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Genetic variation of microsatellite markers D1S117, D6S89, D11S35, APOC2, and D21S168 in the Spanish population

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Summary. We have used PCR amplification to analyse the allele frequency, distribution and heterozygosity of 5 microsatellite markers (D1S117, D6S89, D11S35, APOC2, and D21S168), in a sample of 100 unrelated Spanish individuals. The loci tested exhibit wide allelic variability having 7–17 alleles, PIC (polymorphic information content) between 0.79 and 0.86, and heterozygosity between 0.81 and 0.86. D1S117 and D21S168 have unimodal distribution, APOC2 has 4 common alleles which account for 71% of the total variation, D11S35 has a bimodal distribution and D6S89 is trimodal. The allelic distribution observed for each locus is in agreement with slippage and mispairing as the main mechanisms involved in the evolution of microsatellite alleles. Multiplex amplification of loci D6S89 and APOC2 was possible due to their non-overlapping allele sizes. The rapidity with which microsatellites can be analysed, and the accurate determination of alleles, make these markers very powerful tools for genetic typing. The information obtained for loci D1S117, D6S89, D11S35, APOC2, and D21S168, provides a basis for their use for DNA typing and paternity analysis in the Spanish population.

Key words: Microsatellites – VNTR – Paternity testing – Polymorphisms

Zusammenfassung. Wir haben die PCR-Amplifikation angewandt, um die Allel-Häufigkeit, die Allel-Verteilung und die Heterozygotie bei 5 Mikrosatelliten-Markern (D1S117, D6S89, D11S25, APOC2 and D21S168) in einer Stichprobe von 100 unverwandten spanischen Personen zu untersuchen. Die untersuchten Loci zeigen eine breite Allel-Variabilität mit Häufigkeiten zwischen 7 und 17 Allelen, einem PIC (polymorpher Informationsgehalt) zwischen 0,79 und 0,86 und einer Heterozygotie-Rate zwischen 0,81 and 0,86. D1S117 und D21S168 haben eine unimodale Verteilung, APOC2 hat vier häufige Allele, welche 71% der gesamten Variation ausmachen, D11S35 hat eine bimodale Verteilung und D6S89 ist trimodal. Die Allel-Verteilung, wie sie für jeden Locus beobachtet wurde, stimmt mit der Annahme überein, daß „Slippage" und Fehlpaarung die Hauptmechanismen sind, welche in der Evolution der Mikrosatelliten-Allele involviert sind. Die Multiplex-Amplifikation der Loci D6S89 und APOC2 war möglich aufgrund ihrer nicht-überlappenden Allel-Größen. Die Geschwindigkeit, mit welcher die Mikrosatelliten analysiert werden können und die genaue Bestimmung der Allele machen diese Marker zu mächtigen Werkzeugen für genetische Typisierung. Die Informationen, welche für die Loci D1S117, D6S89, D11S35, APOC2 und D21S168 erhalten wird, schafft eine Basis für ihre Anwendung bei der DNA-Typisierung und bei der Vaterschaftsanalyse in der spanischen Bevölkerung.

Schlüsselwörter: Mikrosatelliten – VNTR – Vaterschaftsuntersuchung – Polymorphismen

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Introduction

Tandem repeat DNA sequences exhibit variation in the number of repeat units. This length variability has been found for long repeats (Motulsky 1988), for sequences of between 6 and 80 base pairs (bp) known as minisatellites (Jeffreys et al. 1985) or VNTRs (variable number of tandem repeats) (Nakamura et al. 1987), and for short repeat sequences between 1 and 5 bp (Smeets et al. 1989; Tautz 1989; Weber and May 1989). These short tandem repeats (STRs), variable number of tandem/dinucleotide repeats (VNTRS/VNDRs), or microsatellites, have been found to be highly polymorphic (Weber and May 1989; Smeets et al. 1989; Litt and Luty 1989). These STR sequences are repetitions of dinucleotides (e.g. CA/GT, TC/AG, TA/AT), trinucleotides (e.g. TTA/AAT), or tetranucleotides (e.g. AATC/TTAG, GATC/CTAG) (Dryja et al. 1989; Litt and Luty 1989; Economou et al. 1990; Edwards et al. 1991).

Table 1. Polymorphic dinucleotide repeat loci analysed

Locus	Chromosome location	Repeat sequence	PCR primers
D1S117	1q23–q25.1	$(TG)_{21}(AG)_{10}$	5′-CCTTTTGCCTCCTTCGT 5′-CTCATTTACAATAGCTACC
D6S89	6p	$(AC)_{14}$	5′-CTTGTTCATCTGCCTTGTGC 5′-ACCTAAGCGACTGCCTAAAC
D11S35	11q22	$(GT)_{17}$	5′-ACAATTGGATTACTACTAGC 5′-TGTATTTGTATCGATTAACC
APOC2	19q12–q13.2	$(TG)_{21}(AG)_7$	5′-CATAGCGAGACTCCATCTCC 5′-GGGAGAGGGCAAAGATCGAT
D21S168	21q22.3	$(GT)_{19}$	5′-ATGCAATGTTATGTAGGCTG 5′-CGGCATCACAGTCTGATAAA

The CA/GT dinucleotide is one of the most commonly analysed microsatellite sequences. The molecular nature of these repeats has been classified into 3 groups: perfect, imperfect, and compound sequences, depending on the percentage of the CA/GT repeat core (Weber et al. 1990a, b). Heterozygosities for microsatellite loci are usually higher than 75% and, on average, they detect more than 6 alleles, making them excellent DNA markers for the diagnosis of genetic diseases.

Although VNTR markers are now being widely used for population genetics, including personal identification and forensics (Budowle et al. 1991), they have several limitations due to the difficult determination of allele sizes by Southern blotting, and the amplifications of large fragments for PCR (Jeffreys et al. 1988). Such problems do not seem to occur with microsatellites, as allele determination is performed at the sequence level.

We have used PCR to analyse the allele frequency distribution and heterozygosity of 5 microsatellite markers, located on chromosomes 1 (D1S117), 6 (D6S89), 11 (D11S35), 19(APOC2), and 21 (D21S168), in a sample of 100 unrelated Spanish individuals. The loci tested exhibit wide allelic variability having 7–17 alleles and heterozygosities higher than 80%. The allelic distribution obtained here, provides the basis for the use of these markers as tools for DNA typing and paternity analysis in the Spanish population.

Materials and methods

High molecular weight DNA was obtained from 10 ml of EDTA peripheral blood, from 100 unrelated healthy individuals from different parts of Spain. The subjects were the partners of patients analysed for different genetic disorders, and consent for genetic analysis was obtained.

The oligonucleotides flanking the repeat blocks used for PCR analysis (Saiki et al. 1988), were obtained using an Applied Biosystems 391 DNA Synthesiser. Table 1 lists the primer sequences used to amplify the 5 loci: D1S117 (Sharma and Litt 1991), D6S89 (Litt and Luty 1990), D11S35 (Litt et al. 1990), APOC2 (Weber and May 1989), and D21S168 (Guo et al. 1990).

For each locus PCR was performed using 200 ng of genomic DNA, 200 μ*M* each of dATP, dCTP, dGTP, and dTTP, 10 pmol of one primer, 1.5 pmol of the other primer, end-labelled with [γ-^{32}P]ATP, 50 m*M* KCl, 10 m*M* Tris (pH 8.3), 1.5 m*M* $MgCl_2$, and 1 unit of *Taq* DNA polymerase (Perkin-Elmer-Cetus) in a total volume of 25 μl. End-labelling of primers was performed as described (Morral et al. 1991) with 30 pmol of primer, 20 μCi [γ-^{32}P]ATP (3000 Ci/mmol) and 5 units of T4-polynucleotide kinase.

PCR was performed for 30 cycles, at 94°C for 15 s; annealing at 50°C for D11S35, 52°C for D1S117, 59°C for APOC2 and D21S168, and 63°C for D6S89, for 40 s; extension at 74°C for 1 min, utilising a Thermal Cycler (Perkin-Elmer-Cetus 9600). An initial incubation step at 94°C for 5 min, and a final incubation step at 74°C for 10 min were performed. A 2 μl aliquot of the reaction products was mixed with 2 μl formamide stop solution (U.S. Biochemical Corp.), loaded onto a 6% polyacrylamide gel, and electrophoresed at 1500 V for 2–4 h. The dried gels were exposed to radiographic films for periods ranging from 20 min to overnight.

Multiplex amplification of loci D6S89 and APOC2 was performed. In this case annealing was 59°C for 40 s, and elongation was 1 min at 74°C. End-labelling of both primers was performed in the same reaction. The size of the alleles was determined using size standards created by dideoxy sequencing ladders, using M13-mp18 as template.

The number of possible genotypes was calculated as follows: k being the number of alleles observed, and the number of possible genotypes being $k(k+1)/2$. Expected heterozygote (H) frequencies were calculated as follows:

$$H = n[1 - \sum_{j=1}^{K} (n_j/n)^2]/(n-1),$$

where $n_1, \ldots, n_k$ correspond to K alleles in a sample of n genes. Standard errors (SE) of allele frequencies were $\sqrt{f(1-f)/2N}$, and of heterozygosity H were $\sqrt{f(1-f)/N}$, where f is the frequency and N is the number of individuals sampled (Nei 1978; Chakraborty et al. 1988; Edwards et al. 1992). PIC (polymorphic information content) was calculated according to Botstein et al. (1980):

$$PIC = 1 - \sum_{1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 P_i^2 P_j^2$$

Results

To determine allelic variation for the 5 microsatellite markers in the Spanish population we amplified DNA from 100 individuals. Mendelian inheritance of alleles was established by the analysis of more than 30 meioses for each locus. Once individual genotypes were deter-

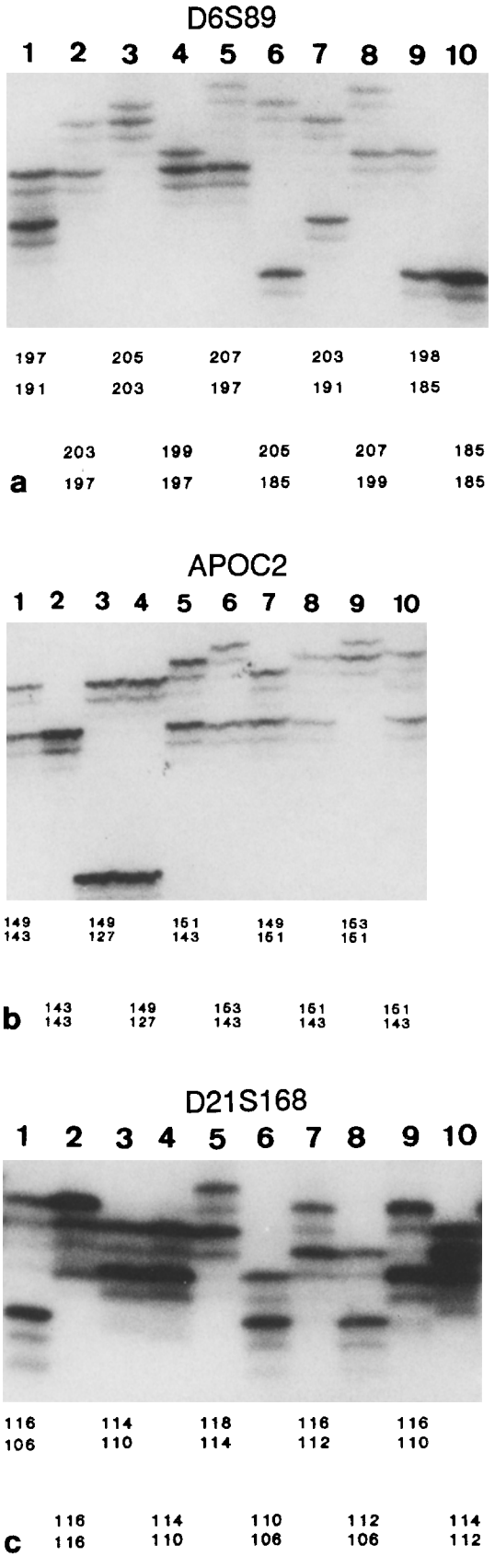

Fig. 1a–c. Analysis of PCR-amplified products containing microsatellite sequences at loci D6S89 **a**, APOC2 **b**, and D21S168 **c**. Lanes 1 to 10 are unrelated individuals. Genotypes for each locus are shown at the bottom of each figure, and are indicated as base pairs (bp)

mined for a wide range of alleles, these samples were then used as allele markers. Figure 1 shows the analysis of 10 unrelated individuals with 3 of the 5 loci analysed (D6S89, APOC2, and D21S168).

Figures 2–4 show the allele frequency distribution for the 5 loci analysed in this work. Both D1S117 (12 alleles) and D21S168 (7 alleles) (Fig. 2) have a unimodal distribution, with the most common alleles being 122 bp and 114 bp for D1S117 (0.34) and D21S168 (0.29), respectively.

The APOC2 locus has 4 common alleles which account for about 71% of the total variation, and which vary in size by one dinucleotide (Fig. 3).

The D11S35 locus has 8 alleles showing a bimodal distribution, each mode differing by 2–4 dinucleotides. The D6S89 locus has 12 alleles, the two commonest alleles being 197 bp and 185 bp (Fig. 4).

Allele frequencies, heterozygosity, calculated standard errors and PIC for each locus, and the previously described allelic distributions, are given in Tables 2–6. PICs were between 0.79 and 0.86, and expected heterozygosities were between 0.81 and 0.86. The population sample is a good representation for Spain, with no differences in allelic distributions compared with other population samples. The observed heterozygosities in the analysed sample were not significantly different from expected values. Multiplex PCR of loci D6S89 and APOC2 is shown in Fig. 5.

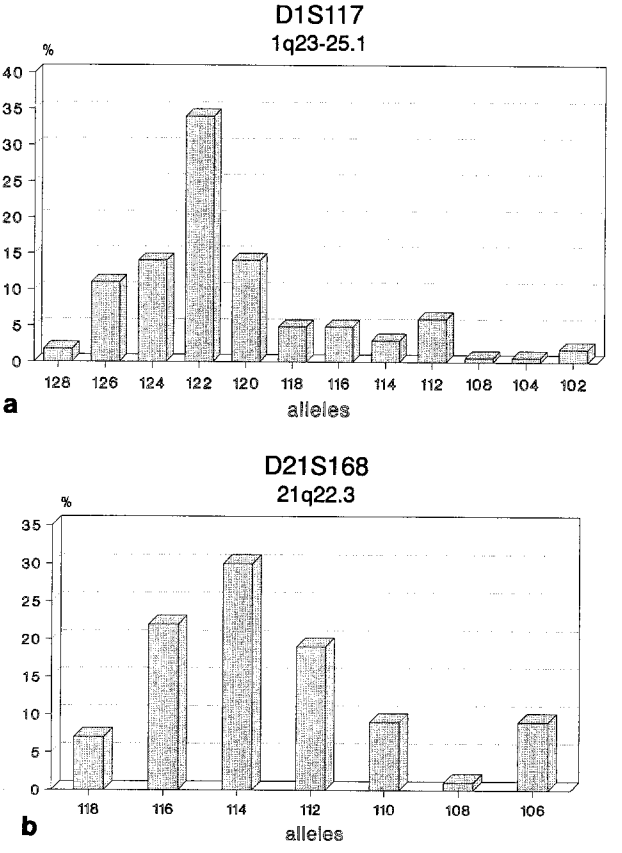

Fig. 2a, b. Frequencies of alleles at D1S117 **a** and D21S168 **b** loci in the Spanish population. The genotypes of 100 individuals were determined. Alleles are the number of base pairs (bp) as in Tables 4 and 6

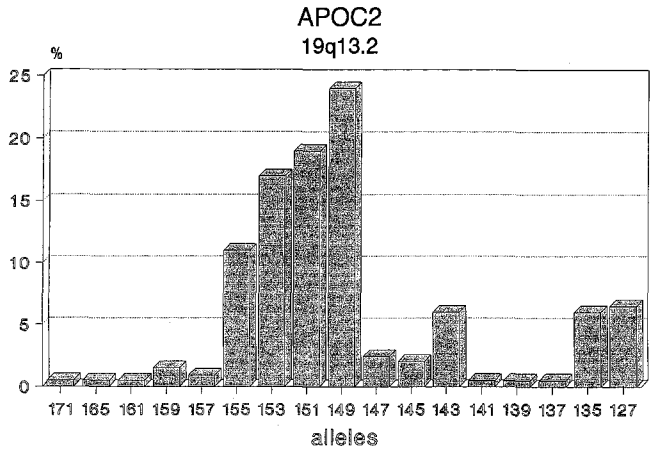

Fig. 3. Frequencies of alleles at locus APOC2 in the Spanish population. The genotypes of 100 individuals were determined. Alleles are the number of base pairs (bp) as in Table 3

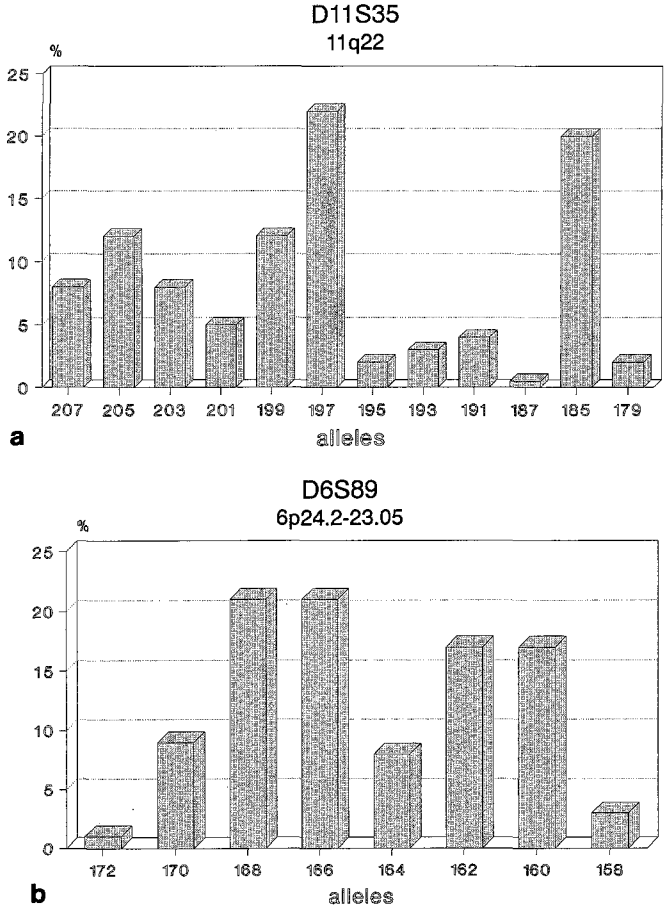

Fig. 4a, b. Frequencies of alleles at D6S89 **a** and D11S35 **b** loci in the Spanish population. The genotypes of 100 individuals were determined. Alleles are the number of base pairs (bp) as in Tables 2 and 5

Discussion

DNA analysis has provided a powerful method for genetic typing, paternity testing, and identification of individuals (Balazs et al. 1989; Budowle et al. 1991). The analysis of DNA polymorphisms by Southern blotting and, in particular, the multilocus minisatellites and single locus multi-allelic VNTR (variable number of tandem repeat) systems, do not provide an accurate and unambiguous estimation of the size of DNA fragments (Balazs et al. 1989). In addition, the low power of resolution for fragments differing by only one repeat unit might be responsible for excess of homozygosity observed for some VNTRs (Devlin et al. 1990). As the measurement error is greater than the repeat unit size, it is difficult to resolve discrete alleles and hence the real allele frequencies in the population. Therefore, a 2.5% window of the

Table 2. Allele frequency distribution, heterozygosity and PIC for locus D6S89

Allele bp	Frequency ± Standard error	Reported[a] frequency	HT	PIC
209	0.000 ± 0.000	0.004	0.86 ± 0.035	0.86
207	0.083 ± 0.019	0.056		
205	0.120 ± 0.023	0.139		
203	0.083 ± 0.019	0.070		
201	0.047 ± 0.015	0.070		
199	0.120 ± 0.023	0.123		
197	0.224 ± 0.029	0.196		
195	0.026 ± 0.010	0.024		
193	0.031 ± 0.012	0.020		
191	0.036 ± 0.014	0.029		
189	0.000 ± 0.000	0.016		
187	0.005 ± 0.005	0.004		
185	0.198 ± 0.028	0.245		
179	0.021 ± 0.010	0.004		

[a] Zoghbi et al. 1991

Table 3. Allele frequency distribution, heterozygosity and PIC for locus APOC2

Allele bp	Frequency ± Standard error	Reported[a] frequency	HT	PIC
171	0.005 ± 0.005	0.000	0.85 ± 0.035	0.85
165	0.005 ± 0.005	0.002		
161	0.005 ± 0.005	0.002		
159	0.015 ± 0.008	0.002		
157	0.009 ± 0.007	0.025		
155	0.109 ± 0.022	0.110		
153	0.168 ± 0.026	0.150		
151	0.188 ± 0.027	0.170		
149	0.238 ± 0.030	0.260		
147	0.025 ± 0.010	0.020		
145	0.020 ± 0.008	0.005		
143	0.064 ± 0.016	0.040		
141	0.005 ± 0.005	0.000		
139	0.005 ± 0.005	0.000		
137	0.005 ± 0.005	0.002		
135	0.059 ± 0.016	0.095		
131	0.000 ± 0.000	0.002		
127	0.069 ± 0.017	0.120		

[a] Fornage et al. 1992

Table 4. Allele frequencies, heterozygosity and PIC for locus D1S117

Allele bp	Frequency ± Standard error	Reported[a] frequency	HT	PIC
134	0.000 ± 0.000	(0.018)	0.83 ± 0.038	0.811
128	0.018 ± 0.010	(0.036)		
126	0.111 ± 0.023	(0.054)		
124	0.149 ± 0.026	(0.125)		
122	0.347 ± 0.036	(0.357)		
120	0.149 ± 0.026	(0.214)		
118	0.049 ± 0.016	(0.018)		
116	0.049 ± 0.016	(0.036)		
114	0.031 ± 0.013	(0.071)		
112	0.062 ± 0.018	(0.036)		
108	0.006 ± 0.006	(0.000)		
104	0.006 ± 0.006	(0.000)		
102	0.018 ± 0.010	(0.036)		

[a] Sharma and Litt 1990; ( ), frequencies for allele sizes which do not correspond (132-100 bp) to those reported here; HT, heterozygosity

Table 5. Allele frequencies, heterozygosity and PIC for locus D11S35

Allele bp	Frequency ± Standard error	Reported[a] frequency	HT	PIC
172	0.011 ± 0.007	(0.00)	0.84 ± 0.037	0.828
170	0.094 ± 0.021	(0.12)		
168	0.217 ± 0.030	(0.26)		
166	0.217 ± 0.030	(0.15)		
164	0.089 ± 0.020	(0.12)		
162	0.172 ± 0.027	(0.18)		
160	0.172 ± 0.027	(0.18)		
158	0.028 ± 0.012	(0.00)		

[a] Litt et al. 1990; ( ), frequencies for allele sizes which do not correspond (152-162 bp) to those reported here; HT, heterozygosity

Table 6. Allele frequencies, heterozygosity and PIC for locus D21S168

Allele bp	Frequency ± Standard error	Reported[a] frequency	HT	PIC
120	0.000 ± 0.000	(0.047)	0.81 ± 0.041	0.794
118	0.074 ± 0.018	(0.063)		
116	0.227 ± 0.030	(0.25)		
114	0.306 ± 0.033	(0.34)		
112	0.198 ± 0.028	(0.22)		
110	0.090 ± 0.021	(0.047)		
108	0.011 ± 0.007	(0.016)		
106	0.090 ± 0.021	(0.016)		

[a] Guo et al. 1990; ( ), frequencies for allele sizes which do not correspond (104-118 bp) to those reported here; HT, heterozygosity

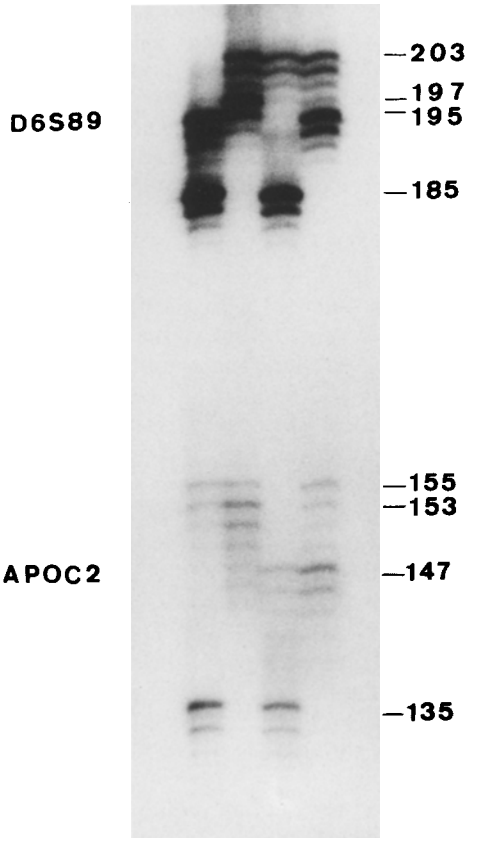

Fig. 5. Multiplex DNA typing of loci D6S89 and APOC2. Simultaneous amplification and electrophoresis of both loci was performed as described (see text). Lanes 1–5 are from unrelated individuals. Genotypes for both loci are shown at the bottom of the figure

mean of 2 fragment sizes has to be used to overcome the difficulty of size measurement in VNTR loci for genetic typing (Budowle et al. 1991).

The analysis of STRs or microsatellite alleles, which is performed using high resolution sequencing gel electrophoresis, gives an accurate and independent determination of allele sizes, which is not normally possible for multi-allelic markers analysed by Southern blotting (Jeffreys et al. 1991). As microsatellites are analysed using PCR, DNA typing has become much quicker allowing degraded material to be analysed.

We have selected 5 microsatellite markers on the basis of their heterozygosity, their localisation on different chromosomes and their feasibility for rapid analysis using PCR. The data obtained has allowed us to detect heterozygosities higher than 80% for each marker and a range of alleles between 102 and 207 bp. The amplification of fragments with non-overlapping allele sizes allows the simultaneous analysis of several loci (Fig. 5). Although a duplex analysis is shown here, triplex and multiplexing of 5 loci could easily be performed (Chamberlain et al. 1988; Edwards et al. 1991; Morral and Estivill 1992). The use of fluorescently labelled primers and an automatic DNA sequencer allows the analysis of several loci in a single experiment (Smith et al. 1986).

The information obtained for the loci D1S117, D6S89, D11S35, APOC2, and D21S168, provides the basis for genetic typing in the Spanish population, as well as a better estimation of the allele sizes and frequencies of these

markers in the general Caucasian population. It is interesting to note that only the data obtained for APOC2 and D6S89 (Tables 2 and 3) are in agreement with some previously reported allele frequencies (Zoghbi et al. 1991; Fornage et al. 1992), whereas for the other markers we have found several discrepancies.

The allele sizes detected for D6S89 are between 207 bp and 179 bp, whereas they were originally reported to be between 227 bp and 199 bp (Litt and Luty 1990). The discrepancy cannot be explained by the population background (both are Caucasians), or by the small sample size of the original study as the distribution of common alleles was completely different. A recent study of D6S89 in autosomal dominant spinocerebellar ataxia families and in CEPH families (Zoghbi et al. 1991) gave an allele distribution between 209 bp and 179 bp, with allele frequencies that match the data obtained here. The discrepancy in the initial report could be due to a sizing differences using *Sau*3A fragments of pBR322 as size markers (Litt and Luty 1990), instead of a M13 sequence ladder, as used here and in the study by Zoghbi et al. (1991). A similar explanation accounts for D11S35, with alleles in the range 172 bp–158 bp in our sample, and 162 bp–152 bp in the previously described 17 unrelated individuals (Litt et al. 1990) (Table 5).

For the other two loci (D1S117 and D21S168) the discrepancies are less critical. The common alleles in our samples correspond in both cases with a lower band in the original description. We have adapted the previously described allele sizes to our data for comparison purposes only (Tables 4 and 6).

The distribution of alleles for the 5 microsatellites analysed here may provide interesting insights into the origin of these polymorphisms. Additions or deletions of one or two repeat units or small addition/deletion events (SADE) and deletions of more than two repeat units, or long deletion events (LDE), (Levinson and Gutman 1987; Freund et al. 1989) are involved in the wide spectrum of microsatellite alleles (Morral et al. 1991). Due to DNA replication errors, SADE may be responsible for alleles detected at loci D1S117, and D21S168, and for the majority of alleles at the other 3 loci, whereas LDE could be responsible for some alleles at loci D6S89, D11S35, and APOC2.

Microsatellites have several advantages compared to "classical" VNTRs. The abundance and even distribution throughout the genome of microsatellites and their suitability for PCR analysis with a range of easily amplifiable allele fragments, avoids some of the problems observed with VNTRs when analysed by PCR (Sajantila et al. 1992). In addition, the simultaneous analysis of several microsatellites improves the rapidity of typing experiments. The difficulty in reading that sometimes occurs for microsatellite dinucleotide markers does not seem to be a problem in genetic typing for linkage analysis, genetic diagnosis or paternity testing. However, the problem of clarity in the scoring of these markers could make their use in forensic identification difficult when mixed samples are analysed. For these situations, the use of trimeric or tetrameric tandem repeat markers provide a more useful tool, which helps to overcome the limitations of VNTR markers in reading real and apparent homozygotes in either PCR or Southern blotting analysis.

Before new markers can be applied to genetic typing they need to thoroughly analysed in the different populations. This study provides the basis for paternity testing and identification using microsatellites in the Spanish population. The rapidity with which microsatellites can be analysed and the accurate determination of alleles, make these type of markers very powerful tools for DNA typing.

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References

Balazs I, Baird M, Clyne M, Meade E (1989) Human population genetic studies of five hypervariable DNA loci. Am J Hum Genet 44:182–190

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Budowle B, Giusti AM, Waye JS, Baechtel FS, Fourney RM, Adams DE, Presley LA, Deadman HA, Monson KL (1991) Fixed-bin analysis for statistical evaluation of continuous distributions of allelic data from VNTR loci, for use in forensic comparisons. Am J Hum Genet 48:841–855

Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT (1988) Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res 16: 11141–11156

Chakraborty R, Smouse PE, Neel JV (1988) Population amalgamation and genetic variation: observations on artificially agglomerated tribal populations of Central and South America. Am J Hum Genet 43:709–725

Devlin B, Risch N, Roeder K (1990) No excess of homozygosity at loci used for DNA fingerprinting. Science 249:1416–1420

Dryja TP, Mukai S, Peterson R, Rapaport JM, Walton D, Yandell DW (1989) Parental origin of mutations of the retinoblastoma gene. Nature 339:556–558

Economou EP, Bergen A, Warren AC, Antonarakis SE (1990) The poly(A) tract of Alu repetitive elements is polymorphic in the human genome. Proc Natl Acad Sci USA 87:2951–2954

Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49:746–756

Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12: 241–253

Fornage M, Chan L, Siest G, Boerwinkle E (1992) Allele frequency distribution of the (TG)n(AG)n microsatellite in the Apolipoprotein C-II gene. Genomics 12:63–68

Freund A-M, Michara M, Fuchs PP (1989) Z-DNA-forming sequences are spontaneous deletion hot spots. Proc Natl Acad Sci USA 86:7465–7469

Guo Z, Sharma V, Patterson D, Litt M (1990) Dinucleotide repeat polymorphism at the D21S168 locus. Nucleic Acids Res 18: 5924

Jeffreys AJ, Wilson V, Thein SL (1985) Hypervariable 'minisatellite' regions in human DNA. Nature 314:67–73

Jeffreys AJ, Wilson V, Newmann R, Keyte J (1988) Amplification of human minisatellites by polymerase chain reaction: towards DNA fingerprinting of single cells. Nucleic Acids Res 16: 10953–10971

Jeffreys AJ, MacLeod A, Tamaki K, Neil DL, Monckton DG (1991) Minisatellite repeat coding as a digital approach to DNA typing. Nature 354:204–209

Levinson G, Gutman GA (1987) High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in Escherichia coli K-12. Nucleic Acids Res 15:5223–5338

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

Litt M, Luty JA (1990) Dinucleotide repeat polymorphism at the D6S89 locus. Nucleic Acids Res 18:4301

Litt M, Sharma V, Luty JA (1990) Dinucleotide repeat polymorphism at the D11S35 locus. Nucleic Acids Res 18:5921

Morral N, Estivill X (1992) Multiplex PCR amplification of three microsatellites within the CFTR gene. Genomics 13:1362–1364

Morral N, Nunes V, Casals T, Estivill X (1991) CA/GT Microsatellite alleles within the cystic fibrosis transmembrane conductance regulator (CFTR) gene are not generated by unequal crossingover. Genomics 10:692–698

Motulsky AG (1988) Normal and abnormal color-vision genes. Am J Hum Genet 42:405–407

Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Culver M, Martin C, Fujimoto E, Hoff M, Kumlin E, White R (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 235:1616–1622

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Saiki RK, Gelfand DH, Stoffel S, Sharf SJ, Higuchi R, Horn GT, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sajantila A, Puomilahti S, Johnsson V, Ehnholm C (1992) Amplification of reproducible allele markers for amplified fragment length polymorphism analysis. BioTechniques 12:16–22

Sharma V, Litt M (1991) Dinucleotide repeat polymorphism at the D1S117 locus. Nucleic Acids Res 19:1168

Smeets HJM, Burnner HG, Ropers HH, Wieringa B (1989) Use of variable simple sequence motifs as genetic markers: application to study of myotonic dystrophy. Hum Genet 83:245–251

Smith LM, Sanders JZ, Kaiser RJ, Hughes P, Dodd C, Connell CR, Heiner C, et al (1986) Fluorescence detection in automated DNA sequence analysis. Nature 321:674–679

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17:6463–6471

Weber JL (1990a) Informativeness of human (dC-dA)n. (dG-dT)n polymorphisms. Genomics 7:524–530

Weber JL (1990b) Human DNA polymorphisms based on length variations in simple-sequence tandem repeats. Genetic and physical mapping, vol 1. Cold Spring Harbor Laboratory Press New York

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Zoghbi HY, Jodice C, Sandkuijl LA, Kwiatkowski Jr. TJ, McCail AE, Huntoon SA, Lulli P, Spadaro M, Litt M, Cann HM, Frontali M, Terrenato L (1991) The gene for autosomal dominant Spinocerebellar Ataxia (SCAI) maps telomeric to the HLA complex and is closely linked to the D6S89 locus in three large kindreds. Am J Hum Genet 49:23–30