BRIEF COMMUNICATION

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A New Extremely Large Allele at the D1S80 (MCT118) Locus

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ABSTRACT: A new extremely large allele at locus D1S80, segregating in a three-generation family is described. The length of PCR-generated allele is approximately 1000 bp. Restriction analysis indicates that this increase is due to an increased number of basic core sequence. The assessed number of tandem repeats is in range 52–55, corresponding to 979–1027 bp exact length of the PCR-generated fragment.

KEYWORDS: D1S80 (MCT118) locus, new allele, polymerase chain reaction (PCR)

The VNTR polymorphism at the D1S80 (MCT118) locus is due to a variable number of 16 bp long repeating units in different individuals. Kasai et al. [*1*] published primer sequences and reaction conditions for effective and rapid PCR-based analysis of this polymorphism. In this paper, the range of PCR-created fragments varied between 387 bp and 723 bp, corresponding to 15 to 36 repeats of 16 bp core unit, respectively. The broadest range of PCR fragments was found in Chinese population, namely from 340 bp to 780 bp, corresponding to number of repeats from 12 to 40, respectively [*2*]. Thanks to the possibility of rather simple and rapid PCR-based analysis, and high informativity of this VNTR (the proportion of heterozygotes in all populations studied so far is about 80%), D1S80 has been widely used not only in population studies, but also in forensic practice for paternity testing and individual identification. For this application, it is important to determine the size range of D1S80 alleles amplified using PCR. This is particularly important in the region of long alleles, which, in combination with shorter ones, may amplify very ineffectively [*3,4*], and thus escape from being identified. The consequences in forensic practice are obvious.

In this contribution we report an extremely large D1S80 allele, which has been identified in connection with exclusion of massive contamination of chorionic villi with maternal tissue in a DNA-based prenatal genetic diagnosis. Figure 1 shows the pedigree of this family and electrophoretic pattern of PCR products of individual members. The new allele, *N*, (band at 1000 bp according to 100 bp ladder in lanes 1 and 8) was present in female I/1, (lane 2, her husband has died), who passed it to one of her sons, II/2 (lane 4), who consequently passed it also to one of his sons, III/2 (lane 7). Mendelian segregation of this band shows that it is an allele rather than a PCR artefact.

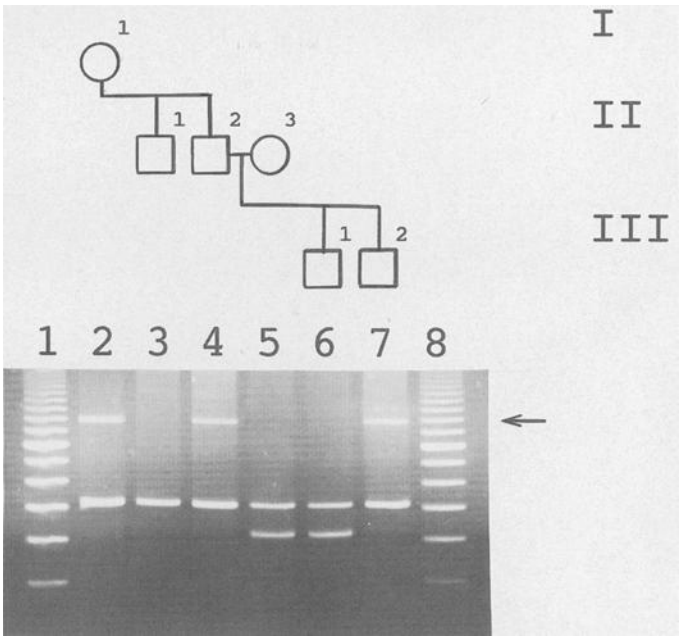

FIG. 1—*Segregation of the new D1S80 allele* (indicated by arrow) *in a three-generation family. Lanes 1 and 8: 100 bp ladder (the lowermost band represents 300 bp), lanes 2 to 7: PCR-pattern of individual family members.*

The PCR reaction was carried out in 50 μL reaction volume containing 200 to 500 ng high molecular template DNA, 10 mM Tris-Cl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.17 mg/mL bovine serum albumin, 0.1% Triton X-100, 400 μM each of dNTPs, 1 μM of primers, and 2 units Taq DNA polymerase per reaction (Fermentas). The enzyme was added to reaction mixture before the 5 min initial denaturation step at 94°C, followed by 30 cycles of denaturation, annealing and extension steps at thermal profile published by Kasai et al. [*1*]. The reaction was completed with incubation at 72°C for 10 min. Incubations were carried out in TECHNE PHC-3 thermal cycler. PCR products were resolved in

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20 cm long 1.5% agarose gel, run in TBE buffer at 140 V constant voltage for 5 hours, and visualized by ethidium bromide staining. To determine the length of the new allele more precisely, samples containing it were run in a 20 cm, 6% PAGE gel in TBE buffer at 170 V for 6 hours, along with a 100 bp ladder.

To improve the efficiency of amplification of the new large allele, various combinations of factors, affecting amplification efficiency (Mg^{2+} concentration, various reaction buffers and thermal profiles, number of cycles, etc.) were tested. Best results, however, were achieved applying reaction conditions described above.

To determine whether the considerable enlargement of allele length was caused by increased number of tandem repeats of basic core sequence, or by insertion of unrelated sequences, restriction analysis of PCR fragments was carried out (Fig. 2). Computer analysis of the published sequence [*1*] revealed that the restriction endonuclease AvaII cuts out from the 5′ end of PCR fragment a 43 bp and 108 bp fragment, and from the 3′ end a 60 bp one. Between these restriction sites, the region built up by various number of repeat units is cut to fragments 32 bp and 16 bp long. The logic behind the restriction analysis was, that if the increased length of the allele was due to increased number of repeat unit, digestion of this allele with AvaII would completely mince the PCR product, except for its longer 5′ and 3′ end fragments. In the case of insertion of unrelated sequence (sized at least 250 bp or more) it is expected, that this sequence (on the basis of random occurrence of restriction sites) will not contain restriction sites for AvaII as frequently as the sequence composed solely of repeat units. Thus, digestion with AvaII will generate also DNA fragment(s) longer than 32 bp, besides of those 108 bp, 60 bp, and 43 bp long. Empirical comparison of electrophoretic pattern of alleles 24 with the new one (Fig. 3) did not reveal any presence of longer DNA fragments. On the basis of these results we suppose that the significantly increased length of the new D1S80 allele is due to increased number of basic repeat units rather than to insertion of unrelated sequences. The number of repeats, on the basis of fragment length, is estimated to be between 52 and 55, corresponding to 979 to 1027 bp exact length of PCR-generated fragment. In a random sample of more than 100 mother-child pairs from the Causasion population of Slovakia, no other D1S80 allele of comparable length was found.

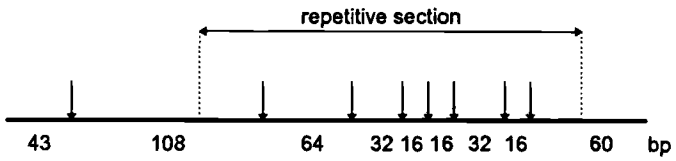

FIG. 2—*Schematic AvaII restriction map of the 387 bp (16 repeating units) long PCR product from the D1S80 locus.* Arrows *indicate the position of AvaII digestion sites.*

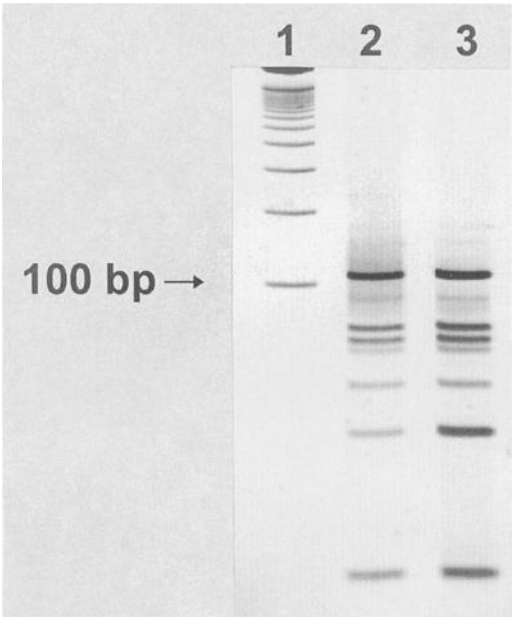

FIG. 3—*AvaII digestion of the allele 24 (lane 2) and of the new allele (lane 3): lane 1–100 bp ladder. Electrophoresis was run in 12% PAGE gel at 170 V constant voltage for 4 hours.*

References

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