CHARACTERIZATION OF THREE MICROSATELLITES AND THEIR APPLICATION IN PATERNITY TESTING.

E. Molteni, F. Laface*, S. Sirchia*, G. Colucci*.
Laboratorio di Biologia Molecolare, Clonit S.p.A.; * Fondazione Centro Studi di Patologia Molecolare Applicata alla Clinica, Milano, Italy.

Introduction

The recent identification of hypervariable sequences interspersed within the human genome has provided new genetic markers useful for the localization of disease genes as well as for biological typing (1).
In previous studies aimed at identifying the parental origin of the extrachromosome found in cases of placental mosaicism, we successfully used several chromosome-specific microsatellites with high polymorphism information content (PIC).
To evaluate their efficacy in paternity testing we selected two CA repeats, D8S85 and D8S88, and one tetranucleotide repeat, D7S460, on the basis of their reported heterozigosity (2, 3). We analyzed their allele distribution and frequency in an italian population and compared it with that available.

Materials and methods

We have collected blood samples from 50 unrelated italian donors. DNA was isolated by standard phenol/chloroform extraction after proteolytic digestion of the cell pellet. 100 to 500 ng of DNA was processed by PCR-mediated amplification using primers specific for D8S85, D8S88 and D7S460. Primer sequences and amplification profiles are illustrated in table 1.
After analyzing amplification products by agarose gel electrophoresis, allelic variants were characterized on a 10-12% non denaturing polyacrilamide gel and subsequently on a modified DGGE based on a 10% polyacrylamide denaturing gel immersed in circulating buffer kept at 60°C.
Allele size was determined by comparison with molecular weight markers, including 10 bp and 100 bp ladders (GIBCO BRL) as well as with an allelic mix previously characterized and provided by J. L. Weber.
Previous reports indicated an heterozigosity of 0.83 for D8S88, 0.75 for D8S85 and 0.95 for D7S460 (2,3). Allele distribution, estimated on a caucasic population, was previously reported only for D8S85 and D8S88 (2).

Results and discussion

Using the modified DGGE, we were able to separate the allelic variants of the highly polymorphic microsatellites and calculate their distribution on 50 italian, unrelated donors (Figures 1-3). Comparing our frequencies to those estimated by Weber et al. (2) on 58 CEPH individuals, we observed significant differencies for D8S88 and D8S85 (Figures 1, 2).

Moreover, we detected an additional 77 bp allele at D8S85 and a 94 bp allele at D8S88. Of the three markers, D8S88 has an high number of alleles with low frequencies and therefore appears as the most informative and potentially useful. On the other hand, being a tetranucleotide, D7S460 was the easiest to resolve and analyse by gel electrophoresis.
The high heterozigosity and potential informativeness of these markers prompted us to evaluate their applicability to paternity testing. In ten cases of mother/father/child trios we observed concordant results with those obtained using three well characterized VNTR, namely D1S80, Apo B and COL21A. These data indicate that D8S85, D8S88 and D7S460 can be valuable, additional markers of biological identity useful for paternity disputes and other forensic medicine applications.

References

1) Fregeau C. J. et al. Biotechniques 15:100, 1993.
2) Weber J. L. et al. Nucleic Acids Res. 18:4038, 1990.
3) Hudson T. J. et al. Genomics 13:622, 1992.

Tables and figures

LOCUS	PRIMERS (5'-3')	CICLES	AMPLIFICATION CONDITIONS
D8S85	AGCTATCATCACCCTATAAAAT AGTTTAACCATGTCTCTCCCG	27	94°C 1 min 55°C 2 min 72°C 2 min
D8S88	TCCAGCAGAGAAAGGGTTAT GGCAAAGAGAACTCATCAGA	27	94°C 1 min 55°C 2 min 72°C 2 min
D7S460	AATACCCCAAGGGGTGGTAA CATTGATGAACAGTTCAAGCA	30	94°C 1 min 55°C 2 min 72°C 2 min

Table 1: Primer sequences and amplification conditions.

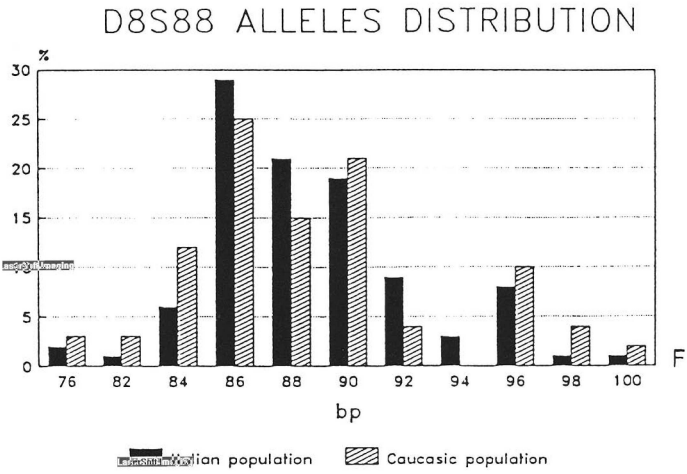

FIGURE 1

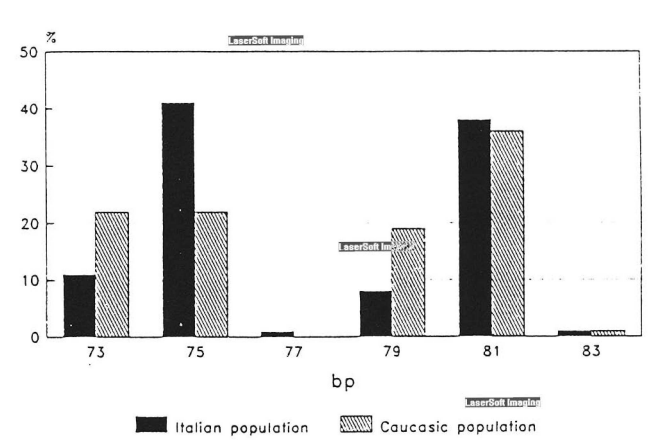

FIGURE 2

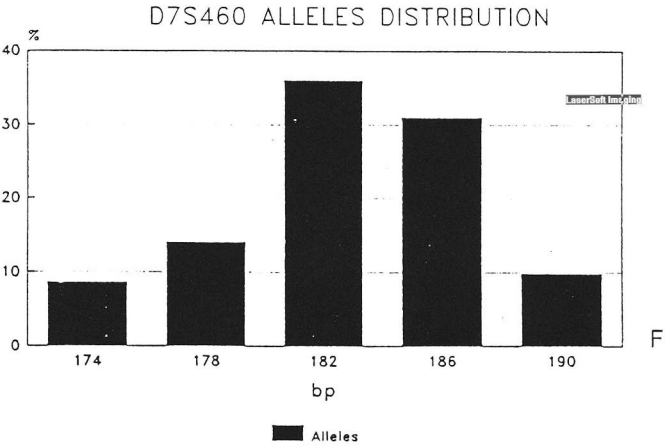

FIGURE 3