

TECHNICAL NOTE

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Population Genetics of the D1S1656, D12S391, and D18S535 Loci in Asturias (North Spain)

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ABSTRACT: Allele and genotype frequencies for three recently described short tandem repeat loci D1S1656, D12S391, and D18S535 were determined in a population sample from Asturias (North Spain). The loci were amplified using a fluorescence based PCR method and were typed automatically. No deviation from Hardy-Weinberg expectations were observed. The three loci proved to be highly discriminating and the allele frequencies observed are similar to those of the other European populations that have been typed for these loci to date.

KEYWORDS: forensic science, short tandem repeats, D1S1656, D12S391, D18S535, population genetics, Asturias

Short tandem repeat (STR) loci are highly polymorphic consisting of simple, compound, or complex (1) tandemly repeated sequences (2–7bp) that are relatively short in length (<400 bp), and are amenable to amplification by the polymerase chain reaction (PCR). STR loci have been used extensively for forensic casework and human identification. However, the selection of STR loci for a laboratory's repertoire should be made judiciously (2). Recently, several STR loci have been identified and proposed as candidates for application to forensic casework routine (3–5).

The aim of this work was to investigate the discrimination power of the STR loci D18S535, D1S1656, and D12S391 and establish databases for these systems in a Spanish population sample residing in Asturias.

Material and Methods

Genomic DNA was extracted by the standard phenol/chloroform extraction procedure.

D1S1656, D12S391, and D18S535 singleplex amplifications were performed as described previously by Lareu et al. (3–4, 6–7). All reactions, together with negative and positive control samples,

were carried out in a Perkin-Elmer DNA Thermal Cyclor 480. Amplified DNA was mixed with internal fluorescent labeled size standards. Sequenced allelic ladders provided by Prof. Carracedo (Institute of Santiago de Compostela, Galicia, Spain) were also run for each system as recommended by the DNA Commission of the International Society of Forensic Haemogenetics (8–9). All fresh PCR products were typed twice.

Separation was carried out on 6% (w/v acrylamide/bisacrylamide) polyacrylamide denaturing high-performance DNA sequencing gel (Ready Mix Gel ALF grade, Pharmacia). 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.

Statistical evaluations were performed as described previously (see Refs 10 and 11 for details).

Results and Discussion

The observed allele frequencies for the three STR loci in the Asturias population are shown in Table 1. A total of 21 genotypes and 8 alleles were observed at the D18S535 locus ($n=159$), 54 genotypes and 11 alleles were observed for D1S1656 locus ($n=160$) and for the D12S391 locus, 46 genotypes and 12 alleles were observed ($n=157$). The results of tests for the correspondence of the genotype frequencies with their HWE proportions are displayed in Table 2. The genotype frequency distributions for the three loci showed no deviations from HWE expectations based on the homozygosity test (except for the D1S1656 locus), the likelihood ratio test and the exact test. When corrected for the number of tests (i.e., Bonferroni correction), the departure at the D1S1656 locus for the homozygosity test is no longer considered significant.

Table 3 shows statistical parameters of forensic interest that reveal the usefulness of these three STR systems for personal identification (cumulated PD=0.999 and total PE=0.974).

An interclass correlation test analysis demonstrated that there was no evidence for correlation between the alleles at any of the pair-wise comparisons of loci (Table 4). Moreover, correlation tests made with previous results obtained in the same sample population for the HLA DQA1 locus, the polymarker set, the D1S80 locus and the STR systems HUMTH01, HUMTPOX, and HUMVWA demonstrated (data not shown) that there is little evidence for departures from independence for the sample population and support the view that the use of the product rule would provide

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TABLE 1—Observed allele frequency distribution for the STR loci: D1S1656, D12S391, and D18S535 in a population sample from Asturias.

Alleles	D1S1656	D12S391	D18S535
9			0.110
10			0.006
11	0.084		0.009
12	0.159		0.233
13	0.072		0.314
14	0.087		0.204
15	0.184	0.051	0.113
15.3	0.069	—	—
16	0.091	0.022	0.009
17	0.084	0.102	
17.3	0.106	—	
18	—	0.223	
18.3	0.041	—	
19	0.022	0.143	
19.3	—	—	
20		0.127	
21		0.131	
22		0.102	
23		0.057	
24		0.032	
25		0.006	
26		0.003	

TABLE 2—Summary of HWE tests on the loci analyzed.

	D1S1656	D12S391	D18S535
Observed homozygosity	16.9%	15.3%	17.0%
Expected homozygosity (unbiased)	11.0%	12.9%	21.8%
Homozygosity test*	0.018	0.371	0.145
Likelihood test*	0.758	0.462	0.316
Exact test*	0.547	0.374	0.219

* These are probability values.

TABLE 3—Parameters of forensic efficiency.

	D1S1656	D12S391	D18S535
PE*	0.7722	0.7361	0.5717
PD*	0.9741	0.9648	0.9047

* Probability of exclusion.

† Power of discrimination.

TABLE 4—Summary of Karlin correlation tests.

Loci	p value
D1S1656/D12S391	0.083
D1S1656/D18S535	0.498
D12S391/D18S535	0.410

TABLE 5—Comparison of different populations for D1S1656, D12S391, and D18S535 loci.

Populations	D18S535	D1S1656	D12S391
Asturias/Galicia (3,4,6,7)	0.4931	0.5271	0.8806
Asturias/Germany (12)	NA	NA	0.8934
Asturias/Portugal (13)	NA	NA	0.8050
Asturias/Catalonia (15)	NA	NA	0.0001
Asturias/Austria (16)	NA	NA	0.8244
Asturias/China (12)	NA	NA	0.0001

NA = Data not available.

a good approximation of the estimate of the rarity of a multiple locus profile.

A comparison of the allele frequencies in the population under study with the only European populations studied to date (3–4, 6–7, 12–16) revealed to significant differences (Table 5), except at the D12S391 locus with Catalonians (15) and with non-Caucasian populations such as Chinese (12).

In conclusion, an Asturian population database has been established for the STRs D18S535, D1S1656, and D12S391. The three STR systems are highly informative, and the data presented can be used to calculate matching probabilities in forensic case work if Asturians are considered as a source of DNA evidence.

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