PATERNITY TESTING IN ITALY USING MINISATELLITE VARIANT REPEAT (MVR)

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MVR-PCR provides an innovative and highly discriminating technique to assess individual variation in human DNA (1). Using MVR specific amplimers to amplify the D1S8 (MS32) locus, a ladder of PCR products corresponding to different classes of repeat units, a-type and t-type are generated to produce a diploid digital code derived from the superimposed maps of specific alleles (1). MVR-PCR can be used in pedigrees examination, forensic analysis and population studies (1,3,4).
We have analysed 23 Italian cases of disputed paternity and compared the results founded by MVR technique using those obtained with a set of mini/microsatellite markers (APOB, HLADQa, YNH24, 3' HVR, D5S127, D1S80) (5).

MATERIALS AND METHODS

Peripheral lymphocytes DNA was extracted according to standard protocols. About 200 ng of genomic DNA was amplified using MVR specific amplimers (32-OR, TAG, 32-TAG-A, 32-TAG-T) (1). Reactions were cycled for 1.2 min at 94°C, 1 min at 66°C and 2 min at 70°C for 19 cycles on a DNA Themal Cycler (Perkin-Elmer Cetus). Aliquots of 14μl were subjected to electrophoresis on agarose-gel (1.5% w/v) for approximately 14 hours. DNA was transferred to a nylon-membrane by Southern blotting, and hybridized with a ^{32}P MS32 probe (insert). The MVR code defined by a-type and t-type alleles was established by a computer program designed by Cellmark Diagnostics (U.K.)

RESULTS AND DISCUSSION

An average of 40 repeat units were resolved in each examined individual (Fig. 1). It was found that 1.7% of repeat units scored from 92 separated alleles were "null "or 0-type repeats, compared to 70.20% a-type repeats, and 28.10% t-type repeats. The diploid code 1(aa), 2(tt), 3(at), 4(a0), 5 (t0), and 6(00) was used for parentage testing. Fourteen exclusions and 9 attributions probated by mini/microsatellite analysis were confirmed without ambiguity, with a mean of 6.8 exclusions in the first 35 repeats. Biostatistical values of paternity calculated with traditional DNA markers (range 98 to 99.45%) increased significantly (>99.999%) using the MVR test in the parentage analysis.

Personal experience proves higher efficiency of the single MVR assay compared to traditional mini/microsatellite DNA analysis for testing genetic relationships.

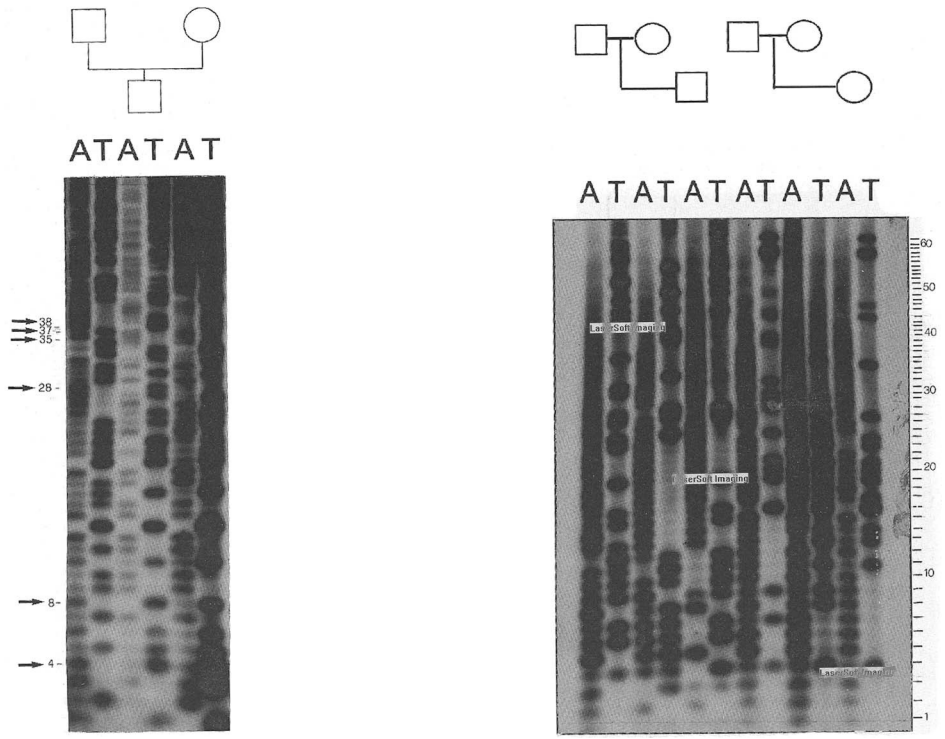

Fig.1. Examples of minisatellite repeat coding by MVR-PCR on total genomic DNA prepared from nuclear families for paternity testing.

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