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Amplified fragment length polymorphism analysis of the VNTR locus D1S80 in central Spain

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Summary. The polymorphism of the D1S80 locus has been analyzed in a population sample of 203 unrelated individuals living in Madrid (central Spain) by PCR and subsequent semi-dry discontinuous polyacrylamide gel electrophoresis (Tris-chloride/Tris-glycine buffer system) followed by silver staining. The electrophoretic system described in this study offers high resolution in the separation of the different D1S80 alleles allowing the detection of microvariability around the allele T22 in the spanish population. Twenty different alleles containing 17–40 repeats of the basic 16 bp unit were distinguished. The alleles T18 and T24 were found to be relatively common in Spain, as in other populations, with frequencies of 0.224 and 0.372, respectively. No evidence of significant deviations from Hardy-Weinberg equilibrium was found in these preliminary population data.

Key words: D1S80 locus – PCR – Discontinuous PAGE – Allele frequencies – Hardy-Weinberg equilibrium

Zusammenfassung. Der Polymorphismus des Locus D1S80 wurde in einer Bevölkerungsstichprobe von 203 unverwandten, in Madrid lebenden Personen untersucht. Die Untersuchung erfolgte mit Hilfe der PCR und nachfolgender, halbtrockener diskontinuierlicher Polyacrylamidgel-Elektrophorese (Tris-chlorid/Tris-glycin-Puffersystem) mit nachfolgender Silberfärbung. Das beschriebene elektrophoretische System dieser Untersuchung bietet eine hohe Auflösung in der Auftrennung der verschiedenen D1S80-Allele und erlaubt den Nachweis einer Mikrovariabilität um das Allel T22 in der spanischen Bevölkerung, welche zwischen 17 und 40 Wiederholungseinheiten der zugrunde liegenden 16 Bp-Einheit enthalten. Die Allele T18 and T24 wurden als relativ häufig in Spanien gefunden, wie auch in anderen Populationen, mit Frequenzen von 0,224 bzw. 0,372. Es fand sich kein Beweis für signifikante Abweichungen vom Hardy-Weinberg-Gleichgewicht in dieser vorläufigen Populationsstudie.

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Schlüsselwörter: D1S80-Locus – PCR – diskontinuierliche PAGE – Allelfrequenzen – Hardy-Weinberg-Gleichgewicht

Introduction

Amplified fragment length polymorphism (AMP-FLP) technology has proved to be a valuable method for identitiy testing (Boerwinkle et al. 1989; Ludwig et al. 1989; Horn et al. 1989; Kasai et al. 1990; Budowle et al. 1991). The analysis of relatively small-sized variable number of tandem repeats (VNTRs) loci by the polymerase chain reaction (PCR) followed by electrophoretic separation of the amplified DNA fragments, offers 2 obvious advantages over the classical restriction fragment length polymorphism (RFLP) analysis via Southern blot. Firstly, PCR enables the rapid analysis of samples containing very small amounts and even degraded DNA. Secondly, AMP-FLP analysis permits the resolution of the different alleles associated with the VNTR locus under investigation into discrete entities.

For forensic purposes, the AMP-FLP analysis has been centered on 4 VNTRs loci: D1S80 (Kasai et al. 1990; Budowle et al. 1991), ApoB (Boerwinkle et al. 1989; Ludwig et al. 1989), D17S30 (Wolff et al. 1988; Horn et al. 1989) and COL2A1 (Wu et al. 1990; Priestley et al. 1990) and more recently on short tandem repeats (STRs) loci (Caskey and Hammond 1992). However, for the majority of these loci, data on genotype distribution and allele frequencies are scarce.

In this report, we present preliminary allelic data for the D1S80 locus in a population sample of 203 unrelated individuals living in Madrid (central Spain). We also describe a semi-dry discontinuous polyacrylamide gel electrophoresis method based on the Tris-glycine/Tris-chloride buffer system (Ornstein 1964; Davis 1964) that offers high resolution in the separation of the different D1S80 alleles.

Materials and methods

Samples. Whole blood was collected in EDTA tubes by venipuncture from 203 unrelated individuals living in Madrid and DNA was extracted by the standard phenol-chloroform extraction procedure (Smith et al. 1989).

Amplification. Amplification of D1S80 was performed with the following primer sequences (Budowle et al. 1991) and conditions:

5′-GAA ACT GGC CTC CAA ACA CTG CCC GCC G-3′
5′-GTC TTG TTG GAG ATG CAC GTG CCC CTT-3′

Temperature: 94/65/72°C
Time: 1/1/1 min.
Cycles: 30 (after the last cycle an additional extension at 72°C for 10 min was performed)

Each amplification sample contained 20–100 ng of genomic DNA, 10 m*M* Tris-HCl, pH 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatine, 2.5 units of Amplitaq (Perkin-Elmer Cetus), 1 μ*M* of each primer, and 200 μ*M* of each dNTP. Each sample was overlaid with 50 μl of mineral oil. The PCR was carried out in a Perkin-Elmer Thermocycler. After amplification, the mineral oil was removed and the samples were stored at −80°C prior to electrophoretic analysis.

Electrophoresis: A semi-dry discontinuous polyacrylamide gel electrophoresis system was employed for the separation of the D1S80 amplified fragments. Ultrathin-layer polyacrylamide gels (5.5% T, 3% C; 400 μm thick) were prepared by mixing the required amounts of acrylamide and piperazine diacrylamide as cross-linker (Budowle et al. 1991) in a solution containing 0.375 *M* Tris-Chloride, pH 8.8 (molarity in respect to Tris). Gels were cast onto Gelbond PAG film (FMC) in the presence of N,N,N′,N′-tetramethylethylenediamine (TEMED) and ammonium persulfate at 45°C for 25 min. The electrode buffer, supplied to the gel from buffer strips (1 × 1 cm) made of 2% agarose, was 0.250 *M* Tris-glycine, pH 8.8 (Olsson and Stalberg 1986). The gel was placed on the cooling plate of the flat bed apparatus (FBE 3000, Pharmacia-LKB) with a drop of water beneath to promote heat transfer. Agarose strips were placed on the top of the gel, one at each end, with a small drop of 0.1% bromophenol blue placed at the border of the gel and the cathodal strip. Amplified samples were applied directly onto the gel surface 1 cm from the cathode by means of fiberglass applicator tabs (Pharmacia-LKB). The platinized titanium electrodes (flat bed apparatus FBE 3000, Pharmacia-LKB) were then placed on top of the agarose strips with an interelectrode distance of 10 cm. Electrophoresis was carried out at constant current (15 mA) with a continuously increasing voltage from 70–400 V during the run. When the bromophenol blue had migrated 1 cm from the sample application point the applicator tabs were removed and the run was then continued until the bromophenol blue reached the anode buffer strip.

Silver stain. Following electrophoresis the DNA fragments were silver stained according to the silver nitrate method developed by Blum et al. (1987), utilizing sodium thiosulfate as image enhancer and background reducer except that the fixation step was carried out in a solution containing 40% ethanol and 12% acetic acid.

Results and discussion

Figure 1 shows 12 D1S80 profiles displaying 17 alleles as analyzed by PCR and subsequent semi-dry discontinuous polyacrylamide gel electrophoresis (Tris-chloride/Tris-glycine buffer system) followed by silver stain. The alleles were classified by comparison with a ladder consisting of 15 alleles (Perkin-Elmer-Cetus) and were designated according to the number of repeat units. As can be seen, the resolution obtained with the Tris-chloride/Tris-glycine buffer system could clearly distinguish alleles differing by only one repeat unit (16 bp) in length and is comparable with the resolution obtained with the Tris-sulfate/Tris-borate buffer system developed by Allen et al. (1989).

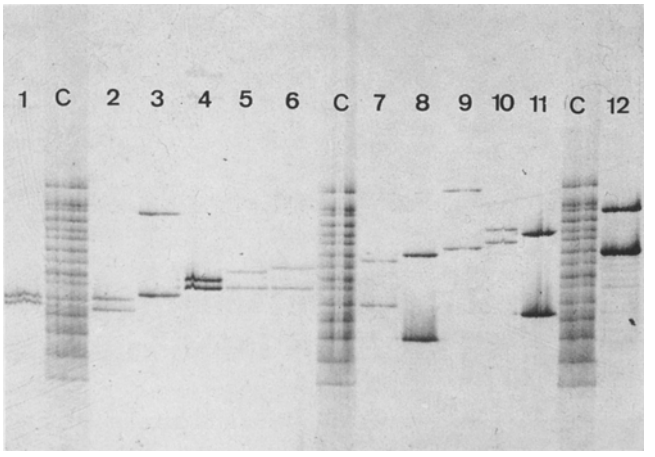

Fig. 1. D1S80 profiles analyzed by PCR and subsequent semi-dry discontinuous polyacrylamide gel electrophoresis (Tris-chloride/Tris-glycine buffer system) followed by silver stain. Samples: (1) T21T22, (C) Allelic ladder, (2) T21T22, (3) T23T36, (4) T24T25, (5) T24T26, (6) T24T27, (C) Allelic ladder, (7) T22T28, (8) T18T29, (9) T30T40, (10) T31T33, (11) T21T32, (C) Allelic ladder, and (12) T29T36. Allelic ladder: T14 T16 T18 T22 T24 T25 T27 T29 T31 T32 T43 T36 T37 T40

The resolution obtained with the electrophoretic system used in this study has also allowed the detection of microvariability around the allele T22 in the spanish population. In lanes 1 and 2 of Fig. 1, both samples are generically designated T21T22. However, the allele T22 in sample 1 aligns with the allele T22 of the allelic ladder, while the allele T22 in sample 2 is slightly cathodal. This phenomenon, as has been previously described (Budowle et al. 1992), could be due to sequence variation or slight differences in the size of alleles. Regardless of the extra variability, alleles were placed into bins such that it reflects the basic repeat unit.

The distribution of phenotypes and allelic frequencies for D1S80 in a population sample of 203 unrelated individuals living in Madrid (central Spain) are shown in Table 1 and Fig. 2, respectively. A total of 20 different alleles in 51 different genotypes was observed. The most common alleles detected are those with 18 and 24 repeat units. Consequently, the most common genotypes observed were the combinations of these 2 alleles, e.g., T18T18 (frequency: 0.064), T18T24 (frequency: 0.172), and T24T24 (frequency: 0.118). All other alleles show frequencies of less than 0.100. The observed heterozygosity was 80.29%.

Since the resolution of D1S80 alleles is discrete testing for deviations from Hardy-Weinberg equilibrium, which is a basic population genetic premise for the application of genetic markers in forensic haemogenetics, can be applied. However, due to the high degree of poly-

Table 1. Distribution of D1S80 genotypes from 203 unrelated individuals living in Madrid (central Spain)

Genotype	Number observed	Genotype	Number observed
T17T18	1	T22T29	4
T17T24	1	T22T36	1
T18	13	T23T25	1
T18T20	4	T23T29	1
T18T21	5	T23T36	1
T18T22	2	T24	24
T18T24	35	T24T25	12
T18T25	4	T24T26	6
T18T29	4	T24T27	3
T18T31	5	T24T28	6
T18T32	1	T24T29	11
T18T34	1	T24T31	13
T18T37	2	T24T32	1
T18T40	1	T24T36	1
T20T21	1	T24T37	2
T20T24	4	T25T29	2
T20T25	1	T25T37	1
T21	1	T26T30	1
T21T22	2	T27T31	1
T21T23	1	T27T32	1
T21T24	3	T29	2
T21T29	1	T29T31	2
T21T31	1	T29T36	1
T21T32	3	T30T40	1
T22T24	5	T31T33	1
T22T28	1		

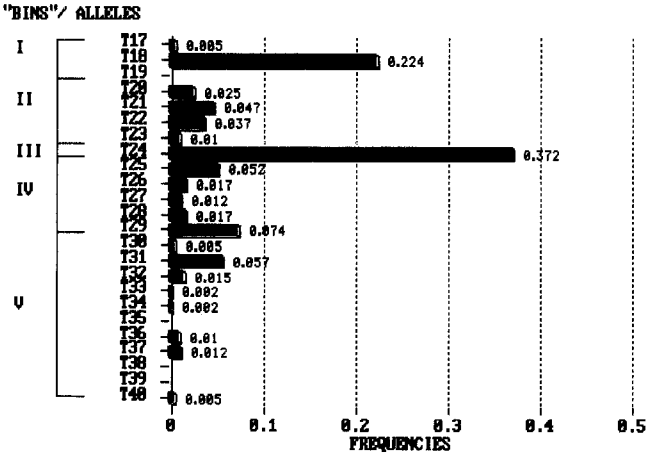

Fig. 2. D1S80 Allele frequencies from 203 unrelated individuals living in Madrid (central Spain). I–V indicate the bins of alleles used to estimate the Hardy-Weinberg equilibrium

morphism that the D1S80 locus displays and the relatively small population sample analyzed, a reliable estimation of deviations from Hardy-Weinberg equilibrium is not possible. In spite of this, the goodness of fit of the distribution of D1S80 genotypes in this preliminary Spanish population data has been carried out using a 5-allele model (15 phenotype classes) following the "allele binning" strategy suggested by Brenner and Morris (1990). The groups of alleles established under the 5-allele model are shown in Fig. 2. No evidence of deviations from the Hardy-Weinberg equilibrium was observed when the numbers of the 15 observed and expected phenotype classes were compared by means of the chi^2 test ($\chi^2 = 16.25$; $df = 10$; $P = 0.093$).

A qualitative comparison of the D1S80 allelic data presented here with those reported in Germany (Rand et al. 1992), Finland (Sajantila et al. 1992) and USA (Budowle et al. 1991) shows that the distribution of alleles in these 4 population samples is bimodal with two peaks at the alleles T18 (with frequencies ranging from 0.307 in Finland to 0.224 in Spain) and T24 (with frequencies ranging from 0.414 in Germany to 0.311 in Finland). The remaining alleles show frequencies of less than 0.100 in all these 4 population samples. It is necessary to analyze larger sample sizes to know if the small differences observed in the distribution of low frequency alleles in these 4 populations are population specific or due to sample size.

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