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TECHNICAL NOTE

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A *Hinf*I polymorphism in the 5′ flanking region of the human VNTR locus D1S80

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Abstract A restriction fragment length polymorphism (RFLP) characterized by the presence (*Hinf*I+) or absence (*Hinf*I–) of a *Hinf*I site has been found in the 5′ flanking region of the VNTR locus D1S80. RFLP-allele frequencies were determined from 82 unrelated individuals: *Hinf*I+ = 0.49, *Hinf*I– = 0.51. The RFLP/VNTR haplotype frequencies show an absolute association between the *Hinf*I+ allele and the VNTR allele of 18 repeat units and an extreme association between the *Hinf*I– allele and the VNTR allele of 24 repeat units. The remaining VNTR alleles associate more randomly with the 2 flanking *Hinf*I alleles.

Key words D1S80 locus · PCR · *Hinf*I polymorphism · RFLP allele frequencies · RFLP/VNTR haplotype frequencies

Introduction

Variable number of tandem repeats (VNTRs) loci are highly informative markers for linkage analysis and identity testing. D1S80 is a highly polymorphic, small-sized (300–900 bp) VNTR locus (repeat unit: 16 bp; core sequence: GNNGTGGG) located on chromosome 1p and amenable to analysis by the polymerase chain reaction (PCR) (Nakamura et al. 1988; Kasai et al. 1990), that has become a well defined genetic marker for routine use in forensic identification and paternity testing (Budowle et al. 1991; Sajantila et al. 1992; Kloosterman et al. 1993).

In this study, we describe a biallelic *Hinf*I+/*Hinf*I– polymorphism in the 5′ flanking region of the D1S80 locus. The haplotype frequencies determined in a spanish population study showed an extreme association between the *Hinf*I+ allele and the VNTR allele of 18 repeat units and between the *Hinf*I– allele and the VNTR allele of 24 repeat units.

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Materials and methods

EDTA blood samples were collected from 82 unrelated spanish individuals and 45 individuals from 15 mother-father-child trios. The DNA was extracted by the standard phenol-chloroform extraction procedure.

PCR amplification of D1S80 was performed using the primer sequences (Forward primer: 5′-GAAACTGGCCTCCAAACACT-GCCCGCCG-3′; Reverse primer: 5′-GTCTTGTTGGAGATGC-ACGTGCCCCTTGC-3′) and PCR time-temperature profiles previously described (Budowle et al. 1991).

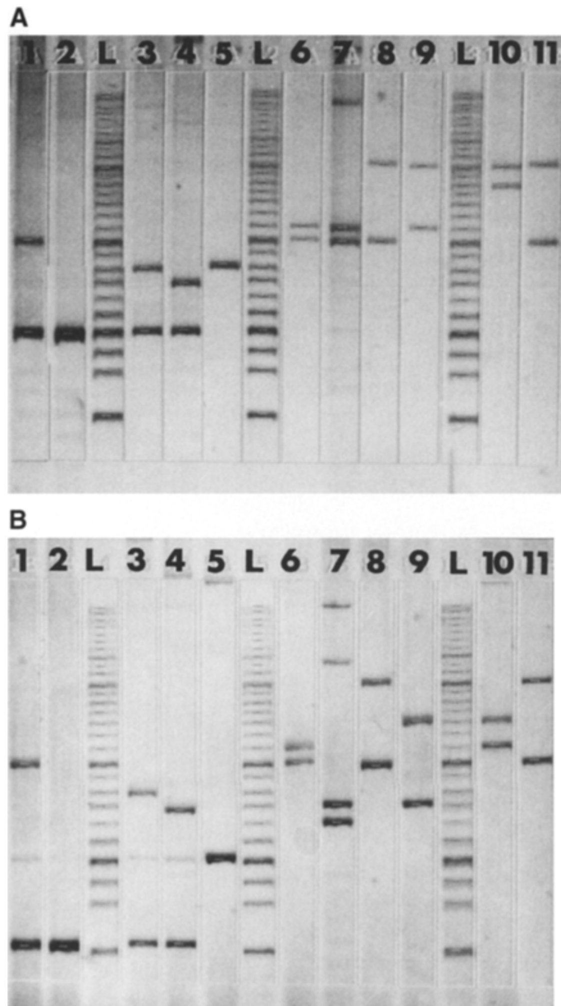
PCR products were analyzed by vertical discontinuous (Tris-glycine/Tris-chloride) polyacrylamide gel electrophoresis followed by silver staining (Alonso et al. 1994) and the VNTR alleles were classified by comparison with a commercial ladder consisting of 27 alleles (allele 14 and alleles 16–41) (Perkin-Elmer).

Restriction of the PCR products was performed in a reaction volume of 15 μl containing 30–40 U *Hinf*I and 1 × restriction buffer (50 m*M* Tris-HCl, 10 m*M* $MgCl_2$, 100 m*M* NaCl and 1 m*M* DTE) overnight at 37° C. The restriction fragments were also separated by discontinuous polyacrylamide gel electrophoresis. The band size comparison between undigested and *Hinf*I-restricted PCR products was performed on polyacrylamide gels by means of a image analyzer (Bioimage, Millipore) using the 27-allele ladder as the size standard.

The localization of the *Hinf*I restriction site polymorphism was performed by agarose (2% Nusieve, FMC) gel electrophoresis in TBE buffer, southern blotting, hybridization with the forward primer conjugated with alkaline phosphatase and chemiluminscent detection.

Results and discussion

Restriction of the PCR-amplified D1S80 VNTR alleles with *Hinf*I identified a biallelic restriction fragment length polymorphism (RFLP) characterized by the presence (*Hinf*I+) or the absence (*Hinf*I–) of a *Hinf*I site (Fig. 1). The *Hinf*I restriction site has been located in the 5′ flanking region by southern blot analysis of the *Hinf*I+ alleles, before and after restriction, using the forward primer con-



◀ **Fig. 1 A, B** D1S80 profiles analyzed by PCR and subsequent discontinuous polyacrylamide gel electrophoresis followed by silver stain. **A** Undigested samples. **B** *Hinf*I restricted samples. (1) 24 – 18 +; (2) 18 + 18 +; (3) 22 – 18 +; (4) 21 – 18 +; (5) 22 + 22 +; (6) 24 – 25 –; (7) 24 + 25 +; (8) 24 – 31 –; (9(25 + 31 +; (10) 29 + 31 +; (11) 24 – 31 –; L allelic ladder

jugated with alkaline phosphatase as the hybridization probe. Only the undigested alleles were detected on the autoradiographs (results not shown). On the other hand, the band size analysis of the restricted *Hinf*I+ alleles revealed the loss of a 60 bp (± 2 bp) constant fragment in comparison to the undigested alleles.

Taking into account these results and the nucleotide sequence of the D1S80 5′ flanking region determined by Kasai et al. (1990), it could be predicted that the presence of the *Hinf*I restriction site could be determined by a C to T transition at the 61 nucleotide from the 5′ end of the forward primer.

RFLP allele frequencies were determined from 164 chromosomes of 82 unrelated spanish individuals: *Hinf*I+ = 0.49; *Hinf*I– = 0.51. The observed heterozygosity was 0.50. The population sample was in agreement with the expections of the Hardy-Weinberg equilibrium ($\chi^2 = 0.009$; df = 1; $p = 0.90$). Codominant Mendelian inheritance was demonstrated in 15 father-mother-child trios.

Figure 2 shows the RFLP/VNTR haplotype frequencies of the D1S80 locus estimated from 164 chromosomes. As can be seen, an absolute association has been found between the *Hinf*I+ allele and the allele of 18 repeat units and an almost absolute association (only one exception of 55 alleles analyzed) between the *Hinf*I– allele and the allele of 24 repeat units, while the remaining alleles associate more randomly with the two flanking *Hinf*I alleles. However, the sample size should be enlarged in order to perform statistical analysis to evaluate the magnitude of the linkage disequilibrium observed be-

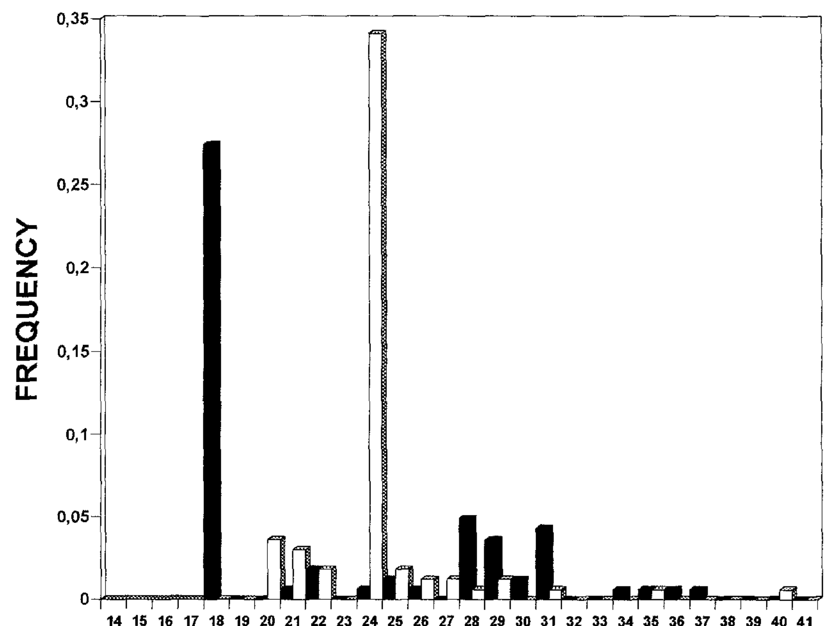


Fig. 2 RFLP/VNTR haplotype frequencies of the D1580 locus estimated from 82 unrelated spanish individuals. (■) *Hinf*I +/VNTR haplotypes; (□) *Hinf*I –/VNTR haplotypes

tween both loci. Further research is also necessary to explain the extreme disequilibrium in the frequencies of the *Hinf*I+/18 and *Hinf*I–/24 haplotypes observed in this study, and especially to evaluate the possible role of genetic drift in determining this disequilibrium.

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