TECHNICAL NOTE

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Allelic Frequencies for the HLA-DQA1, D1S80, HUMTHO1, HUMTPOX, HUMCSF1PO and HUMVWA Loci in Cantabria (Middle North Spain)

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ABSTRACT: Allele frequencies for six DNA polymorphisms have been studied in a population sample from Cantabria (middle north Spain) using the polymerase chain reaction. The HLA-DQA1 locus was analyzed by the reverse dot-blot technique and the other five by polyacrylamide gel electrophoresis followed by silver staining. Six alleles were found for HLA-DQA1, 15 alleles for D1S80, 6 alleles for HUMTHO1 and HUMCSF1PO, 7 for HUMTPOX and 8 alleles for HUMVWA. The 21 repeat allele in HUMVWA had not previously been reported in a Spanish population. The genotype distributions met Hardy-Weinberg expectations for all the systems and some statistical parameters of forensic interest were calculated. Comparisons with other populations revealed significant differences for HLA-DQA1, HUMVWA and HUMTHO1, with interracial differences being more pronounced than between Spanish populations. The HUMVWA system showed the highest forensic efficiency of the six polymorphisms studied.

KEYWORDS: forensic science, DNA typing, population genetics, HLA-DQA1, D1S80, THO1, TPOX, CSF1PO, VWA, polymerase chain reaction, short tandem repeat, Spain

We studied a sample of the Cantabrian (middle north Spain) population to obtain frequencies for six DNA-based polymorphisms (HLA-DQA1, D1S80, HUMTHO1, HUMTPOX, HUMCSF1PO and HUMVWA) for use in forensic genetic diagnosis.

Materials and Methods

Blood samples were obtained from healthy unrelated individuals (N = 130 to 133) living in Cantabria. DNA was extracted from EDTA blood using the Chelex method (1) or the phenol-chloroform procedure described by Valverde et al. (2).

Amplification and typing of HLA-DQA1 was carried out with an AmpliType^R HLA-DQα Kit (Perkin-Elmer) according to the manufacturer's recommendations. The PCR was performed in a Perkin-Elmer 2400 Thermal Cycler.

The D1S80 locus was amplified using an AmpliFLP™ Kit (Perkin-Elmer) and was typed by horizontal polyacrylamide gel electrophoresis (PAGE) followed by silver staining. The crosslinker used was piperazine-diacrylamide (T = 6%, C = 3%) and the distance between the electrodes was 24 cm. Electrophoresis was performed at 40 mA and a constant current of 1000 V and it was stopped when the bromophenol blue front (0.01%) reached the anodal wick.

The STR loci were amplified using the Geneprint™ STR Systems Kit (Promega): monoplex (HUMVWA) and multiplex (HUMTHO1, HUMTPOX and HUMCSF1PO). The STR alleles were analyzed by vertical denaturing PAGE (T = 6 to 4%, C = 5%, 7 M urea, TBE × 1) for 2 h 30 min, at a constant voltage (1500 V). The differents bands were visualized by silver staining. Allelic ladders supplied by the manufacturers were included in each gel.

Hardy-Weinberg (HWE) expectations were tested for with conventional Pearson's chi-square methods (χ^2) for the HLA-DQA1 and for the D1S80 loci; the latter was also studied after regrouping the alleles into new bins, as described by Rand et al. (3) (data not shown). For the other polymorphisms, the statistical evaluations were performed using an HWE-Analysis software package (C. Puers, Institute of Legal Medicine Münster). Analyses included the possible divergence from HWE expectations, the likelihood ratio test (G test) (4) and exact test (5).

Other parameters of forensic interest were calculated: the chance of exclusion (CE) (6), the allelic diversity values (h) (7) and the single and combined discrimination power (PD) (8). The possible associations between loci were tested using the computer program GDA (Genetic Data Analysis, Po Lewis and D Zaykin). An R × C contingency table was used to test for homogeneity between various Spanish and European populations.

Results and Discussion

The allele frequencies and some parameters of forensic interest for the six systems in the Cantabrian population are shown in Tables 1 and 2.

The results of the different procedures testing for correspondence of the genotypes frequencies with HWE expectations are shown in Table 3. All the loci were in HWE equilibrium based on the χ^2 test, likelihood ratio test (G test) (4) and exact test (5). An interclass correlation test analysis demonstrated that there was no detectable evidence for correlation between the alleles at any of the loci in the pair-wise comparisons (Table 4).

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TABLE 1—*Allele frequencies and some parameters of forensic interest for the HLA-DQA1 and D1S80 in the population of Cantabria (middle north Spain).*

	HLA-DQA1 (n = 130)		D1S80 (n = 130)	
	Allele	Frequency	Allele	Frequency
	DQA1*0101	0.1846	18	0.2077
	DQA1*0102	0.1538	19	0.0038
	DQA1*0103	0.0577	20	0.0538
	DQA1*0201	0.2038	21	0.0462
	DQA1*0301	0.1308	22	0.0385
	DQA1*0401	0.2692	23	0.0038
			24	0.3615
			25	0.0692
			26	0.0115
			27	0.0038
			28	0.0615
			29	0.0385
			30	0.0231
			31	0.0731
			39	0.0038
CE		0.6170		0.6397
PD		0.9292		0.9362
h		0.8109		0.8066

CE = chance of exclusion, PD = power of discrimination, h = allelic diversity values.

TABLE 2—*Allele frequencies and some parameters of forensic interest for STR loci in a Cantabrian population sample.*

Allele	HUMTHO1 (n = 130)	HUMTPOX (n = 130)	HUMCSF1PO (n = 130)	HUMVWA (n = 133)
6	0.1923	0.0038		
7	0.2231			
8	0.0731	0.5885		
9	0.2038	0.0885	0.0346	
9.3	0.2885			
10	0.0192	0.0346	0.3115	
11		0.2500	0.3308	
12		0.0308	0.2462	
13		0.0038	0.0654	
14			0.0115	0.1316
15				0.1654
16				0.2406
17				0.2406
18				0.1541
19				0.0489
20				0.0150
21				0.0038
CE	0.5711	0.3416	0.4812	0.6275
PD	0.9148	0.7793	0.8567	0.9369
h	0.7858	0.5835	0.7301	0.8162

CE = chance of exclusion, PD = power of discrimination, h = allelic diversity values.

TABLE 3—*Hardy-Weinberg equilibrium tests on the loci analyzed.*

	DQA1	D1S80	THO1	TPOX	CSF1PO	VWA
χ^2 test	0.7275	0.0965	0.1765	0.7500	0.0505	0.9575
G test	0.6100	0.1890	0.2130	0.7995	0.1290	0.9560
Exact test	0.7310	0.1990	0.1490	0.8335	0.0980	0.9310

TABLE 4—*Two-loci interclass correlation test for the six analyzed polymorphisms (p-values).*

	CSF1PO	TPOX	THO1	DQA1	D1S80	VWA
CSF1PO	⋯	0.3235	0.3420	0.9630	0.9220	0.3765
TPOX		⋯	0.0625	0.8600	0.7935	0.9665
THO1			⋯	0.4345	0.1370	0.5535
DQA1				⋯	0.4495	0.5390
D1S80					⋯	0.3420
VWA						⋯

Number of random shufflings performed: 2000

Of particular interest was the high value for the allele DQA1 *0201 in this Cantabrian sample, with a range similar to that found in other Northeast Spanish populations: Basque Country (9), Aragon (10) and Catalonia (11).

For the D1S80 locus, the population of Cantabria displays a high frequency for alleles 18 and 24, which agrees with other reported populations (12,13). Some alleles were not found in our survey, which may be due to the small sample size.

For the STR systems, the most frequent alleles were 9.3 for HUMTHO1, 8 for HUMTPOX, 11 for HUMCSF1PO and 16/17 for HUMVWA. All the observed alleles appeared to migrate with alleles present in the allelic ladder—differing in size by one repeat unit, except for the HUMTHO1 allele 9.3, which had a single base deletion.

Our population showed a low frequency for allele 8 (f = 0.0731) and a high frequency for allele 7 (f = 0.2231) for the HUMTHO1 locus. The seven different alleles found for the HUMTPOX system included allele 13, which had only been found previously in a population from Catalonia (11) and in Japan (14). The HUMCSF1PO locus showed the same range of variability as other populations. For the HUMVWA STR, we found an allele with 21 repeats, which has been reported in samples from European countries (15,16) but has not previously been reported in a Spanish population, although it has been identified (GEP-ISFH, 1998—unpublished results) (17).

A comparison of the allele frequencies in the population under study with those of previous studies by means of χ^2 contingency tables revealed significant differences with populations from Northwest Spain (10,12) and North Europe (18) and with U.S. black and U.S. Hispanic populations (19) for HLADQA1, but only slight or no differences for the D1S80 locus (12,13). Significant differences were observed for the HUMTHO1 system between the Cantabrian population and other samples from Spain (11,20) and Europe (21,22). Comparisons with Chinese (23), Japanese (14), Afro-American and Hispano-American (24) populations showed that interracial differences are more pronounced. Thus the allele frequency distributions were different between the racial groups, suggesting the usefulness of these alleles as race markers. Differences were also found for the HUMTPOX and HUMCSF1PO loci. For HUMVWA, the differences found were with North European (15,16), Japanese (14) and Hispano-American (24) populations.

The allelic diversity and the power of discrimination for HUMVWA (Table 2) reveal that this system has the highest forensic efficiency of the six DNA polymorphisms analyzed.

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