MINISATELLITE DNA PROBE MZ 1.3: APPLICATION IN PATERNITY TESTING AND ESTIMATE OF THE NUMBER OF GENETIC LOCI

P.M. Schneider[1], U. Schacker[1], K. Braunbeck[1], T. Breidbach[1], G. Rittner[2], B. Holtkamp[3], C. Rittner[1]

[1]Institut für Rechtsmedizin, Johannes Gutenberg-Universität Mainz, FRG;
[2]Institut für Rechtsmedizin, Kaiserslautern, FRG;
[3]Biotest AG, Offenbach, FRG

The use of hypervariable DNA minisatellite probes recognizing repetitive genomic DNA sequences has become a valuable and powerful tool in paternity testing as well as in forensic stain analysis (Jeffreys et al. 1985, 1987; Werrett et al. 1988). It has been shown that bacteriophage M13 DNA can also be used to obtain hypervariable DNA restriction fragment patterns in humans and other species (Vassart et al. 1987). To obtain more informative and specific fragment patterns for the DNA 'fingerprint' analysis in man, we have used M13 DNA as a probe to screen a human genomic library. Thus, we have isolated the minisatellite DNA probe MZ 1.3 (Schacker et al., in press). MZ 1.3 is a 1.9 kb fragment containing a 27 bp core sequence which is repeated 40 times. Alignment of the MZ 1.3 repeat with the 15 bp repeat of the M13 protein III gene has revealed identity at 8-11 positions, i.e. 53 - 73 %. No significant homology >70% was found by comparison of MZ 1.3 to the core repeat sequences of 33.6 and 33.15 described by Jeffreys et al. (1985).

With MZ 1.3, hypervariable restriction fragment patterns can be obtained by using the restriction enzymes Bst NI, Hae III, Hinf I, Mbo I, Pst I/Pvu II, and Rsa I. With Hinf I, we compared the fragment patterns of unrelated individuals as well as of parents and their offspring. The number of informative bands > 4.3 kb varied between 12 and 25 with an average of 18 bands. The band sharing frequency among unrelated individuals was 23.8 ± 7.2 % and 59.9 ± 7.8 % in parent-child comparisons. We were able to solve a number of special paternity cases with MZ 1.3, in which the conventional blood group analysis was either not informative or not applicable. These include a number of incest cases involving father, son and daughter/ sister within the same family, as well as paternity testing post mortem or after abortion (Schacker et al., in press; Rittner et al. 1989a, 1989b). Besides conventional labelling by radioisotopes, MZ 1.3 can also be used in a non-radioactive detection system (Fig. 1; B.E.S.T. Probe MZ 1.3, Biotest AG, Dreieich, FRG). It was found that a sequence-specific increase in binding enhances the sensitivity of the probe (Holtkamp et al., this volume).

Using minisatellite probes recognizing multiple loci throughout the genome, information on the number and linkage relationship of individual DNA fragments is difficult to obtain, as identical fragments may be derived from independent loci and allelic fragments are not recognizable. To get an idea about the number of genetic loci detected by MZ 1.3, we studied families with

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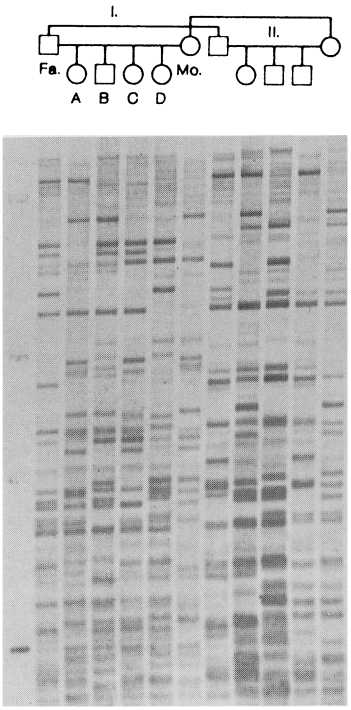

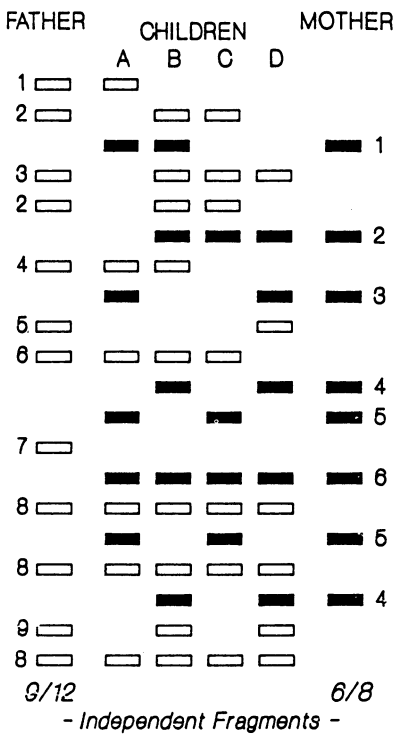

FIGURE 1 (left):
DNA fingerprint analysis of two related families (I. and II.) with the minisatellite probe MZ 1.3. Genomic DNA was digested with the enzyme Hinf I, subjected to electrophoresis in a 0.8 % agarose gel of 25 cm length for 40 h at 40 V, blotted onto nylon membrane and hybridized with the biotinylated probe MZ 1.3. Specific fragments were developed using enzymatic detection with streptavidin and horseradish peroxidase according to the manufacturer's instructions (Biotest AG, Dreieich, FRG). The family pedigrees are shown above. DNA size markers are in the left lane (top to bottom): 23.1 kb - 9.6 kb - 4.3 kb.

FIGURE 2 (right):
Schematic interpretation of unique parental DNA fragments of family I. (Fig. 1). Only informative fragments are shown, i.e. fragments which are clearly inherited either from the father or the mother. Parental fragments of identical size are omitted. The numbers of the both sides indicate unique segregation patterns for father and mother, e.g. pattern 1 (father) is found in child A, whereas pattern 2 from the father is found twice in children B and C. In the latter case, linkage has to be assumed due to cosegregation of the two paternal fragments. The total number of fragments as well as the number of independently segregating fragments is shown below for both parents.

three or four offspring as shown in Fig. 1. For this analysis, paternal and maternal fragments of identical size due to band sharing were not included, as they are not informative. For each segregating fragment which was clearly inherited either from father or mother, we determined the distribution pattern within the offspring. Individual distribution patterns were taken as evidence for independent loci, whereas identical segregation patterns were assigned to the same or a closely linked locus. Using this approach in two unrelated families with 4 children, we found in both evidence for at least 9 independent loci which are recognized by MZ 1.3. In another family with three children (Fig. 1, Fam. II.), five independently segregating fragments were identified.

An example is given in Fig. 2, which represents a schematic interpretation of the informative fragments of family I.(Fig. 1). in Fig. 1. Twelve paternal and eight maternal fragments are informative in this family. Parental fragments of identical size are not shown. In the father, nine different segregation patterns are found and six in the mother. Patterns 2 and 8 into the father and 4 and 5 in the mother are represented at least twice suggesting that these are either linked or could be split in additional patterns by studying larger pedigrees. This method does not allow, however, to distinguish clearly between independent and allelic fragments. On the other hand, it provides a simple way to estimate the number of informative loci represented in a DNA fingerprint analysis.

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