ANALYSIS OF D1S80 (pMCT 118) LOCUS POLYMORPHISM IN AN ITALIAN POPULATION SAMPLE BY THE POLYMERASE CHAIN REACTION

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INTRODUCTION

The D1S80 locus, identified by the probe pMCT118, is suitable for forensic haemogenetic purposes because of its wide polymorphism, the high rate of heterozygosity and the easiness and speed of the analysis method (Nakamura et al. 1988; Kasai et al.1990; Budowle et al. 1991).
Further studies are necessary to increase the number of observations, to check geographic distribution and possible genetic variations among various populations, and to make a data-base for practical application in paternity testing and individual identification. For these reasons and because of the lack of data on Italians, we studied a suitable sample of subjects living in Ancona (Central Italy).

MATERIALS AND METHODS

Whole blood collected from125 unrelated healthy subjects was stored in EDTA microcentrifuge tubes at -70° C until use.
The DNA extraction was performed by the phenol-isoamyl method described by Budowle and Baechtel (1990).
Amplification was carried out in a DNA Amplifier (Violet) with 30 cycles consisting of 1 min at 95° C for denaturation, 80 sec at 65° C for primer annealing and 7 min at 70° C for extension. The reaction mix was made up as recommended by Perkin Elmer Cetus with minor modifications in a final volume of 50 microliters. The amount of DNA sample we used was 0.2 micrograms. The amplification was performed using the primers described by Kasai et al. (2):
1) 5'GAAACTGGGCTCCAAACACTGCCCGCCG3'
2) 5'GTCTTGTTGGAGATGCACGTGCCCCTTGC3'
Electrophoresis of 10 microliters of the amplified product with 2 microliters of loading buffer (0.25 % bromophenol blue, 0.25 % xylene cyanol FF, 30% glycerol in water) was achieved on 8% polyacrylamide gel (90 min at 150 V), visualizing the bands with ethidium bromide under UV light at 300 nm. (Fig. 1). The gel image, recorded by a video camera, was fed into a computer (Macintosh IIx) using a digitizing card (Color Capture). After image processing and enhancement, the size of alleles was calculated by comparing the band mobilities with those of a marker (PGEM-Promega) using the local reciprocal method of Elder and Southern (1983).

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RESULTS AND DISCUSSION

In our study we have found fourteen alleles ranging in size from ≈ 385 to ≈ 600 bp.
Allele distribution is represented in Fig. 2. The most frequent was the 467 bp allele, which corresponds to the 20 repeats in the classification of Kasai et al. The other most frequent alleles were the 21, 22 and 16 repeats. Compared with previous works we have a remarkably different distribution of the alleles and the lack of those of bigger size.
The observed heterozygosity was 79.2 %. The number of observed homozygotes was greater than expected, perhaps due to a less efficient amplification for the largest alleles. Alleles differing by only one repeat occasionally appeared as homozygotes, but they were conveniently separated after rerunning in more concentrated gel. When the attribution between similar alleles was in doubt, we reran the samples in neighboring lanes.
Finally, extra bands were occasionally present, but they have not caused any difficulties in typing the MCT region because they were located in a far position, near 2kb.

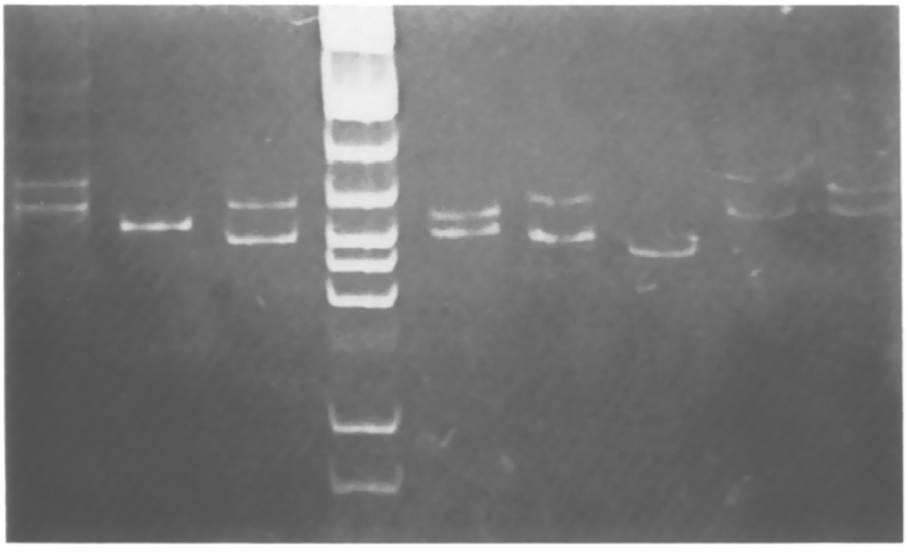

FIGURE 1. Ethidium-bromide stained polyacrylamide gel observed under UV at 300 nm after electrophoresis of 10 microliters of PCR-amplified products of D1S80 locus. From left to right: lane 1: 22/26; lane 2: 20/20; lane 3: 18/22; lane 4: pGEM Marker; lane 5: 19/21; lane 6: 18/23; lane 7: 16/16; lane 8: 21/27; lane 9: 21/24

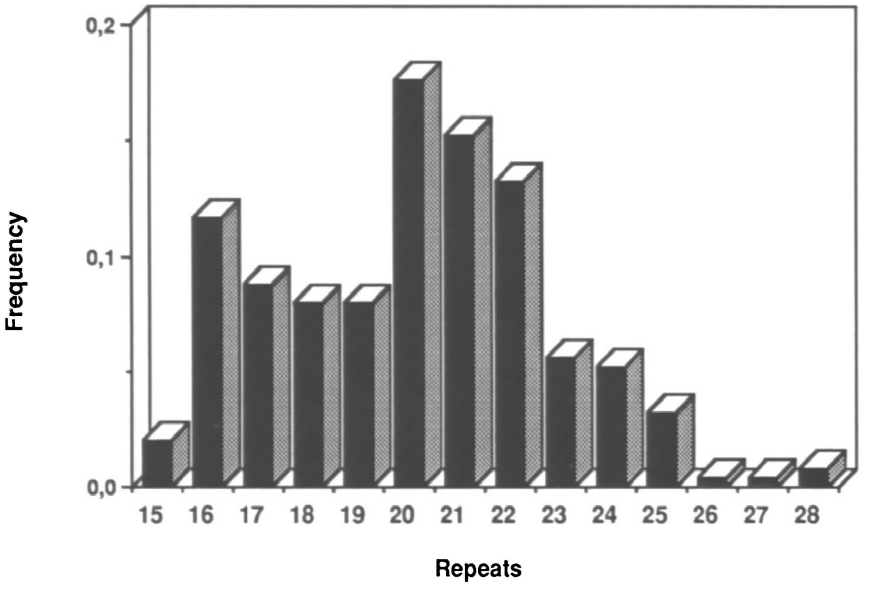

FIGURE 2. Distribution of MCT118 alleles among 125 unrelated Italians

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