TECHNICAL NOTE

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Description of Three New Alleles at the D1S80 (MCT118) Locus

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ABSTRACT: The minisatellite locus D1S80 is a variable number of tandem repeat (VNTR) locus which is commonly typed for forensic and paternity testing in a large number of laboratories all over the world. Here we describe three new alleles at the D1S80 locus; one small allele composed of 13 units of the repeated 16 mer, a further large allele with approximately 45 repeat units which we found two times in our population sample and another large allele with approximately 50 repeats.

KEYWORDS: forensic science, VNTR, D1S80, rare alleles, paternity testing

Hypervariable loci within the human genome are useful tools for forensic identification and paternity testing. One of these loci is located on chromosome 1 and is called D1S80 (MCT118). This PCR-based variable number of tandem repeat (VNTR) marker can be easily typed from small amounts of DNA and offers a high degree of discrimination power (1,2). Usually the PCR-amplified fragments are separated by gel electrophoresis and classified in comparison with an allelic ladder. In our population sample of Southern German origin, we found three new alleles of D1S80 not represented in the allelic ladder. This paper describes the analysis carried out to estimate the repeat size of the newly identified D1S80 alleles.

Materials and Methods

DNA was extracted from blood samples as described by Miller et al. (3). PCR-amplification was performed using the primers described by Kasai et al. (1990). Each sample contained approximately 100 ng DNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 250 nM of both primers and 2.5 units of Taq-DNA-Polymerase (Pharmacia Biotech, Uppsala, Sweden) in a final volume of 50 μL. Amplification conditions after hotstart using Ampliwax™ gems (Perkin Elmer, Langen, Germany) were as follows: 1 min at 95°C, 30 cycles with 1 min 92°C, 1 min 64°C, 1 min 72°C in a Thermal Cycler (Quatro TC40) from Biosystems Inc. (Manchester, UK). For visualization, the PCR-products were run on a 2% MetaPhor® agarose-gel (FMC, Rockland, ME) with 1 × TBE-buffer at 1 V/cm for 16 h in comparison with an allelic ladder (Perkin Elmer, Langen, Germany) and stained with ethidium bromide. The size of the fragments were analyzed by careful visual estimation.

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The purified PCR-Products (PCR-purification Kit from Qiagen, Hilden, Germany) were cloned in E. coli JM109 applying the Sure Clone Kit® (Pharmacia Biotech, Uppsala, Sweden) as recommended by the manufacturer. After the isolation of the plasmid DNA with a commercially available Kit (Macherey & Nagel, Düren, Germany), sequencing reactions were performed with the ALFexpress™ AutoRead™ Sequencing Kit (Pharmacia Biotech, Uppsala, Sweden) and run on an ALFexpress™ DNA Sequencer (Pharmacia Biotech, Uppsala, Sweden).

Results and Discussion

After PCR-amplification of the D1S80 locus from the genomic DNA of our probands and separation of the allelic products by high resolution MetaPhor™ agarose-gel electrophoresis, we observed two enlarged and one smaller fragments which were not represented in the allelic ladder. One of the enlarged fragments has been found two times in obviously unrelated individuals. The other new fragments occurred only once. These unassigned PCR-products, amplified from three of the probands, were cut out of the gel, ligated into the pUC18 plasmid vector and transfected into E. coli JM109 cells, enabling further detailed analysis of the putative D1S80 alleles.

PCR-amplification with D1S80 primers amplified exactly the same PCR-products with both, the recombinant plasmid clones and the genomic DNA of the respective probands. The plasmid clones should therefore carry the original allelic fragments representing the genomic amplification product of the probands. The smallest of the fragments analyzed could be easily typed as a D1S80 allele with 13 repeat units by sequence analysis of the whole insert of the respective recombinant plasmid clone (data not shown).

We failed to analyze the complete sequence of the two larger alleles because we did not manage to read through the whole insert of the plasmid clones. This was not surprising since the two strands of the D1S80 sequence exhibit an extreme bias of base composition with very small portions of thymidine and adenine, respectively. In large alleles these repeated sequences can spread over a whole kilobase, leading to an interruption of proper incorporation of nucleotides in the sequencing reactions; in our hands after approximately 500 bp of length.

Nevertheless, partial sequence analysis proceeding from both ends of these large repeats confirmed the amplification of true alleles of the D1S80 locus from the DNA of the two probands. The number of repeat units in those extremely large alleles could be finally estimated by a high resolution gel electrophoresis (2% Meta Phor™ agarose gel) applying a 20 bp ladder (Roth, Karlsruhe, Germany) as size standard. This gel allowed us to separate all fragments of the size standard even between 900 and 1000 bp as shown in Fig. 1. To test the relia-

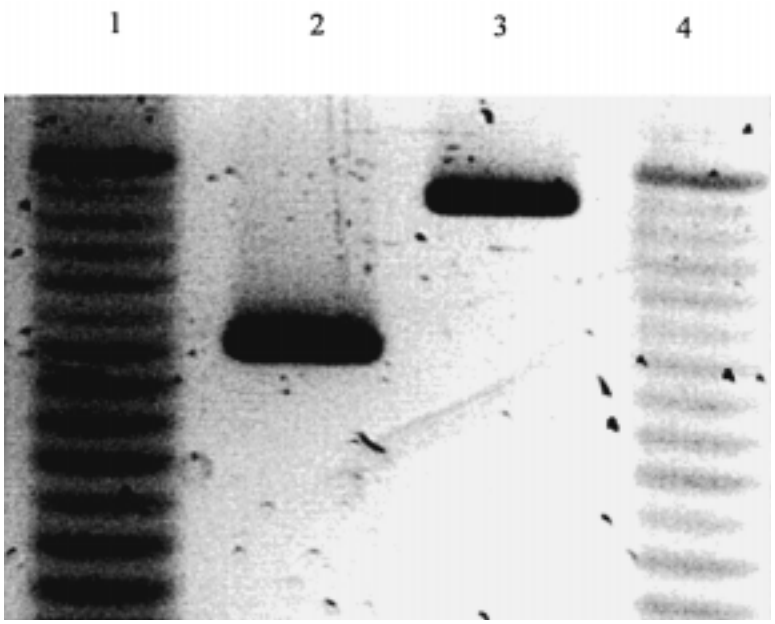

FIG. 1—*High resolution agarose gel-electrophoresis of the enlarged D1S80 alleles found in this study. A 20 bp DNA ladder was loaded in lane 1 and 4. The uppermost band of the DNA ladder represents a size of 1000 basepairs. Insert A was cut out of the respective plasmid clone and loaded in lane 2, insert B in lane 3. The DNA fragment in lane 2 could be estimated to a size of 880 basepairs, the DNA fragment in lane 3 cosegregates with the 980 basepair fragment of the size standard.*

bility of the size estimation with the 20 bp ladder, two PCR-products of known alleles of the D1S80 locus (18 and 24) were run on the same gel as the plasmid clones with the newly identified alleles (data not shown). As described by the supplier of the allelic ladder (Perkin Elmer, Langen, Germany) the PCR-products of these alleles have a calculated size of 433 bp (allele 18) and 529 bp (allele 24): 147 bp for the unique region flanking the repeat unit, 14 bp for the first repeat and $(n - 1) \times 16$ bp for the core repeat structure. With gel electrophoresis we found the PCR-products of allele 18 to migrate between the 420 and 440 bp fragments of the size standard, the PCR-products of allele 24 migrated between the 520 and 540 bp marker fragments. Therefore, the DNA marker seems to give a rather good estimate for the length of the D1S80 alleles. The inserts of the two plasmid clones with the newly identified large alleles (A and B) co-migrated approximately with the 880 and 980 bp fragments of the DNA-ladder. The sizes of the polylinker region and the unique regions flanking the repeats were determined precisely for each of the two inserts by sequence analysis. This was necessary since plasmid A showed a small deletion in the unique flanking sequence at one end of the insert, most probably the result of a cloning artifact. After subtraction of 36 bp for the polylinker region of the plasmid vector (both clones) as well as 131 bp for the unique region flanking the repeats in case of the smaller allele (insert A) and 146 bp in case of the larger allele (insert B), 713 bp for the repeat structure of the smaller of the two alleles and 798 bp for the larger one were left. Considering that the first repeat unit of this locus is composed of 14 bp while the remaining repeat units have 16 bp, as published in (2), the number of repeat units can be estimated to 45 for the smaller, and 50 for the larger allele, respectively. Such alleles have not been described for the D1S80 locus before. Klintscher et al. (4) reported two new alleles in an Austrian population sample with a size of approximately 47 to 49 repeats, but these fragments have not been analyzed exactly so that the real size of the alleles is still not known. Another group (5) found a new extremely large allele with approximately 52–55 repeats but also its exact size has not been determined. Here we describe three new alleles of the D1S80 locus found in our population sample, one small allele with 13 repeats and two enlarged alleles estimated at 45 and 50 repeat units. A transmission of the newly identified alleles from parent to offspring was observed in two families. We are not able to give a frequency estimate for these new alleles since our sample size is too small to provide an adequate calculation. Taken together 281 individuals have been typed in our laboratory for D1S80 since we introduced this VNTR in our panel of applied polymorphisms in paternity testing.

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