CASE REPORT

Keiji Tamaki,[1] M.D.; Xiu-Lin Huang,[1] M.D.; Masaki Mizutani,[1] M.D.; Toshimichi Yamamoto,[1] Ph.D.; Ryu Katsumata;[1] Rieko Uchihi,[1] Ph.D.; Yoshinao Katsumata,[1] M.D.; and Alec J. Jeffreys,[2] D.Phil.

The Potential Contribution of MVR-PCR to Paternity Probabilities in a Case Lacking a Mother

REFERENCE: Tamaki K, Huang X-L, Mizutani M, Yamamoto T, Katsumata R, Uchihi R, Katsumata Y, Jeffreys AJ. The potential contribution of MVR-PCR to paternity probabilities in a case lacking a mother. J Forensic Sci 1999;44(4):863–867.

ABSTRACT: Minisatellite variant repeat (MVR) mapping using the polymerase chain reaction (PCR) was applied to a paternity case lacking a mother to evaluate the paternity probability. After three flanking polymorphic sites at each of MS31A and MS32 loci were investigated from the child and alleged father, allele-specific MVR-PCR was performed using genomic DNA. It was confirmed that one allele in the child was identical to that in the alleged father at both loci. Mapped allele codes were compared with allele structures established from population surveys. No perfect matches were found although some motifs were shared with other Japanese alleles. The paternity index and probability of paternity exclusion at these two MVR loci were then estimated, establishing the power of MVR-PCR even in paternity cases lacking a mother.

KEYWORDS: forensic science, pathology and biology, DNA typing, paternity test, motherless case, minisatellite, MVR-PCR

Since the initial development of DNA fingerprinting (1), DNA typing techniques have been applied to paternity testing. Compared with conventional genetic markers, hypervariable minisatellite loci have enormous numbers of alleles resulting in very high heterozygosity and thus, unprecedented superiority of discrimination. Since most DNA typing systems can type co-dominant alleles, the paternity index (PI) or the probability of exclusion (PE) can easily be calculated by pedigree analysis algorithms (2,3). However, the mother is sometimes unavailable for testing in paternity cases (4,5). Evaluations of PI and PE from genotypes of the child and alleged father in these motherless cases have been specifically addressed (6–8).

Minisatellite variant repeat mapping using PCR (MVR-PCR) (9) was devised to reveal the interspersion pattern of subtle repeat variants along minisatellite tandem arrays. So far, it has been successfully applied to several human minisatellite loci (10–12). MVR-PCR has revealed enormous diversity of allele structures at D1S8 (MS32) and D7S21 (MS31A) loci in various populations including the Japanese (13,14). Preliminary forensic applications for identification by MVR-PCR have also been described (15–18). MVR-PCR is the best approach to exploit the potential of hypervariable minisatellite loci because mapping is unambiguous, precise sizing of alleles is not required, and the digital code data obtained is suitable for computer analysis. It also reveals far more variability than can be resolved by allele length analysis.

As part of a research exercise, MVR-PCR was used to establish the paternity of a case lacking a mother and to evaluate its potential by comparing the paternity probability with those yielded by other DNA systems.

Materials and Methods

Sample Preparations

Venous blood was collected from a one-year-old child and the alleged father at the family's request. DNA was extracted as described previously (19). The DNA concentration was determined fluorometrically by a TKO 100 Dedicated Mini Fluorometer (Hoefer Scientific Instruments, CA).

Genotyping at 12 DNA Loci

HLA-DQA1 and five PM™ loci (LDLR, GYPA, HBGG, D7S8, and GC) were typed using AmpliType PM+DQA1 kit (Perkin Elmer, NJ) according to the manufacturer's protocol. Six STR loci (HUMLIPOL, HUMVWFA31, HUMFXIIIB, HUMTH01, HUMTPOX, and HUMF13A01) were analyzed by the GenePrint STR system (Promega Corporation, WI). The amplified products were separated in 6% denaturing polyacrylamide gels and visualized by silver staining. The allelic ladders supplied with the kit were electrophoresed for determining allele size.

Calculations

For calculating paternity index (PI), the probability of paternity (W), and the probability of exclusion (PE), established allele frequencies reported previously on the loci in the Japanese population were used (19–21). All calculations were performed using Microsoft Excel Version 5.0 software. In order to compare efficiencies between loci, we used the arithmetic mean PE and the geometric mean PI (22). The mean PE was calculated as follows: a value of PE for a particular set of child-mother genotypes in a trio

[1] Associate professor, postgraduate students, associate professor, student, assistant professor and professor, respectively, Department of Legal Medicine, Nagoya University School of Medicine, Nagoya 466-8550, Japan.

[2] Professor, Department of Genetics, University of Leicester, Leicester, LE1 7RH, U.K.

Received 7 Aug. 1998; and in revised form 27 Oct. 1998; accepted 2 Nov. 1998.

(or in a child genotype in a motherless case) was multiplied by its estimated proportion under Hardy-Weinberg equilibrium, and values were simply added for all possible combinations of mother-child-father (or child-father) genotypes. The geometric PI was calculated as $\prod PIi^{fi}$, where *PIi* is the PI value of a particular trio or child-father (motherless case) genotype combination, and fi is its estimated proportion in the population.

Allele-Specific MVR-PCR at D1S8 (MS32) and D7S21 (MS31A) Loci

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 (23). In order to perform allele-specific MVR-PCR, flanking polymorphisms at MS31A and MS32 were investigated as described previously (11,14,23). A total of 100 ng of genomic DNA was used for PCR amplification to genotype the three flanking variants of MS32 (H1, Hf, and H2) (23) and MS31A (−221G/C, −109C/T, and −4A/G) (11,14). Allele-specific MVR-PCR was then performed using 100 ng of genomic DNA, MVR-specific primers and an appropriate allele-specific primer directed to a heterozygous site flanking the minisatellite. The start position of the allele code was determined by reference to a standard genomic DNA sample of known code. Allele codes of each individual at both loci were compared with those in the allele database by using dot matrix analysis (using software written by A. J. J. in Microsoft Quick Basic) to identify related alleles which share extensive regions of internal structural similarity (9,11,13,14,24,25). Details of the allele database may be obtained from Alec Jeffreys upon request. The parameters used for this analysis are as follows: MS32, perfect nine repeat matches and at least 25 matching positions over the best two diagonals searched; MS31A, perfect eight repeat matches and at least 20 matching positions.

Results

The typing results of 12 DNA markers for the child (**C**) and alleged father (**AF**) are summarized in Table 1. The resulting accumulated PI was calculated as 157 and the combined PE was estimated at 97.12%. The probability of paternity [$W = 1/(1 + PI)$] was then estimated at 99.36% assuming a 50% prior probability of paternity.

To map each allele of **C** and **AF** by allele-specific MVR-PCR, three flanking single nucleotide polymorphisms at each of MS31A and MS32 were investigated. At MS31A, **C** was found to carry the flanking haplotypes G-C-G and C-C-G [−221G/C(11), −109C/T(14), and −4A/G (11) site, respectively], while **AF** was typed as G-C-G and G-T-G. If **AF** is the real biological father of **C**, they should thus share the allele with the flanking haplotype G-C-G. Allele-specific MVR-PCR detecting the −221G allele (31HgaI+ as the allele-specific primer) (11) on **C** and the −109C allele (Psp1406I+ primer) (14) on **AF** can therefore map single alleles selectively. For MS32, both **C** and **AF** are heterozygous for H1, while Hf and H2 are homozygous. Therefore, the H1 site was used for allele-specific MVR-PCR (primers H1G and H1C) (23).

Single allele mapping by allele-specific MVR-PCR using primers at MS31A and MS32 was performed from genomic DNA; examples are shown in Fig. 1. Allele-specific primers ignore the alternative allele, and only one allele is amplified when the individual is heterozygous for the flanking polymorphic site. As a result, the pattern of PCR products from **a**-type repeats was complementary to that from **t**-type repeats while a few 'null' repeat positions were also scored (24). The allele codes at both loci could be determined without difficulty although some fluctuations in the intensity of the bands were observed in MS31A.

The number of repeat units coded was determined by reference to a standard sample (Fig. 2). All alleles could be coded for more than 40 positions. At both loci, **C** and **AF** share not only a flanking haplotype but also an identical allele MVR code (C1 and AF1 alleles at both loci in Fig. 2) consistent with paternity. Mapped allele codes obtained from this case at both loci were then compared with others previously established from population diversity surveys. At MS31A, no matches were found with the 149 Japanese alleles. The other MS31A alleles carried by **C** and **AF** (31-C2 and 31-AF2) are both unique and yet share some motifs (31-C2: 39 repeat units, 31-AF2: 27 repeats, see underlined codes) with three and seven Japanese alleles, respectively (data not shown). In contrast, the allele shared by **C** and **AF** has no significant similarity to any other allele in the database. For MS32, an allele code database contain-

TABLE 1—*Typing results of a motherless paternity test at 12 DNA markers and two MVR loci and their paternity probabilities.*

Locus	Child	Alleged Father	PI	acm. PI	PE(%)	acm. PE(%)
AmpliType (PM + DQA1)						
HLA-DQA1	1.1/4.1	4.1/4.2 or 4.3	3.79	3.79	67.9	67.9
PM LDLR	BB	BB	1.23	4.66	3.5	69.0
GYPA	AA	AB	0.88*	4.12	18.9	74.9
HBGG	AB	AB	1.22	5.04	0	74.9
D7S8	AB	AB	1.06	5.34	0	74.9
GC	BB	BB	1.94	10.4	23.5	80.8
STR						
HUMLIPOL	10, 10	10, 10	1.40	14.6	8.3	82.4
HUMVWFA31	18, 17	17, 17	1.76	25.6	25.3	86.8
HUMFXIIIB	10, 10	10, 9	0.69*	17.7	7.6	87.8
HUMTH01	9, 9	9, 9	2.62	46.3	38.2	92.5
HUMTPOX	8, 8	8, 8	2.21	103	30.2	94.8
HUMF13A01	3.2, 3.2	6, 3.2	1.53	157	45.2	97.1
MVR-PCR		(Allele frequency†)				
MS31A		0.02	12.4	1,950	92.1	99.8
MS32		0.009	26.5	51,600	96.3	99.992

* PI values not increasing the accumulated PI values.

† Frequency of the allele shared by the child and the alleged father is the upper 95% limit (see Discussion).

PI, paternity index; acm. PI, accumulated paternity index; PE, probability of exclusion; acm. PE, accumulated probability of exclusion.

ing 1072 allele codes from 15 different ethnic groups (including 318 Japanese alleles) was examined; all codes in the database could be distinguished from those mapped in this case. However, the allele shared by **C** and **AF** has a motif (underlined in 32-C1 and 32-AF1) similar to several Japanese alleles in a large group of related alleles (107 alleles from 9 different ethnic groups including 52 Japanese alleles). The 32-C2 allele also shares some motifs with a group of three other Japanese alleles.

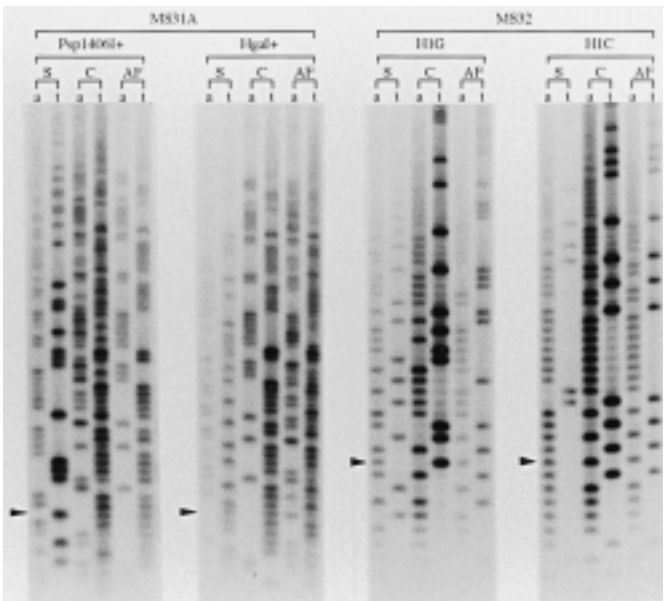

FIG. 1—*Example of allele mapping results of the motherless case at MS31A and MS32 by allele-specific MVR-PCR, using allele-specific primers PSP1406I+ (−109C allele), HgaI+ (−221G allele), H1G, and H1C. The 10th positions of MVR codes are marked (>). Allele mapping of AF by Psp1406I+ and C by HgaI+ in MS31A, and AF and C by H1C in MS32 show indistinguishable alleles shared by AF and C. In MS31A, diploid maps are shown of AF by Psp1406I+ and C by HgaI+ since they are homozygous for these positions. AS: standard allele; C: child; AF: alleged father.*

Discussion

We previously reported the application of MVR-PCR for identity purposes to the remains of an infant and placenta found in an incinerator (18). The present study reports the first application of MVR-PCR to questions of parentage, in particular a paternity case lacking a mother.

Using MS32 and MS31A databases, we estimated the frequency in the Japanese population of each of the MVR codes generated in this paternity case. Since Poisson analysis predicts both loci to have very large numbers of different alleles in Japanese [MS31A: >5000 alleles (14), MS32: >12,000 (unpublished data)], exact frequencies cannot be determined from the relatively small databases [MS31A = 149 alleles (14), MS32 = 318 alleles (25)]. It is therefore not appropriate to use 1/5000 or 1/12,000 as the frequency of the MVR haplotype shared by the child and the alleged father. In a database of N alleles containing no alleles identical to that shared by the child and alleged father, the upper 95% C.I. of the allele frequency (f) is given by $(1 - f)^N = 0.05$, based on the binomial distribution. Since N is large (i.e., $N > 100$), f can simply be calculated as $3/N$. The conservative frequency of the allele shared between **C** and **AF** at each locus is therefore 0.020 (3/149) for MS31A and 0.009 (3/318) for MS32. These estimated allele frequencies are likely to be gross overestimates of the true frequencies.

We then calculated PI and PE using the MVR data. Given the extreme variability in MVR codes generated at MS31A and MS32, any two unrelated individuals will usually both be heterozygous and share no alleles at either locus. Similarly, the vast majority of child-father pairs will share only one allele at each minisatellite locus. This genotype combination of child and father has less discriminatory power compared with any other combinations (7), but with systems of high allelic diversity it is possible to obtain high PI values. The PI in the present case is $1/4p$ (7), where p is the frequency of the allele shared by the child and the alleged father. PE is estimated by determining the frequency of individuals in the population who do not share either of the child's alleles: $PE = (1 - p - q)^2$, where p and q denote the frequencies of the child's alleles, conservatively estimated as above. The results are summarized in Table 1. The com-

MS31A

```
          -221 -109  -4    1        *        *        *        *        50       *        *

31-C1      G    C    G    ataatttttttttattttattttttaaattaaaaaattttttaatttttttaataaaaatatttaaa....
31-C2      C    C    G    ?tttataatttttaattttttataaatataat?tatatatat?tataa?tatat.................

31-AF1     G    C    G    ?taatttttttttattttattttttaaattaaaaaattttttaatttttttaataaaaatattt.......
31-AF2     G    T    G    ?tttaattatattatttatattaatattatttaatttttttatttaaatttataattaatatat......
```

MS32

```
          H1   Hf   H2    1        *        *        *        *        50       *

32-C1      C    +    C    atataaaatatatataaaaaaaataataataaaataaaaaatatataaata.........
32-C2      G    +    C    aaaaaaa0atattaaaaattatataaaataaaataaaaaatataat...............

32-AF1     C    +    C    atataaaatatatataaaaaaaataataataaaataaaaaatatataaata.........
32-AF2     G    +    C    ?ataaatatatataaatataaaattaatttaaaaaatttaatataaa..............
```

FIG. 2—*MVR allele maps at MS31A and MS32 in the present case. 0 = null or unamplifiable repeat. Underlined regions are motifs which are shared by other related Japanese alleles.*

TABLE 2—*Geometric mean paternity index and the mean probability of exclusion at 6 STR loci and two MVR loci in a Japanese trio or motherless case.*

		Geometric mean PI				Mean PE (%)	
Locus	No. Alleles	M+	(%PI < 1*)	M−	(%PI < 1*)	M+	M−
STR							
HUMLIPOL	6	1.58	(18.7)	1.33	(32.1)	23.8	10.3
HUMVWFA31	8	2.92	(0.0)	2.02	(11.0)	59.6	41.8
HUMFXIIIB	5	1.53	(17.8)	1.30	(31.1)	21.7	9.3
HUMTH01	6	2.21	(6.3)	1.66	(17.7)	45.8	28.9
HUMTPOX	6	2.04	(3.9)	1.59	(14.4)	39.8	24.0
HUMF13A01	6	1.75	(22.3)	1.43	(36.7)	31.7	17.9
MVR-PCR							
MS31A†	>5,000	24.5	(0.0)	12.8	(0.0)	95.9	92.1
MS32†	>12,000	53.0	(0.0)	27.0	(0.0)	98.1	96.3

* %PI < 1: proportion of the PI values being less than 1.0 in all possible combinations of trios or motherless cases.

† Numbers of alleles were estimated from population diversity data. PI and PE were estimated from upper 95% C.I. limit of allele frequencies of 0.02 (MS31A) and 0.009 (MS32), assuming that the paternal allele in the child is not matched by any allele in the population database (see Discussion).

M+, a paternity trio (mother, child, and alleged father); M−, a motherless case (child and alleged father).

PI, paternity index; PE, probability of exclusion.

bined PI with just two MVR loci is 329, compared with 157 for 12 other loci including 6 STRs. The PI over all loci is 51,600, which gives a probability of paternity (W) of 99.998% if the prior probability is 50%. The total PE in the case is estimated at 99.992%, again with most statistical power provided by the two MVR systems. These values are worthy of judicial consideration. The potential contribution of MVR to PI in a motherless case was compared to that for a paternity trio. In MVR analysis, since most mother-father pairs will not share any alleles, any offspring will be heterozygous sharing one allele with the mother and the other with the father. PI in a trio is therefore estimated at twice that in a motherless case ($^1/_2p$ vs $^1/_4p$). The relative importance of this difference becomes very minor when the allele frequency is very small.

Finally, we compared the power of the MVR loci to that of STR markers by estimating the geometric mean PI and mean PE values in both the trio and motherless case (Table 2). For the STRs, the geometric mean PI is based on all possible combinations of genotypes using the allele frequency distribution for the Japanese population. The values of PI in paternity trios derived from MVR loci are far higher than those from STR loci. Even in motherless cases, MVR loci show very high PI values as a consequence of large numbers of rare alleles. In paternity testing, if the PI value at a locus is less than 1.0, it will decrease the accumulated PI even if the locus does not exclude paternity (see GYPA or HUMFXIIIB in Table 1). To evaluate the extent of this phenomenon, we estimated the proportion of such cases for each locus. Amongst trio cases, all the STR loci except HUMVWFA31 can yield a PI value of <1.0 depending on the precise genotype combination. In motherless cases this is more pronounced and all six STRs can give a PI value of <1.0 (Table 2). In contrast, because of the large numbers of rare alleles, MVR loci always contribute positively to establishing paternity when **C** and **AF** share an allele. We conclude that MVR-PCR is a useful tool for paternity cases especially those lacking a mother. We note however that the power of MVR will be reduced by population stratification, particularly in isolated communities, and if the true father is related to a falsely accused man.

Hypervariable minisatellites such as MS31A and MS32 can show significant germline mutation rates to new length alleles which can generate false exclusions in paternity cases (26–28). The paternal mutation rate at MS31A and MS32 is 1.0 and 0.8% respectively (29). Since these loci are unlinked, with no evidence of correlation of mutations between the loci, paternity cases showing mutant paternal alleles at both loci will be rare (8×10^{-5}). However, 1.8% of cases will show paternal mutation at one or other of the loci. In such cases, allele length measurement does not allow distinction of non-paternity from mutation. In contrast, detailed knowledge of mutation processes coupled with MVR analysis of allele structure (30–32) can help distinguish mutation from non-paternity. For example, considerable information exists on MS32 mutants analyzed in single sperm (30). 93% of mutation events involve the gain or loss of less than 20 repeats, with 74% showing gains. 90% of gain mutants show extreme polarity, with repeat segments being added within the first few repeat units of the progenitor allele. Since MVR-PCR allows information to be recovered from at least 40 repeat units, then a mutant paternal allele will usually show extensive structural identity with the progenitor paternal allele except over the first few repeats, and as a result will tend to be more similar to the paternal allele than to any other allele typed in the population database. Preliminary data on MS31A again suggests polar mutation with small allele length changes in the male germline (30), allowing in principle the development of empirical rules that would assist in distinguishing non-paternity from true paternity with germline mutation.

Acknowledgments

We thank Charles Brenner and Yuri Dubrova for statistical advice, and Celia May for critical reading of the manuscript and for helpful comments. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan. AJJ is supported in part by an International Research Scholars Award from the Howard Hughes Medical Institute and in part by grants from the Wellcome Trust, Medical Research Council and Royal Society.

References

1. Jeffreys AJ, Wilson V, Thein SL. Hypervariable 'minisatellite' regions in human DNA. Nature 1985;314:67–73.
2. Ihm P, Hummel K. Ein Verfahren zur Ermottlung der Vaterschaftswahrscheinlichkeit aus Blutgruppenbefunden unter beliebiger Einbeziehung von Verwandten. Z Immun Forsch 1975;149:405–16.

3. Brenner CH. Symbolic kinship program. Genetics 1997;145:535–42.
4. Asano M, Minakata K, Hattori, H. General formulas of the estimated likelihood ratio Y/X in the diagnosis of paternity of a deceased putative father. Z Rechtsmed 1980;84:125–33.
5. Henke J, Baur MO, Hoffman K, Henke L. Application of highly informative single-locus DNA minisatellite probes to complicated deficient paternity cases. Arztl Lab 1991;37:175–9.
6. Garber RH, Morris JW. General equations for the average power of exclusion for genetic systems of codominant alleles in one-parent and no-parent cases of disputed parentage. In: Walker RH. editor. Inclusion probabilities in parentage testing. Arlington, Virginia: American Association of Blood Banks, 1983;277–80.
7. Brenner CH. A note on paternity computation in cases lacking a mother. Transfusion 1993;33:51–4.
8. Chakraborty R, Jin L, Zhong Y. Paternity evaluation in cases lacking a mother and nondetectable alleles. Int J Leg Med 1994;107:127–31.
9. Jeffreys AJ, MacLeod A, Tamaki K, Neil DL, Monckton DG. Minisatellite repeat coding as a digital approach to DNA typing. Nature 1991;354: 204–9.
10. Armour JAL, Harris PC, Jeffreys AJ. Allelic diversity at minisatellite MS205 (D16S309): evidence for polarized variability. Hum Mol Genet 1993;2:1137–45.
11. Neil DL, Jeffreys AJ. Digital DNA typing at a second hypervariable locus by minisatellite variant repeat mapping. Hum Mol Genet 1993;2:1129–35.
12. Buard J, Vergnaud G. Complex recombination events at the hypermutable minisatellite CEB1 (D2S90). EMBO J 1994;13:3203–10.
13. Tamaki K, Yamamoto T, Uchihi R, Kojima T, Katsumata Y, Jeffreys AJ. DNA typing and analysis of the D1S8 (MS 32) allele in the Japanese population by the minisatellite variant repeat (MVR) mapping by polymerase chain reaction (PCR) assay. Nippon Hoigaku Zasshi 1992;46:474–82.
14. Huang X-L, Tamaki K, Yamamoto T, Suzuki K, Nozawa H, Uchihi R, et al. Analysis of allelic structures at the D7S21 (MS31A) locus in the Japanese, using minisatellite variant repeat mapping by PCR (MVR-PCR). Ann Hum Genet 1996;60:271–9.
15. Yamamoto T, Tamaki K, Kojima T, Uchihi R, Katsumata Y. Potential forensic applications of minisatellite variant repeat (MVR) mapping using the polymerase chain reaction (PCR) at D1S8. J Forensic Sci 1994;39: 743–50.
16. Hopkins B, Williams NJ, Webb MB, Debenham PG, Jeffreys AJ. The use of minisatellite variant repeat-polymerase chain reaction (MVR-PCR) to determine the source of saliva on a used postage stamp. J Forensic Sci 1994;39:526–31.
17. Yamamoto T, Tamaki K, Kojima T, Uchihi R, Katsumata Y, Jeffreys AJ. DNA typing of the D1S8 (MS32) locus by rapid detection minisatellite variant repeat (MVR) mapping using polymerase chain reaction (PCR) assay. Forensic Sci Int 1994;66:69–75.
18. Tamaki K, Huang X-L, Yamamoto T, Uchihi R, Nozawa H, Katsumata Y. Applications of minisatellite variant repeat (MVR) mapping for maternal identification from remains of an infant and placenta. J Forensic Sci 1995;40:695–700.
19. Tamaki K, Yamamoto T, Uchihi R, Katsumata Y, Kondo K, Mizuno S, et al. Frequency of HLA-DQA1 alleles in the Japanese population. Hum Hered 1991;41:209–14.
20. Nagai A, Yamada S, Watanabe Y, Bunai Y, Ohya I. Analysis of the STR loci HUMF13A01, HUMFXIIIB, HUMLIPOL, HUMTH01, HUMTPOX, and HUMVWFA31 in a Japanese population. Int J Leg Med 1996; 109:34–6.
21. Watanabe Y, Yamada S, Nagai A, Takayama T, Hirata K, Bunai Y, et al. Japanese population DNA typing data for the loci LDLR, GYPA, HBGG, D7S8, and GC. J Forensic Sci 1997;42:911–3.
22. Brenner CH, Morris JW. Paternity index calculations in single locus hypervariable DNA probes: validation and other studies. In: Data acquisition and statistical analysis for DNA typing laboratories. Proceedings for The International Symposium on Human Identification; 1989; Madison, Wisconsin: Promega Corporation, 1990;21–53.
23. Monckton DG, Tamaki K, MacLeod A, Neil DL, Jeffreys AJ. Allele-specific MVR-PCR analysis at minisatellite D1S8. Hum Mol Genet 1993; 2:513–9.
24. Tamaki K, Monckton DG, MacLeod A, Neil DL, Allen M, Jeffreys AJ. Minisatellite variant repeat (MVR) mapping: analysis of 'null' repeat units at D1S8 [published erratum appears in Hum Mol Genet 1992;1:558]. Hum Mol Genet 1992;1:401–6.
25. Tamaki K, Huang X-L, Yamamoto T, Uchihi R, Nozawa H, Katsumata Y, et al. Characterization of MS32 alleles in the Japanese population by MVR-PCR analysis. In: Sawaguchi A, Nakamura S, editors. DNA Polymorphism. Tokyo: Toyo Shoten, 1995:137–43.
26. Brinkmann B, Möller A, Wiegand P. Structure of new mutations in 2 STR systems. Int J Leg Med 1995;107:201–3.
27. Chakraborty R, Stivers DN. Paternity exclusion by DNA markers: effects of paternal mutations. J Forensic Sci 1996;41:671–7.
28. Gunn PR, Trueman K, Stapleton P, Klarkowski DB. DNA analysis in disputed parentage: The occurrence of two apparently false exclusions of paternity, both at short tandem repeat (STR) loci, in the one child. Electrophoresis 1997;18:1650–2.
29. Jeffreys AJ, Royle NJ, Wilson V, Wong Z. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 1988;332:278–81.
30. Jeffreys AJ, Tamaki K, MacLeod A, Monckton DG, Neil DL, Armour JAL. Complex gene conversion events in germline mutation at human minisatellites. Nature Genet 1994;6:136–45.
31. May CA, Jeffreys AJ, Armour JAL. Mutation rate heterogeneity and the generation of allele diversity at the human minisatellite MS205 (D16S309). Hum Mol Genet 1996;5:1823–33.
32. Buard J, Bourdet A, Yardley J, Dubrova Y, Jeffreys AJ. Influences of array size and homogeneity on minisatellite mutation. EMBO J 1998;17: 3495–502.

Additional information and reprint requests:
Keiji Tamaki
Department of Legal Medicine
Nagoya University School of Medicine
65 Tsuruma-cho, Showa-ku
Nagoya 466-8550
Japan