CASE REPORT

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Applications of Minisatellite Variant Repeat (MVR) Mapping for Maternal Identification from Remains of an Infant and Placenta

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ABSTRACT: Minisatellite variant repeat (MVR) mapping using the polymerase chain reaction (PCR) at D1S8 (MS32) was applied to a practical forensic case of an infant and placenta found in an incinerator. They were thought to be left for a few days postmortem, and the infant was severely burnt when found. DNA was extracted from the infantile muscle and maternal placental hematoma. MVR-PCR analysis as well as other common DNA typing (D1S80, HLA-DQA1) were performed on both DNA samples. Both MVR diploid codes were matched although some extra faint bands in the ladder were observed from the maternal placental sample, which probably indicated superimposing of an allele derived only from the mother, and not the infant. In order to detect the original maternal alleles, three flanking polymorphic sites were typed and allele-specific MVR-PCR was performed. Finally, one maternal allele not inherited by the infant and two alleles from the infant were typed. Two alleles suggested the infant and/or mother was Japanese. The two diploid codes including one possibly from the mother were deduced and compared with other codes in the databases for evaluating the discriminating power.

KEYWORDS: pathology and biology, minisatellite, polymerase chain reaction, MVR-PCR, DNA typing

MVR-PCR at D1S8 (MS32) [*1*] is an approach for analyzing individual variations in human DNA. The process is simple, rapid and can give unambiguous and digital code information ideal for computer databasing and analyses. MS32 alleles have been previously shown to contain two major classes of repeat units [*2*], designated **a-** type and **t-** type, which differ by a single base substitution within a repeat unit, and show highly diverse dispersion patterns within alleles [*3*]. MVR-PCR has many advantages over current DNA typing systems used in forensic investigation involving analysis of allele length measurements: MVR-PCR does not require standardization of electrophoretic systems, does not involve error-prone allele length measurements, nor requires side-by-side comparisons [*1*]. Some potential forensic applications of MVR-PCR have reported on the evaluation of this technique using forensic specimens [*4,5*] or developing a non-isotopic method [*6*].

MVR-PCR has been successfully applied to two additional human minisatellites, MS205 (locus D16S309) [*7*] and MS31A (locus D7S21) [*8*], and MS32 and MS31A can be used to generate MVR diploid codes simultaneously in "duplex MVR-PCR." This approach may be very effective for individual identification from degraded DNA samples because it increases the amount of information from the limited number of repeat units which can be scored. Furthermore, an allele-specific MVR-PCR [*9*] has also been developed. This method can map single alleles from the total genomic DNA using allele-specific PCR primers directed at polymorphic sites in DNA flanking the minisatellite. It may be a very powerful method for the analysis of mixed human DNA samples through the selective amplification of one or two alleles from only one individual.

In the present study, we have applied the MVR-PCR approach to analyze DNA samples extracted from remains of a newborn and placenta found in an incinerator, and determined three allelic codes. We then deduced two unique diploid codes, one of which was the mother's. This may help identify an individual in a large database.

Materials and Methods

DNA Samples

In December 1993, the badly burnt remains of a male infant were found in an incinerator near a small factory (Fig. 1). The infant was estimated to be a full-term fetus at 40 weeks' gestation, 1680 g in weight, having a crown-heel length of 48 cm, and a foot length of 22 cm. Although the infant was covered with a small bath towel, the right half of the infant was severely charred, and heat coagulation of the tissues was observed in the remaining surfaces (Fig. 1*a*). Some parts of his trunk were discolored greenish-brown or purplish-brown due to putrefaction after suspectedly being left in the open air for a few days postmortem. His umbilical cord was 21 cm long and burnt away at the end. Although most internal organs suffered heat coagulation or were burnt away, some muscles near the vertebral column were fairly well preserved. From the left psoas major of the infant (0.3 g), DNA was extracted as previously described [*5*]. Under the infant's body, placenta (185 g) also covered with a small towel was found (Fig. 1*b*). The

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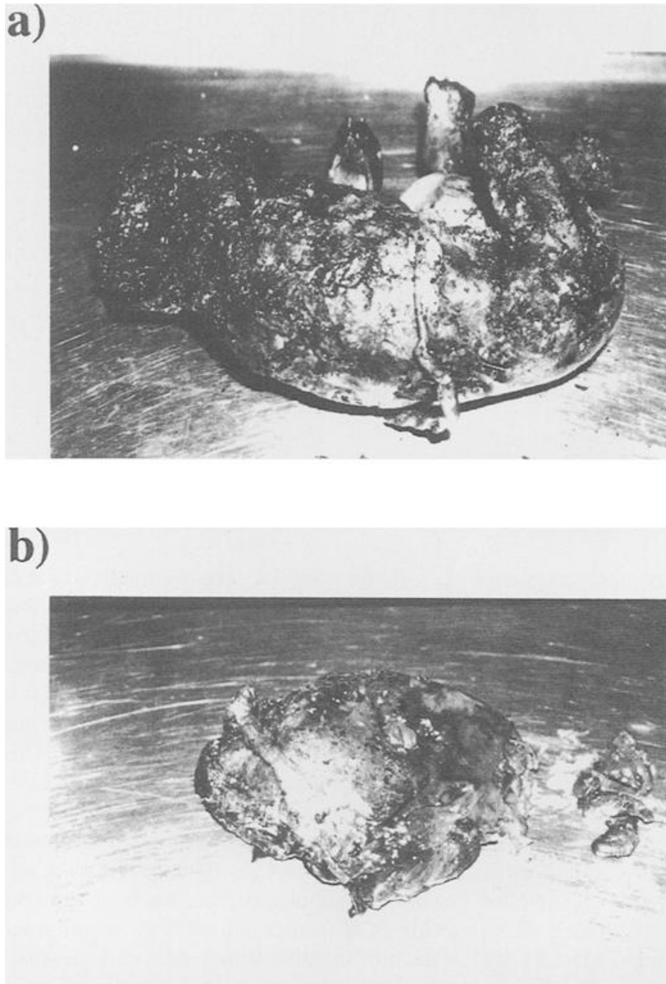

FIG. 1—(a) *The charred remains of the right half of the newborn and* (b) *the maternal side of the placenta found in an incinerator.*

umbilical cord connected to the placenta was only 11 cm long, and the fetal side of the placenta was putrefied and discolored greenish-brown. Half of the maternal side of the placenta (maternal placenta) was mostly heat coagulated and there was a hen's egg-sized hematoma in the remaining surface. From 0.15 g of the hematoma, DNA was isolated using the same method as above. Each extracted DNA sample was fluorometrically quantified as previously described [5].

Blood Group Typing and Standard DNA Typing

From the muscle and the hematoma, ABO blood group was typed by an absorption-elution test. As an example of a single locus probe (SLP) analysis, the sizes of MS32 alleles were determined according to the protocol of Wong et al. [2]. The allelic numbers of D1S80 were typed by the method of Kasai et al. [*10*] with side-by-side electrophoresis of allelic ladder marker (AmpliFLP™ D1S80 Allelic Ladder, Perkin-Elmer, Roche Molecular Systems, Branchburg, NJ). HLA-DQA1 genotypes were also determined as previously described [*11,12*].

MVR-PCR and the Allele-Specific MVR-PCR at D1S8 (MS32)

Each 100 ng DNA sample was placed into one A-tube or T-tube for PCR, and MVR-PCR was performed according to the protocol of Jeffreys et al. [*1*] using the primers 32O, TAG and 32-TAG-A or 32-TAG-T. Three base-substitutional polymorphisms in the DNA flanking the MS32 were typed as previously described [9], and the allele-specific MVR-PCR was performed using allele-specific primers designated 32Hf2+ or 32Hf2-, TAG, and 32-TAG-A or 32-TAG-T [9], changing the number of PCR cycles from 18 to 30. In order to obtain allelic MVR information from the infantile DNA, two alleles from the infant, designated infant-1 and infant-2, were separated by agarose gel electrophoresis and purified by electroelution onto a dialysis membrane [*13*]. MVR-PCR was performed using separated allelic DNA equivalent to 100 ng of genomic DNA.

Matching Diploid Codes and Allelic Codes in the MS32 Database

In order to find similarities with other alleles, the three mapped allele codes were compared with 1018 allele codes in the allelic database by dot matrix analysis [*1*]. Two deduced diploid codes were also compared with the diploid database of 799 different individuals. Both databases and analyzing programs were kindly supplied by Prof. Sir Alec J. Jeffreys.

Results

Although both the infant and the placenta were severely damaged due to putrefaction and burning, high molecular DNA was obtained from the psoas major from the infant and the hematoma of the placenta (data not shown). From both infantile and maternal placental DNA samples, standard DNA analyses were performed (Table 1). By SLP analysis (MS32), two bands were observed in both samples, and their lengths in both samples were estimated to be the same, that is, 2.8 kbp and 3.6 kbp. Therefore, the infant and the placenta seemed to come from the same mother. No extra bands, which would have indicated the hematoma contained some maternal blood, were observed in the sample from the placenta. D1S80 clearly showed two bands in both samples. The numbers of repeats were determined as 30 and 31 using the allelic ladder marker. No extra bands were observed in the placental sample. On the other hand, the HLA-DQA1 genotype showed that both samples contained DQA1*0301, and the placental DNA contained an extra allele to be possibly derived from maternal DNA. The generic type of the allele was determined by dot blot analysis (DQA1*01), however, the subtypes (DQA1*0101, DQA1*0102, and DQA1*0103) could not be determined due to a lack of dot

TABLE 1—*Forensic typing results of the infant and the placental hematoma.*

	Infantile muscle	Maternal placental blood
ABO	A	A
SLP(MS32) (kbp)	2.8/3.6	2.8/3.6 (no extra bands)
HLA-DQA1	*0301/*0301	*0301/*0301 and *01?
AmpFLP (D1S80) (allelic No.)	30/31	30/31 (no extra bands)
MS32 flanking polymorphism		
Hump1	C/C	C/C
Hf	+/+	+/−
Hump2	C/C	C/C

blot marks which determine subtypes. This may have been caused either by a lack of maternal DNA or low sensitivity of the probes.

Standard MVR-PCR using the 320 flanking primer was performed (Fig. 2). The MVR-coding ladders in both samples were similar although that from the placental sample (P) had some faint bands in the A-track (positions indicated by +), and also in the T-track (*). Three polymorphisms of the DNA flanking MS32 (Hump1, Hf and Hump2) were typed (Table 1). Hump1 and Hump2 matched in both samples, but Hf was typed as +/+ in the infant's sample, while a faint Hf- band was also observed in the placental sample. Therefore, Hf in the placental sample was typed as heterozygous (+/−). Allele-specific MVR-PCR of the placental sample using the 32Hf2+ flanking primer to detect only Hf+ alleles

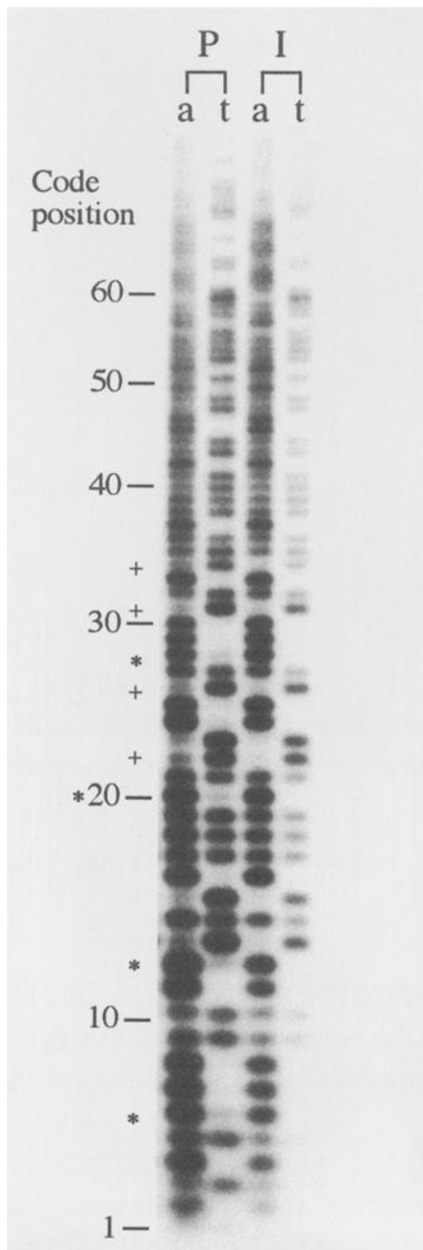

FIG. 2—*Diploid code mapping by MVR-PCR from the infant (I) and the placenta (P). Code positions which show faint bands observed in the placenta indicated by "+" and "*" for a-types and t-types, respectively.*

showed the same diploid codes obtained from the infantile DNA by the standard MVR-PCR, and no extra faint bands were observed even though the number of PCR cycles were increased up to 30 cycles (Fig. 3*a*). Allele-specific MVR-PCR using the Hf2-flanking primer detected the Hf- allele clearly when the number of PCR cycles was more than 24 (Fig. 3*b*). Although signals in the T-track were weaker than those in the A-track, the allelic code of this sample was finally determined by one week exposure of four MVR mappings. In order to obtain allelic MVR information from the infantile DNA, two alleles from the infant, designated infant-1 and infant-2, were separated by agarose gel electrophoresis, purified by electroelution onto a dialysis membrane, and MVR mapping was performed using separated allelic DNA.

The three allelic codes involved in this case are shown in Figs. 4*a*, 4*b* (infant-1, infant-2 and mother). Using either allelic code from the infant and one from the placenta, two possible codes of the mother were deduced (Fig. 4*c*), and they were compared with 230 unrelated Japanese diploid codes in the database. No individuals were found that shared the same diploid codes up to 50 repeat units. The three alleles were also compared with others in the allelic database which contained 1018 allelic codes from 15 different ethnic groups. Alleles infant-1 and mother shared the same motifs to nine and six alleles derived from Japanese, respectively (Fig. 5). Allele infant-2 shared no same motifs to alleles in the present database.

Discussion

MVR-PCR [*1*] provides a new and powerful method for individual identification from human DNA, and it offers digital information which allows for exact matching, thereby avoiding the considerable debate over the evaluation of allele length estimates. In our previous study, reliable MVR coding ladders were obtained with DNA samples extracted from various human tissues and stains [*5*]. Mixed human DNA samples are also an important subject for forensic analysis and MVR-PCR can be applied to these samples. Preliminary experiments have shown that for a 10%/90% mixed DNA sample, the 10% target DNA can be detected [*1*]. Allele-specific MVR-PCR is more powerful for analyzing mixed DNA samples because it can be used to map single alleles for mixtures containing as little as 1% admixture of DNA [*9*]. Based on the combined heterozygosity from the haplotype frequencies of three flanking polymorphisms in MS32, more than 60% of Japanese individuals can have single alleles mapped by allele-specific MVR-PCR [*9* and Tamaki et al. unpublished data].

In the present case, we have used a few common DNA typing systems as well as MVR-PCR. From the infant sample, SLP (MS32), HLA-DQA1 and D1S80 were typed clearly. The diploid code of the infant was determined from the genomic DNA by standard MVR-PCR, and MVR-PCR also enabled his allelic code to be determined using separated allelic DNA. Furthermore, HLA-DQA1 indicated the possibility of an extra allele which was only derived from the mother contained in the DNA sample isolated from the maternal placental hematoma, while a PCR-based method of AmpFLP (D1S80) and a SLP analysis (MS32), which does not use PCR and results in lower sensitivity, failed to show any extra bands. In both analyses, the authors suggest missing or very weak signals from maternal DNA, or possible overlapping of both infant and maternal alleles may have occurred, thereby producing only one set of results.

The standard MVR-PCR from the placental sample also managed to detect some extra bands which seemed to indicate the

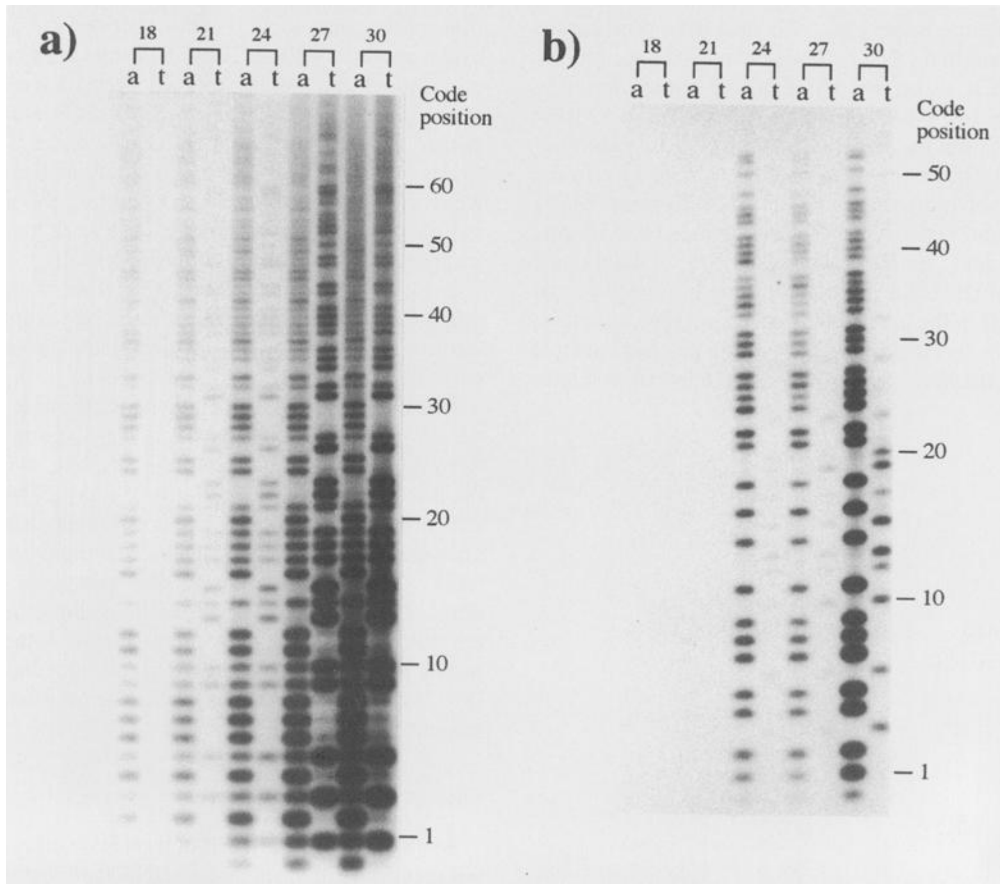

FIG. 3—*Allele mapping using* (a) *Hf2+ primer and* (b) *Hf2− primer by allele-specific MVR-PCR. Numbers on the top indicate the number of PCR cycles.*

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                        1       .        .        .        .        50      .
a)      diploid infant  31313111331123213331322112311123153313333133113313133 3......
        allele  infant 1  aatataaattaatttaaaaatttaataaaattattaaaattattaattaaataa......
                infant 2  taaaaaaaaaaatatatttaattaattaaataa0atattaaaaaaaaaataatt......

b)      allele  mother    aataataaatattatatattaataaaataaataaaaataaaataaatattataa......

c) [I]  diploid mother    1121331132132321313333211313113213311313312311233312 11......
        allele  infant 1  aatataaattaatttaaaaatttaataaaattattaaaattattaattaaataa......
                mother    aataataaatattatatattaataaaataaataaaaataaaataaatattataa......

   [II] diploid mother    31311311131321212323132113331133141312311131113132133 3......
        allele  infant 2  taaaaaaaaaaatatatttaattaattaaataa0atattaaaaaaaaaataatt......
                mother    aataataaatattatatattaataaaataaataaaaataaaataaatattataa......
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FIG. 4—*Synthesizing maternal diploid codes. Six scoring codes are used: 1(a,a), 2(t,t), 3(a,t), 4(a,0), 5(t,0) and 6(0,0).* (a) *the infantile diploid codes mapped from infantile muscle DNA and allelic codes mapped using separated allelic DNA.* (b) *allelic code of a maternal allele not inherited by the infant mapped by allele-specific MVR-PCR.* (c) *two maternal diploid codes deduced by synthesizing either infant allele with the maternal allele not inherited by the infant.*

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  JAP121 1   ...attattaattaaaaaattaaaataaaaattaTTAAAAATTTAATAAAATTATTAATTAAATAA.....................
  JAP550 2   ...taaaatatatataaatataaataaAAATTAATTTAAAAATTTAATAAAATTAT...............................
  JAP521 2    >taaaatatatataaatataaataaAAATTAATTTAAAAATTTAATAAAATTATTAATTAA..........................
  JAP518 1   ...aataataaatataaatataataattAATTAATTTAAAAATTTAATAAAATTATTAATTAAATAA.....................
  JAP554 2                  >ttataatatTAAATTAATTTAAAAATTTAATAAAATTATTAATTAAAT-AA0ATATTAAAAAAA..........
  JAP538 2                        >ATATAAATTAATTTAAAAATTTAATAAAATTATTAATTAAAT-AA0ATATTAAAAAAAAATAATTT..
* infant 1                         >aATATAAATTAATTTAAAAATTTAATAAAATTATTAAattattaattaaataa..............
  JAP600 2        >0aaaaatataaatatttATATAAATTAATTTAAAAATTTAATAAAATTATTAAT---AT-AA0ATATTAAAAAAAAATAATTT..
  JAP123 1               >ataaataaaaaATAAATTAATTTAAAAATTTAATAAAATTATTAAT---AT-AA0ATATTAAAAAAAAATAATTT..
  JAP539 1  ...ttataatatataaatttattataatatataAATTTAAAAATTTAATAAAATTATTAA----ATAAA0ATATTAAAAAA...........


* infant 2    >taaaaaaaaaaatatatttaattaattaaataa0atattaaaaaaaaataatt...


  JAP547 2    >aaaaaa0taaAAATAATATTATATATTAAT-AAAATAAAT-AAAAATAAAATAaAATATTATAATT<
  JAP117 2         >tatATAAATAATATTATATATTAAT-AAAATAAAT-AAAAATAAAATA-AATATTAT....<
* mother             >aATAATAAATATTATATATTAAT-AAAATAAAT-AAAAATAAAATA-AATATTATAA..<
  JAP577 1           >tATAAATAATATTATATATTAATtAAAATAAAT-AAAAATAAAATA-AATATTATAATT<
  JAP575 1             >atAATAATATTATATATTAAT-AAAATAAAaaAAAAATAAAATA-AATATTATAATT<
  JAP318 2          >ttaaaataAATATTATATATTAAT-AAAATAAAT-AAAAATAAAATA-AATATTATAATT<
  JAP272 2            >aaaaattaatataaaaataAAT-AAAATAAAT-AAAAATAAAA-A-AATATTATAATT<
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FIG. 5—*MVR maps of three alleles involved in this case and related alleles found in the database. Alleles infant-1 and mother have very similar motifs to nine and six alleles to only Japanese in the present database, respectively. These two alleles are placed next to alleles sharing the most common characteristics for ease of viewing. Allele infant-2 has no similar alleles in the present database. Segments sharing related sections are shown in uppercase and the "−" marks are for alignment. The ">" and "<" signs indicate the termination of alleles.*

existence of a maternal allele not inherited by the infant. If the sample was known to contain only three alleles, or not contaminated by other human DNA, the allelic information could be obtained by subtracting the infant's diploid code from the placental diploid code even though there still would remain many unscorable code positions. Additionally, if the admixture of DNA from maternal blood were enough to show an extra band in the Southern blot analysis, an allelic MVR map of the allele not inherited by the infant could be obtained following separation of the alleles by electrophoresis and purification by electroelution, such as performed in the infant's sample. However, this sample did not seem to fit these conditions. Allele-specific MVR-PCR using the Hf2-flanking primer was performed and only one Hf- allele was clearly detected (Fig. 3*b*). Fortunately, the DNA sample isolated from the placental hematoma proved to contain only three alleles. In other words, it was not contaminated by other human DNA, but contained DNA from only the infant and mother. The MVR ladder obtained from very small amounts of DNA (< 10 ng of genomic DNA) may have random fluctuations in band intensity due to stochastic loss of PCR products from the small number of target molecules [*1*]. In fact, the allele seemed to have few **O**-type repeats near the upper end of the allele. We typed this allele for four times and determined the authentic allelic code by longer exposure of the autoradiographs.

Two possible mother's codes using either code from the infant and one from the placenta were deduced (Fig. 4), and they were compared with 230 unrelated Japanese diploid codes in the database. No individuals were found who shared the same diploid codes up to 50 repeat units. The probability of unrelated individuals who share the same diploid code as the mother's code is less than 0.4% (1/230), provided that the present Japanese database reflects the Japanese population. It is estimated that there are more than 3500 different MS32 alleles in the Caucasian population [*1*] (that is, more than 6.0×10^6 different diploid codes can exist). Although the number of different diploid codes in the Japanese population may be slightly smaller due to geographical and historical reasons, the chance that a diploid code could match a record in the database would be almost zero since even one pair of identical diploid codes have not been observed in unrelated individuals. MVR-PCR of MS31A is another powerful tool for individual identification, and their combined analyses of two loci as "duplicate MVR-PCR" [*8*] can make the power of discrimination even greater.

Analysis of large numbers of MS32 alleles in various ethnic populations [*1,13*] and Jeffreys et al. unpublished data] has revealed allelic diversity with no common alleles. Even one pair of 'identical' alleles defined by two-state (standard) MVR-PCR observed in very different populations showed differences in 5′ flanking haplotypes and two MVR positions by four-state MVR-PCR [*14*]. Nevertheless, alleles show internal regions of significant similarity, suggesting recent common ancestry of those allele segments. Around half of the alleles thus far mapped can be classified into groups [*1,13,14*], and alleles are largely population-specific, except in cases of recent admixture. All three allelic codes from these samples were compared with others in the allelic database which contains 1018 allelic codes from 15 different ethnic groups. Alleles infant-1 and mother shared the same motifs to nine and six alleles in the database, respectively, whereas allele infant-2 was found to have no similar matches (Fig. 5). All those alleles from the database were from only Japanese. Therefore, it is thought that the infant and/or the mother were possibly Japanese, although alleles of other Asian populations which have not been typed should be compared to confirm this result.

Although further statistical study is needed to evaluate the power of discrimination, our study clearly shows that MVR-PCR can be applied as an extremely effective tool to practical forensic samples for individual identification. MVR analysis should become even more effective if allele-specific MVR-PCR can be applied to mixed

DNA samples since alleles can be selectively mapped and some alleles are largely population-specific.

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