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SHORT COMMUNICATION

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Population genetics of the D12S391, CSF1PO and TPOX loci in Catalonia (Northeast Spain)

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Abstract Allele and genotype frequencies for three short tandem repeat loci were determined in a population sample from Catalonia (NE Spain). After denaturing PAGE electrophoresis, 11 alleles were identified for D12S391 (n = 167), 9 alleles for CSF1pO (n = 282) and 6 alleles for TPOX (n = 283). No deviation from Hardy-Weinberg equilibrium was found. The allele frequencies observed are similar to those of other compared European populations.

Key words D12S391 · CSF1PO · TPOX · Population genetics · Paternity testing

Introduction

Short tandem repeat (STR) loci are polymorphic markers in the human genome [1, 2], which can be amplified by the polymerase chain reaction (PCR) [3]. They can be used in forensic analysis and in paternity testing. In order to obtain allele frequencies for D12S391 [4], CSF1PO [5] and TPOX [6] loci for forensic genetic diagnoses, a sample of an Eastern Iberian Peninsula population (i.e. Catalonia, N.E. Spain) was studied.

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Material and methods

DNA was extracted from healthy unrelated individuals living in Catalonia, using the organic phenol-chloroform-isoamyl alcohol method [7]. Duplex amplification of CSF1PO and TPOX was achieved using primers described by Huang et al. [8]. PCR reactions were carried out in a 25 µl volume containing 5–10 ng genomic DNA template, 200 µM each dNTP, 3 pmols of each primer, buffer at 1.5 mM $MgCl_2$, and 1 unit of Taq DNA polymerase. Temperature cycles used were according to manufacturer's recommendations (Promega) for the GenePrint Kit. D12S391 singleplex reactions were performed as described previously by Lareu et al. [9]. All reactions together with negative and positive control samples were performed in a MJ200 Thermocycler.

Separation of the amplicons was carried out on 6% (w/v acrylamide/bisacrylamide) polyacrylamide denaturing high-performance DNA sequencing gels (Ready Mix Gel ALF grade, Pharmacia). All fresh PCR products were typed twice. The electrophoresis was carried out on the Automated Laser Fluorescent (ALF) DNA Sequencer (Pharmacia) at 1450 V, 38 mA, 45 W and 50° C with laser power at 3 mW for 220 min.

Amplified DNA was mixed with internal fluorescent labeled size standards and external lane ladders were also used for adjustment. Sequenced allelic ladders were used for each system as recommended by the DNA Commission of the International Society of Forensic Haemogenetics [10, 11].

A standard χ^2 goodness-of-fit was calculated to assess Hardy-Weinberg expectations. Where expected absolute genotype frequencies were less than five, the classes were pooled according to the system outlined by Dickinson-Gibbons [12]. Possible divergence from Hardy-Weinberg equilibrium (HWE) also was determined by calculating the exact test proposed by Guo and Thompson [13]. An interclass correlation criterion for two-locus associations for detecting disequilibrium between the STR loci were calculated using the updated version of GENEPOP [14]. From a forensic point of view, the power of discrimination (PD) [15], heterozygosity value (h) [16] and the "a priori" chance exclusion value (CD) [17] were calculated. The Catalonia data were compared with Spanish and other populations using a RxC contingency table χ^2 test for homogeneity.

Results and discussion

The genotype and allele frequencies for the three STR systems in the population analysed are shown in Table 1. A total of 46 genotypes and 11 alleles were observed in 167 individuals typed at the D12S391 locus, 22 genotypes and 9 alleles were observed for CSF1PO, of which alleles 10, 11 and 12 occurred at the highest frequency. For TPOX 14 genotypes and 6 alleles were observed in 283 individuals. The distribution of the genotypes at all three loci were in Hardy-Weinberg equilibrium. An interclass correlation test analysis demonstrated that there was no detectable evidence for correlation between the alleles at any of the pair-wise comparisons of loci (Table 2).

Table 1 Allele frequency distributions for D12S391 (n = 167), CSF1PO (n = 282) and TPOX (n = 283) in a Catalonian population and statistical parameters for the three STR systems

Allele	D12S391	CSF1PO	TPOX
06			0.002
08		0.009	0.514
09		0.016	0.132
10		0.264	0.067
11		0.326	0.252
12		0.314	0.032
13		0.062	
14		0.005	
15	0.027	0.002	
16	0.045	0.002	
17	0.117		
18	0.191		
19	0.138		
20	0.131		
21	0.138		
22	0.123		
23	0.051		
24	0.036		
25	0.003		
HWE-Exact Test:	P = 0.6879	P = 0.3210	P = 0.5242
System	H	PD	CE
D12S391	90.41	0.970	0.742
CSF1PO	70.92	0.870	0.469
TPOX	64.66	0.827	0.406

H = Heterozygosity value, PD = Power of discrimination, CE = Chance of exclusion

Table 2 Two loci interclass correlation test for D12S391, CSF1PO and TPOX loci

Loci	Two-sided probability
D12S391/TPOX	0.295
D12S391/CSF1PO	0.997
TPOX/CSF1PO	0.223

The data from Catalonia for the D12S391 locus were compared with the only European populations studied up at present, from Galicia (NW Spain) [9] and Germany [9] and no significant differences were observed.

For CSF1PO and TPOX, the allele frequencies from Catalonia do not differ significantly from Swiss [18] and Spanish [19] populations. In contrast, significant differences were observed with the populations from China [8] and Japan [20]. The data suggest that there is a general uniformity for the three STR systems in the European populations compared.

From a forensic point of view, theoretical values were calculated from gene frequencies obtained in our population (Table 1). The observed heterozygosity and the power of discrimination for D12S391 reveal that this system has the highest forensic efficiency of the three loci.

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