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CASE REPORT

Evaluation of the paternity probability on an application of minisatellite variant repeat mapping using polymerase chain reaction (MVR-PCR) to paternity testing

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ABSTRACT Minisatellite variant repeat (MVR) mapping using polymerase chain reaction (PCR) was applied to a practical case of paternity testing to evaluate the paternity probability. In order to obtain single allele mapping by allele-specific MVR-PCR, three flanking polymorphic sites for each of the MS31A and MS32 loci were investigated and all three individuals were typed as heterozygous for at least one flanking polymorphic site at each locus. Allele-specific MVR-PCR was then performed using genomic DNA. It was confirmed that one allele in the child was identical to that from the mother and the other one in the child was identical to that from the alleged father. Mapped allele codes were also compared with those in the database by dot-matrix analysis, and no identical allele was found although some motifs were shared with Japanese alleles. The paternity index and the probability of paternity exclusion in the case at these two MVR loci were calculated using the presumed values of the allele frequencies. These studies seem to illustrate the practical value of MVR mapping of MS31A and MS32 loci in paternity testing.

KEY WORDS: Minisatellite, MVR-PCR, Paternity testing, Paternity index, Paternity probability

Introduction

Before the application of DNA techniques, paternity testing was mainly based on the traditional serological markers, such as the red blood cell antigen systems, the isoenzyme markers, red cell protein markers, serum protein markers and human leukocyte antigens (HLA), to determine whether the alleged father is the true biological father. Since the successful development of DNA fingerprinting in 1985[1], DNA typing techniques have been applied in forensic science. Compared with other genetic markers, DNA markers such as hypervariable minisatellite loci have enormous numbers of alleles resulting in a much higher heterozygosity and unprecedented superiority of discrimination.

Minisatellite variant repeat mapping using PCR (MVR-PCR)[2] was invented to map the interspersion pattern of variant repeat units along the tandem repeat array of hypervariable minisatellite. So far it has been successfully applied to several hypervariable human minisatellite loci[3]~[5]. Using the MVR-PCR method, we reported considerable diversity of Japanese allele structures at D1S8 (MS32)[6] and D7S21 (MS31A)[7] loci. Potential forensic applications were also shown by mapping from bloodstains[8] or a used postage stamp[9], by a rapid detection method[10] and by maternal identification from remains of an infant and placenta[11]. MVR-PCR is the best approach to exploit the potential of hypervariable minisatellite loci because of the unambiguous mapping and digital code data suitable for computer analysis. Furthermore, an allele-specific MVR-PCR method[12] has been developed which can map single

alleles from total genomic DNA using allele-specific PCR primers directed at polymorphic sites in the regions flanking the minisatellite. This method is very convenient since it does not entail the time-consuming separation of the alleles on an agarose gel by electrophoresis.

This paper reports an application of MVR information to establish paternity in a practical case by allele-specific MVR-PCR and to evaluate the paternity by comparing the paternity probability with the probabilities yielded by conventional markers and DNA markers. Although results from MVR-PCR were not used in the report to the court, the detection of allele diversity by MVR-PCR seems very effective.

Materials and Methods

Sample preparation

Venous blood was collected from a paternity trio (mother, child and alleged father) and, DNA was extracted as described previously[13]. The DNA concentration of each sample was determined fluorometrically using a TKO 100 Dedicated Mini Fluorometer (Hoefer Scientific Instruments, CA) and DNA was diluted to a concentration of 100 ng/μl.

Genetic markers

For each individual, six conventional markers were typed: two red blood cell antigens (ABO, Rh), the red blood cell enzymes phosphoglucomutase 1 (PGM1), acid phosphatase (AcP), esterase D (EsD), and haptoglobin (Hp). The DNA markers, HLA-DQα, five PM loci (LDLR, GYPA, HBGG, D7S8 and GC) and six STR loci (HUMLIPOL, HUMVWFA31, HUMFXIIIB, HUMTH01, HUMTPOX, and HUMF13A01) were analysed. For calculating the paternity index (PI) or the probability of paternity (W), and the probability of exclusion (PE), established allele frequencies reported previously in the Japanese population were used[13)-16)].

Allele-specific MVR-PCR at D1S8 (MS32) and D7S21 (MS31A) loci

Allele-specific MVR-PCR is based on the selective amplification of single alleles from genomic DNA which is heterozygous for flanking polymorphisms. In order to perform allele-specific MVR-PCR, flanking polymorphisms at MS31A and MS32 were investigated as described previously[4)7)12)]. A total of 100 ng of genomic DNA was used for PCR amplification to determine the three flanking haplotypes of MS32 and MS31A. Allele-specific MVR-PCR was then performed using 100 ng of genomic DNA, MVR-specific primers and one of the allele-specific primers. Allele codes of each individual at both loci were compared with those in the allele database by using dot matrix analysis (software written by A. J. Jeffreys in Microsoft Quick basic) to identify related alleles which share extensive regions of map similarity[2)7)17)]. The parameter values used for this analysis are as follows: MS32; perfect nine repeat matches at least 25 matching positions over the best two diagonals search, MS31A; perfect eight repeat matches at least 20 matching positions.

Results and Discussion

The typing results of the six conventional markers and the 12 DNA markers are summarised in Table 1. The resulting accumulated PI was calculated as 3590 and the combined PE was estimated at 99.93%. The probability of paternity (W) was then estimated at 99.97% assuming a 50% prior probability of paternity.

In order to map each allele of the trio, the three flanking polymorphisms at each of MS31A and MS32 were investigated. Table 2 summarises the flanking haplotypes and their frequencies observed in the Japanese population. All three individuals were typed as heterozygous for at least one flanking polymorphic site at both MS31A and MS32. This corresponds to the fact that 79% and 61% of Japanese individuals are expected to be heterozygous at the polymorphic sites at each locus[7)12)]. In MS31A, allele-specific MVR-PCR using the −4A/G site (31AluI+ as the allele-specific primer) on the mother and the child and using the −221G/C site (31 HgaI+ primer) on the alleged father can map single alleles selectively. With respect to MS32, allele-specific MVR-PCR using the Hf site can selectively map each of the alleles since all the members of the trio are heterozygous at the site.

The single allele mapping by allele-specific MVR-PCR using primers at MS31A and MS32 was per-

Table 1. Typing results of a paternity trio at six conventional markers and 12 DNA markers and their paternity probabilities

Locus	Mother	Child	Alleged father	PI	PE (%)	ave. PE (%)
conventional markers						
ABO	A	A	A	1.39 (–)	0 (0)	19.2
Rh	CcDEe	CcDEe	CCDee	1.31 (1.82)	0.6 (0.6)	23.7
PGM1	2+1+	2+1+	2+1+	1.17 (2.13)	2.1 (2.7)	27.3
AcP	BA	AA	BA	2.35 (5.01)	61.9 (63.0)	14.0
EsD	2–2	2–2	2–1	1.37 (6.88)	40.5 (77.9)	18.8
Hp	2–1	2–1	2–2	1.00 (6.88)	0 (77.9)	15.6
DNA markers						
HLA-DQa	1.2, 3	3, 3	3, 3	2.46 (16.9)	35.2 (85.7)	54.8
PM LDLR	BB	BB	AB	0.62 (10.4)*	3.5 (86.2)	12.9
GYPA	AB	BB	AB	1.15 (12.0)	32.0 (90.6)	18.5
HBGG	BB	BB	BB	1.41 (16.9)	8.4 (91.4)	16.6
D7S8	AB	AA	AA	1.62 (27.2)	14.5 (92.7)	18.0
GC	BC	BC	BC	1.36 (36.9)	6.9 (93.2)	33.6
LPL	12, 10	10, 10	10, 10	1.40 (51.8)	8.3 (93.7)	23.8
vWA	18, 17	18, 14	16, 14	2.77 (144)	67.2 (97.9)	59.6
F13B	10, 10	10, 8	10, 8	7.65 (1100)	87.3 (99.73)	21.7
TH01	9, 7	9, 7	7, 6	0.75 (822)*	11.0(99.76)	45.8
TPOX	9, 8	11, 9	11, 11	2.87 (2360)	42.4 (99.86)	39.8
F13A01	6, 6	6, 3.2	4, 3.2	1.53 (3590)	45.2 (99.93)	31.7

*PI values not cantributing to the accumulative PI values, PI: paternity index, PE: rate of exclusion, ave. PE: average rate of exclusion at the locus. Values in parentheses are accumulated.

Table 2. Results of MS31A and MS32 flanking polymorphic sites in the case studied

Individual	allele	MS31A haplotype				MS32 haplotype			
		–221	–109	–4	frequency*	Humpl	Hf	Hump2	frequency*
Mother	1	C	C	A	0.054	G	–	C	0.130
	2	C	C	G	0.072	G	+	C	0.575
Child	1	C	C	A	0.054	G	–	C	0.130
	2	G	T	G	0.348	C	+	C	0.200
Alleged father	1	C	T	G	0.159	G	–	C	0.130
	2	G	T	G	0.348	C	+	C	0.200

*Haplotype frequencies of MS31A and MS32 flanking polymorphisms in the Japanese population were reported previously[7] and by Monckton et al.[12], respectively.

formed from genomic DNA. The results of some examples of allele-specific MVR-PCR are shown in Fig. 1. The allele-specific primers completely discriminated against the alternative type alleles at both loci and only one allele was amplified when the individual was heterozygous for the flanking polymorphic site. As a result, the bands in the A-lane of the allelic ladder were complementary to those in the T-lane except for some 'null' repeat positions[17]. The allele codes at both loci could be determined without difficulty although some fluctuations in the intensity of the bands were observed in MS31A. On the other hand, no ladders were observed in the alleged father or the mother when mapping using the 31AluI+ primer (the –4A site specific) and the 31 HgaI+ primer (the –221A site specific), respectively, indicating that they were homozygous for the flanking polymorphic sites. Using 32-Hf2+ and 32-Hf2- primers which distinguish the Hf polymorphic site, all six alleles from the trio were mapped by allele-specific MVR-PCR at MS32.

Mapped alleles were coded with reference to a standard sample (lanes S in Fig. 1). All alleles could be coded up to at least 50 positions and four shorter

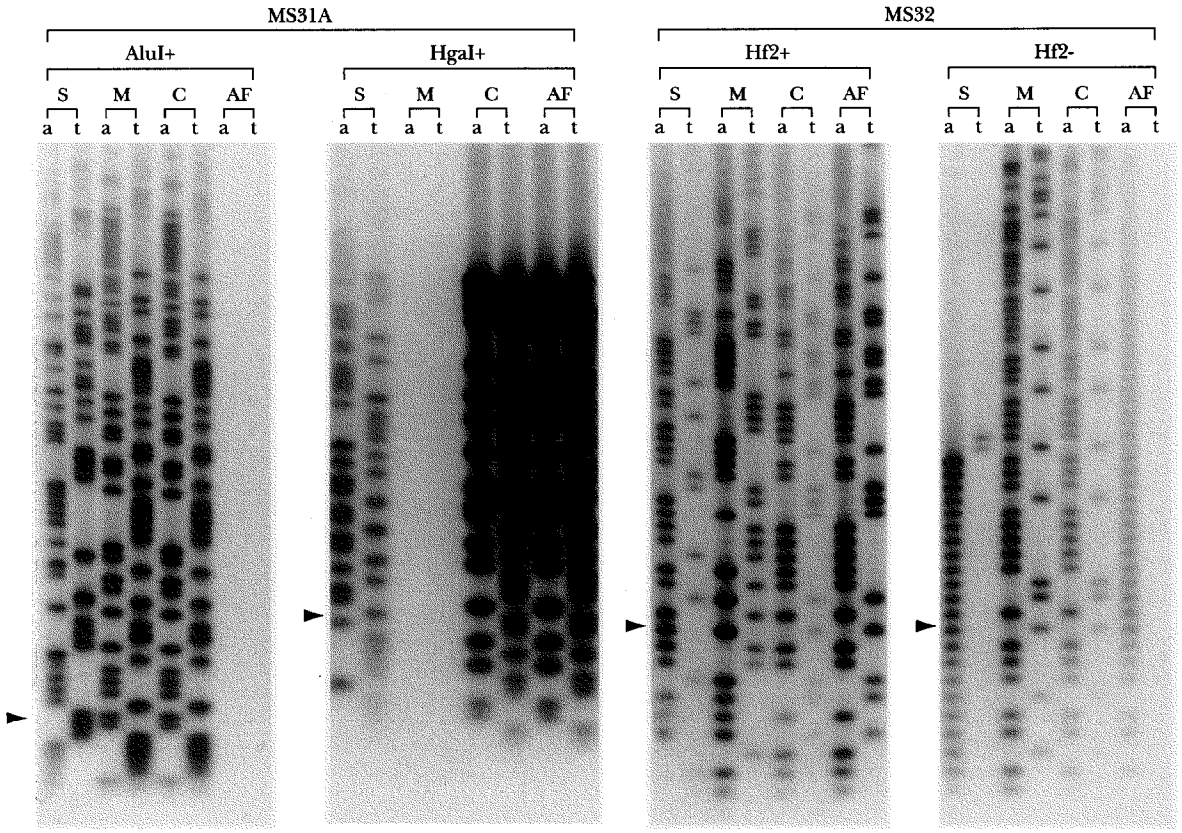

Fig. 1. Allele mapping of the trio at MS31A and MS32 using allele-specific MVR-PCR. AluI+, HgaI+, Hf2+ and Hf2– were the allele-specific primers. The arrowheads indicate the 10th positions of the standard allele. S: standard allele, M: mother, C: child, AF: alleged father

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D7S21(MS31A)
          1       .       .       .       .       50      .       .       .
M-1       ?tatatttttaataaatattataataatttttataattaatatatttttaataaatataaataaaaaaaaaaatatt....
M-2       ?tttataaatataattaaaatattataataattattatattatttaatatattatattattatatataataa........

C-1       ?tatatttttaataaatattataataatttttataattaatatatttttaataaatataaataaaaaaaaaata......
C-2       ttaattatattatttatattaatatttatttattatttatttttata0a0a<

AF-1      ??aaatta?tttatttatatataataataaattattattaattaatatataaattaatatt.....................
AF-2      ttaattatattatttatattaatatttatttattatttatttttata0a0a<


D1S8(MS32)

          1       .       .       .       .       50      .       .       .
M-1       aataataaatattaaaaataaataaaataaataaaaataaaataaatattataatt<
M-2       aataaaattatatatttattaaaattttaaaaattattaaaattttaaaatatatataataatta.............

C-1       aataataaatattaaaaataaataaaataaataaaaataaaataaatattataatt<
C-2       aaatattaatataaaaatttaataaaattattaattaaataa0atattaaaaaaa.......................

AF-1      taa0aa0aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa.........................
AF-2      aaatattaatataaaaatttaataaaattattaattaaataa0atattaaaaaaaaataattt.............
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Fig. 2. MVR allele maps at MS31A and MS32 in the present case, < indicates the termination of alleles.

alleles were mapped over their entire lengths (Fig. 2). In MS31A, the code of the child's allele-1 (C-1) was identical with that of the mother's allele-1 (M-1), while the code of child's allele-2 (C-2: short allele) was identical with that of alleged father's allele-2 (AF-2). The haplotypes flanking the child's alleles were also identical with those flanking the parental alleles. Therefore, the child and the alleged father shared the identical allele. They also shared an identical allele in MS32 (C-2 and AF-2).

Each of the four allele codes of the trio at both loci was compared with the allele codes in MS31A and MS32 databases. Comparison of the MS31A allele codes of the trio with 149 Japanese alleles showed no identical (indistinguishable) codes although the short allele shared by the child and the alleged father which terminates with an **"a0a0a"** motif at the 3′ end, appeared to be a related to 23 short alleles in the database[7]. Similarly at MS32, no identical allele codes were found for the four alleles in the database containing 1018 codes from 15 different ethnic groups (including 318 Japanese alleles), while the allele shared by the child and the alleged father was found to belong to a group consisting of 18 Japanese alleles out of 23 alleles (unpublished data).

We then considered the frequency of each MVR allele code in both loci in the Japanese population. According to the present database, since two MS31A alleles were detected twice and the remaining 145 alleles were detected only once in 149 mapped Japanese alleles[7], the observed maximum frequency at this locus was therefore estimated at 2/149 or 0.013. For MS32, the maximum frequency was similarly estimated at 5/318 or 0.016[18]. In fact, it is not possible to determine accurately the frequency of MVR haplotype from the relatively small databases, as both loci have large numbers of the different alleles in the Japanese population: *i.e.* MS31A: >5000 alleles[7], MS32: >12000 (unpublished data) by Poisson analysis. However, it does not seem practical to use 1/5000 or 1/12000 as the frequency of the MVR haplotype shared by the child and the alleged father.

Since in the database of N alleles there are no alleles which are identical to those shared by the child and the alleged father, the value of the upper limit of 95% C.I. (f) is given by solving $(1-f)^N=0.05$, which is based on a binomial distribution. Unless N is small, f will be calculated almost exactly at $3/N$ regardless of the sample size. Therefore, the allele frequency and the average probability of exclusion at each locus was calculated as follows: MS31A: 3/149 (0.020); 96.0 % (no. of different alleles (n)=49), MS32: 3/318 (0.009); 98.1 % (n=106). These values of the probability of exclusion by both estimations are extremely high compared with some DNA markers (see average PE in Table 1), although their estimated allele frequencies are far larger than the real ones and they may decrease as the number of databases increases.

We finally estimated PI and PE by MVR analysis at MS31A and MS32 using these presumed frequencies. Since the heterozygosities at the two loci are so high, the trio should consist of different heterozygous types, sharing one allele of the child with the mother and the other one with the alleged father, *i.e.* M (A, C), C (A, B) and AF (B, D). The value of PI and PE at each locus is therefore calculated as $1/2p$ and $(1-p)^2$, where p denotes the frequency of the allele shared by the child and the alleged father. The results are summarised in Table 3. The combined PI was then 4,730,000 giving the probability of paternity (W) as

Table 3. Contribution towards paternity probabilities by MVR-PCR at MS31A and MS32

locus	PI	accum. PI.	W (%)	accum. W (%)	PE (%)	accum. PE (%)
total 18 markers*	—	3590	—	99.97	—	99.93
MVR-PCR						
MS31A	24.8	89,200	96.13	99.9989	96.01	99.997
MS32	53.0	4,730,000	98.15	99.99998	98.12	99.99995

*6 conventional genetic markers and 12 DNA markers (see Table 1). PI: paternity index, W: probability of paternity if a prior probability is 50%, PE: rate of exclusion

99.99998% if the prior probability was 50% and the total PE in the case was estimated at 99.99995%.

In conclusion, the application of MVR-PCR to paternity testing is extremely useful since its potential is so high as to identify a single individual in the population. Hypervariable minisatellite loci such as MS31A and MS32 have germline mutation rates as high as 1%[19]. Germline mutation in tandem repeats has also been discussed in practical paternity cases[20][21]. Therefore, one should be wary of rejecting paternity when only the size of the allele inherited from the father is different from that of the alleged father. However, MVR information might help to reject paternity unless these two alleles share any motifs of the MVR haplotypes which are used for grouping[2].

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