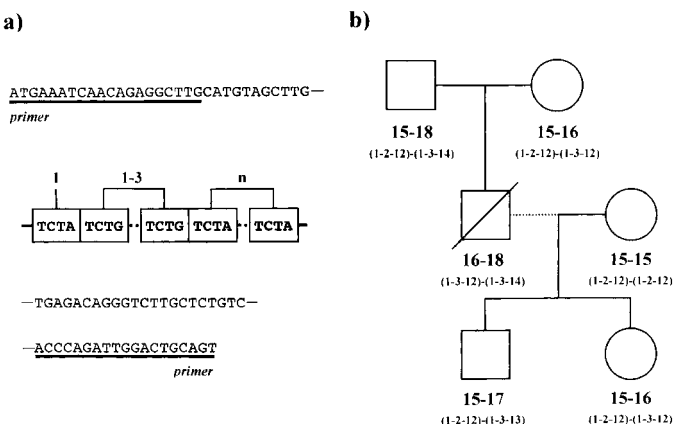

FIG. 2—(a) *Sequence structure at locus D3S1358.* (b) *The pedigree, genotypes, and the allele structures of the locus in the present case. Each allele was expressed as three consecutive numbers corresponding to repeat number in the three repetitive regions;* $(TCTA)(TCTG)_{1-3}(TCTA)_{12-14}$.

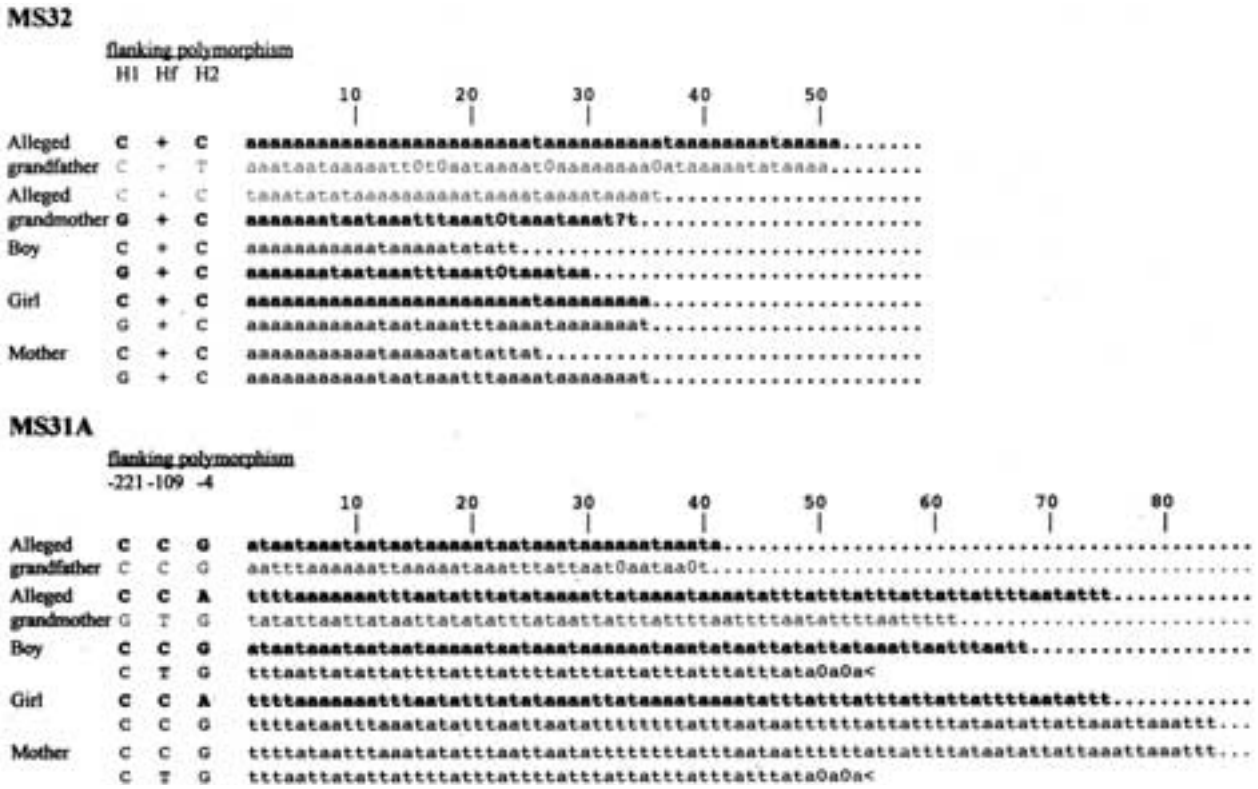

FIG. 3—*The results of each of three flanking polymorphisms and MVR mapping at MS32 and MS31A in the present case. The alleged father could not be analyzed by this means because of the minute amount of DNA extracted from the bloodstain in his medical record. The ends of short alleles are marked (<).*

false paternal exclusion or not. The analysis of MVR-PCR clearly showed for both loci that the boy shared not only a flanking haplotype but also an identical allele MVR code with the alleged grandparents (Fig. 3). The girl and one of the grandparents also shared an identical MVR allele code at both loci. Alleles defined by MVR-PCR at MS32 and MS31A are extremely variable in Japanese as well as other populations, and unrelated persons seldom share MVR allele codes (13,14,17). Using allele code databases at both loci, we have previously estimated allele frequencies in the Japanese population based on the binomial distribution (17). The values of paternity probabilities became far higher when both MVR loci were included than not (Table 2). From the accumulated PI of the boy (PI = 7130), W was 99.986% at 50% prior probability. Consequently, we conclude that allele 17 in the boy at D3S1358 arose by mutation of one of the alleged father's alleles, and that there is full biological relationship between not only the girl but also the boy and the alleged father.

Multiplex STR typing has recently become a common forensic practice (1–3, 23). Although STRs are suitable for personal identification, caution should be taken when applying them to paternity testing since germline mutation (6) is not negligible when the number of STRs is increased for paternity testing. Such single exclusion cases need to be distinguished from real exclusion of paternity. We could increase the number of STRs and/or minisatellite loci for testing to confirm whether the kinship is excluded, although the risk of the mutation also increases. In such cases, MVR-PCR could be useful by virtue of its enormously high discriminating power as shown in the present study. We note, however, that the power of MVR will be reduced by population stratification (i.e., isolated communities), and that the MVR system will also be affected by minisatellite mutation although knowledge of its mutation process (24) may help to distinguish non-paternity from mutation.

The present study indicates that STR analysis is very useful for paternity testing and that MVR-PCR is one of the most powerful tools to determine false exclusions arising through single STR mismatches.

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