

SPANISH POPULATION DATA ON 13 PCR-BASED SYSTEMS

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INTRODUCTION

The enzymatic amplification of polymorphic DNA-loci by PCR (Saiki et al. 1985) offers a number of distinct advantages in forensic DNA typing. First a rapid and specific typing of genetic markers. Second, amplification and detection of target sequences may allow reliable typing results from very small amounts of even degraded DNA. These advantages make the PCR-based typing one of the methods of choice for forensic identification of body fluids and human remains and for paternity testing.

This study presents allele/genotype frequency data in a Spanish population sample (n=187-244) for 7 Short Tandem Repeat (STR) loci and 6 sequence polymorphism loci. The loci are: FES/FPS (15q25-qter), VWA (12p12-pter), TH01 (11p15.5), F13B (1q31-q32.1), CSF1PO (5q33.5-q34), F13A1 (6p24-p25), TPOX (2p13), HLA-DQA1 (6p21.3), LDLR (19p13.1-p13.3), GYPA (4q28-q31), HBG (11q), D7S8 (7cen-q22) and Gc (4q11-q13).

MATERIALS AND METHODS

Population Sample and Sample Preparation

EDTA-blood samples were collected from a total of 244 unrelated Spanish individuals living predominantly in the Communities of Madrid, Castilla-La Mancha, Castilla-León (Central-Spain) and Valencia (Eastern-Spain). The DNA was extracted by the standard phenol/chloroform extraction procedure.

PCR and Typing

The amplification of HUMFES/FPS, HUMVWA, HUMTH01, HUMF13B, HUMCSF1PO, HUMF13A1 and HUMTPOX was performed separately according to the manufacturer's recommendations using the Gene Print STR system (Promega Corporation, Madison, WI, USA) in a 480 Perkin-Elmer thermal cycler. PCR products were typed by denaturing polyacrylamide gel electrophoresis and stained with silver as described previously (Budowie et al. 1991).

The amplification and typing of HLA-DQA1 and PM systems were performed according to the manufacturer's recommendations using the amplitype PM and HLA-DQA1 forensic DNA amplification and Typing kits (Perkin-Elmer Corporation, Norwalk, CT).

Statistical Analysis

The frequency of each allele for each locus was calculated from the numbers of each genotype in the sample. Unbiased estimates of expected heterozygosity were computed as described by Edwards et al. (1992). The expected numbers of distinct homozygous and heterozygous genotypes and their standard errors (SE) were calculated according to the method described by Chakraborty et al. (1988, 1991). Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (Chakraborty et al. 1988; Nei et al. 1974; Nei et al. 1978), the likelihood ratio test (Edwards et al. 1992; Chakraborty et al.

1991; Weir 1992) and the exact test (Guo et al. 1992). An interclass correlation criterion (Karlin et al. 1981) for two-locus association was used for detecting disequilibrium between loci. Independence across all loci was determined by examining whether or not an observed variance of the number of heterozygous loci in the population sample is outside its confidence interval under the assumption of independence (Brown et al. 1980; Chakraborty 1984; Chakraborty et al. 1994). When appropriate, the Bonferroni procedure was used to correct for multiple analysis to determine whether or not HWE or equilibrium between loci holds in the population.

RESULTS AND DISCUSSION

The observed allele frequencies for 13 PCR-based systems in the Spanish population sample are shown in Table 1. The results of the different test procedures for testing the correspondence of the genotype frequencies with their HWE expected proportions are shown in Table 2. Except for LDLR ($p=0.02$), the genotype frequency distributions for the loci do not deviate from HWE based on the homozygosity test, the likelihood ratio test and the exact test. The departure from HWE for LDLR, which is not highly significant, is most likely due to sampling variance, and the difference in LDLR genotype estimates using the product rule or the counting method is small. This suggests that the use of the product rule for estimating LDLR genotype frequencies would still provide a valid estimate for forensic or human identity purposes. These data also show that departures from HWE generally have little impact on genotype frequency estimates when independence is assumed.

An analysis was performed to determine whether or not there were any detectable associations between any of the 13 PCR-based loci. An interclass correlation test analysis demonstrated that there were only 3 departures out of 78 tests (F13B/CSF1PO, $p=0.015$; LDLR/Gc, $p=0.037$; and TH01/HLA-DQA1, $p=0.043$), this is less than 5% of the observations. Thus, the amount of departures was no more than expected. Also, with a Bonferroni correction (used for correcting when multiple tests are performed on a population sample), there was no evidence for departures from expectations for pair-wise comparisons of PCR-based loci.

In conclusion, a Spanish population database has been established for 13 PCR-based loci and it has been shown that the allele frequency data can be used in identity testing to estimate the frequency of a multiple PCR-based DNA profile in the Spanish population.

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Table 1. Allele frequencies for 13 PCR-based systems in a Spanish population sample.

HLA-DQα (n=474) Allele / Freq.	FES/FPS (n=382) Allele / Freq.	VWA (n=444) Allele / Freq.	TH01 (n=488) Allele / Freq.	F13B (n=392) Allele / Freq.	CSF1PO (n=374) Allele / Freq.	F13A1 (n=398) Allele / Freq.	TPOX (n=436) Allele / Freq.
1.1 / 0.116	8 / 0.008	14 / 0.106	6 / 0.217	6 / 0.115	8 / 0.003	3.2 / 0.085	6 / 0.002
1.2 / 0.162	9 / 0.003	15 / 0.144	7 / 0.176	7 / 0.010	9 / 0.011	4 / 0.043	8 / 0.546
1.3 / 0.063	10 / 0.319	16 / 0.227	8 / 0.166	8 / 0.250	10 / 0.267	5 / 0.206	9 / 0.096
2 / 0.154	11 / 0.382	17 / 0.286	9 / 0.178	9 / 0.189	11 / 0.275	6 / 0.256	10 / 0.060
3 / 0.133	12 / 0.246	18 / 0.149	9.3 / 0.256	10 / 0.431	12 / 0.374	7 / 0.372	11 / 0.268
4 / 0.371	13 / 0.039	19 / 0.074	10 / 0.006	11 / 0.005	13 / 0.067	8 / 0.013	12 / 0.028
	14 / 0.003	20 / 0.014			14 / 0.003	13 / 0.013	
						14 / 0.005	
						15 / 0.008	

LDLR (n=404) Allele / Freq.	GYP A (n=404) Allele / Freq.	HBGG (n=404) Allele / Freq.	D7S8 (n=404) Allele / Freq.	Gc (n=404) Allele / Freq.
A / 0.450	A / 0.527	A / 0.498	A / 0.564	A / 0.297
B / 0.550	B / 0.473	B / 0.493	B / 0.436	B / 0.163
		C / 0.010		C / 0.540

n = Number of alleles.

Table 2. HWE test for independence on 13 PCR-based systems in a Spanish population sample

	FES/FPS	VWA	TH01	F13B	CSF1PO	F13A1	TPOX
Obs. Homozygosity	30.9%	16.2%	22.5%	28.1%	28.9%	24.1%	36.7%
Exp. Homozygosity ^a	30.8%	19.2%	20.2%	29.6%	29.0%	25.4%	38.2%
Homozygosity Test ^b	0.989	0.266	0.354	0.648	0.967	0.677	0.644
Likelihood Ratio Test ^b	0.757	0.443	0.288	0.695	0.371	0.406	0.713
Exact Test ^b	0.634	0.392	0.296	0.763	0.550	0.282	0.373

	HLA-DQα	LDLR	GYP A	HBGG	D7S8	Gc
Obs. Homozygosity	19.4%	58.4%	53.0%	42.6%	52.5%	42.1%
Exp. Homozygosity ^a	22.2%	50.4%	50.0%	48.9%	50.7%	40.5%
Homozygosity Test ^b	0.310	0.022	0.402	0.072	0.615	0.639
Likelihood Ratio Test ^b	0.695	0.027	0.478	0.194	0.675	0.920
Exact Test ^b	0.817	0.018	0.478	0.142	0.675	0.877

a- Expected homozygosity is an unbiased estimate

b- These values are probability values