TECHNICAL NOTE

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Distribution of Types for Six PCR-Based Loci; LDLR, GYPA, HBGG, D7S8, GC and HLA-DQA1 in Central Pyrenees and Teruel (Spain)

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ABSTRACT: The PCR-based DNA loci LDLR, GYPA, HBGG, D7S8, GC and HLA DQA1 are widely used in forensic casework analyses. Population data on the distribution of allele frequencies are desired to estimate the rarity of a DNA profile. We studied the allele distributions at these forensically important DNA markers in two Spanish populations (Central Pyrenees and Teruel). Results were in agreement with Hardy-Weinberg expectations. Furthermore, there was little evidence for departures from expectation of independence between loci within the two sample populations. Tests for homogeneity were carried out between the two Spanish populations and a U.S. Caucasian population.

KEYWORDS: forensic science, DNA typing, population genetics, LDLR, GYPA, HBGG, D7S8, GC, HLA-DQA1, Central Pyrenees, Teruel, Spain

Amplification of polymorphic DNA loci by the Polymerase Chain Reaction (PCR) offers a number of distinct advantages in forensic DNA typing. First this approach provides a means of rapid and specific typing of genetic markers. Second, amplification and subsequent detection of target sequences enables reliable typing of samples containing small amounts of DNA, even when the samples are substantially degraded.

HLA-DQA1 is the most characterized PCR-based forensic system (1–8), and the test can be performed using a commercially available kit (AmpliType™ HLA DQ α Forensic DNA Amplification and Typing Kit, Perkin-Elmer, Roche Molecular Systems, Inc., Branchburg, NJ). Subsequently, the AmpliType PM PCR Amplification and Typing Kit (Perkin-Elmer, Roche Molecular Systems, Inc., Branchburg, NJ) was introduced for forensic DNA casework analysis. This kit includes reagents that enable multiplex amplification of six loci: HLA-DQA1, Low Density Lipoprotein Receptor (LDLR) (9), Glycophorin A (GYPA) (10), Hemoglobin G Gammaglobin (HBGG) (11), D7S8 (12) and Group Specific Component (GC) (13). After amplification, LDLR, GYPA, HBGG, D7S8 and GC (PM loci) are typed simultaneously using a reverse dot blot technology and sequence specific oligonucleotide (SSO) probes. The HLA-DQA1 PCR product is typed independently but in a manner similar to that of PM loci (i.e., reverse dot blot format and SSO probes). The AmpliType PM PCR Amplification and Typing kit also has proven useful for forensic identification (14)(15).

In order to use genetic loci in identity testing some population data are needed. This paper presents the first report of allele/genotype frequency data for the loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and GC in a Pyrenean population (Spanish Central Pyrenees), and a Spanish population in Teruel. Additional aims were to test whether or not the allele frequencies conform to Hardy-Weinberg expectations, to obtain some statistics of medico-legal interest such as the allelic diversity value, the power of discrimination and the chance of exclusion in paternity cases, and to compare the results obtained with other population data.

Materials and Methods

Samples

Blood samples were obtained from 205 healthy unrelated individuals: 106 from Central Pyrenees and 99 from Teruel. DNA was extracted with Chelex™ 100 using the method described by Walsh et al. (16). The extracted DNA from each sample was quantified either by 0.8% agarose gel or using the slot-blot procedure described by Way et al. (17). One to five ng of DNA were used for PCR.

Typing

The DNA samples were amplified and typed for the PM loci using the AmpliType PM PCR Amplification Typing Kit (Perkin Elmer Corporation, Norwalk, CT). The HLA-DQA1 locus was amplified and typed using the Amplitype HLA DQ α Forensic DNA Amplification and Typing kit (Perkin Elmer Corporation, Norwalk, CT). Amplification was carried out in a Perkin-Elmer DNA Thermal Cycler 480. The conditions were those recommended by the manufacturer (18)(19).

Statistical Analysis

The frequency of each allele for each locus was calculated from the numbers of each genotype in the sample set. Unbiased estimates

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of expected heterozygosity were computed as described by Edwards et al. (20). Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (21–23), the likelihood ratio test (20,24,25), and the exact test (26). An interclass correlation criterion (27) was used for detecting disequilibrium between loci.

A RxC contingency table exact test was used to generate a G-statistic (1000 shuffling experiments) (28,29) to test for homogeneity between the sample populations, as well as a U.S. Caucasian sample population.

Other statistical parameters of genetic and medico-legal interest were calculated: the power of discrimination (PD) (the probability that two individuals chosen at random from a given population have different genotypes) (30); the CE (probability of excluding a falsely accused man as father in paternity testing) (31) and the heterozygosity value (h) (22).

Results and Discussion

The distributions of observed allele frequencies for the PM and HLA-DQA1 loci in the two sample populations are shown in Tables 1 and 3. The genotype frequency distributions for all loci except D7S8 in the Pyrenees do not deviate from HWE based on the homozygosity test, likelihood ratio test, and the exact test (Tables 2 and 3). Observing one locus that departs from HWE expectations is not unexpected and may be due to chance or sampling. Moreover, there is a deficiency of observed homozygotes at the D7S8 locus, which does not indicate that population substructure is a likely basis for this departure. However, the difference in the D7S8 allele frequencies between the Pyrenees and the other populations (Teruel and U.S. Caucasian) is significant (see Table 5). Another sample for typing at the D7S8 locus would be interesting.

An interclass correlation test analysis was performed to determine whether or not there were any detectable associations between any pairs of the loci (Table 4). There was only one significant (highly) departure between the loci GYPA and HLA-DQA1 in the Teruel sample ($p < 10^{-3}$). In total, there was only one departure out of 30 interclass correlation tests in the two populations, which is less than 5% of the comparisons. Thus, the amount of departure is no more than expected. The data suggest that overall there is little evidence for departures from independence for the sample populations.

There were some significant differences in allele frequencies for the loci HBGG ($p = 0.048$), which is borderline, and D7S8

TABLE 1—*Observed allele frequency distributions for PM loci in 106 unrelated individuals from the Pyrenees and 99 unrelated individuals from Teruel.*

Allele	Pyrenees	Teruel
LDLR A	0.459	0.449
LDLR B	0.505	0.551
GYPA A	0.524	0.535
GYPA B	0.476	0.465
HBGG A	0.524	0.404
HBGG B	0.472	0.586
HBGG C	0.005	0.010
D7S8 A	0.462	0.566
D7S8 B	0.538	0.434
Gc A	0.354	0.379
Gc B	0.165	0.111
Gc C	0.481	0.510

TABLE 2—*Test for independence on PM loci.*

Allele	Obs. Homozyg	Exp. Homozyg*	Homozyg Test†	Likelihood Ratio Test†	Exact Test*
LDLR					
Pyrenees	44.35	49.8%	0.264	0.255	0.319
Teruel	52.5%	50.3%	0.652	0.836	0.677
GYPA					
Pyrenees	50%	49.9%	0.979	1.000	1.000
Teruel	51.5%	50%	0.763	0.835	0.835
HBGG					
Pyrenees	52.8%	49.4%	0.484	0.502	0.502
Teruel	56.6%	50.4%	0.220	0.659	0.425
D7S8					
Pyrenees	39.6%	50.1%	0.032‡	0.040‡	0.040‡
Teruel	49.5%	50.6%	0.824	0.827	0.827
Gc					
Pyrenees	40.6%	38.1%	0.601	0.571	0.457
Teruel	40.4%	41.3%	0.855	0.444	0.721

*Expected homozygosity is an unbiased estimate.
†These values are probability values.
‡Statistically significant.

TABLE 3—*HLA-DQA1 observed allele frequencies and tests for independence values in 106 unrelated individuals from Pyrenees and 99 unrelated individuals from Teruel.*

Allele	Pyrenees	Teruel
1.1	0.137	0.187
1.2	0.217	0.162
1.3	0.066	0.076
2	0.160	0.172
3	0.170	0.126
4	0.250	0.278

Pyrenees: Observed Homozygosity = 13.2%; Expected Homozygosity (unbiased) = 18.3%; HWE-Homozygosity Test ($p = 0.172$). Likelihood Ratio Test ($p = 0.557$), Exact Test ($p = 0.515$).
Teruel: Observed Homozygosity = 17.2%; Expected Homozygosity (unbiased) = 18.5%; HWE-Homozygosity Test ($p = 0.729$). Likelihood Ratio Test ($p = 0.658$), Exact Test ($p = 0.534$).

TABLE 4—*Two locus interclass correlation test for unrelated individuals from the Pyrenees and Teruel.*

Alleles	Pyrenees	Teruel
LDLR/GYPA	0.776	0.356
LDLR/HBGG	0.493	0.544
LDLR/D7S8	0.509	0.758
LDLR/Gc	0.771	0.662
LDLR/DQA1	0.309	0.973
GYPA/HBGG	0.053	0.704
GYPA/D7S8	0.232	0.539
GYPA/Gc	0.564	0.709
GYPA/HQA1	0.189	$<10^{-3}$*
HBGG/D7S8	0.453	0.219
HBGG/Gc	0.177	0.630
HBGG/DQA1	0.315	0.079
D7S8/Gc	0.573	0.954
D7S8/DQA1	0.363	0.974
Gc/DQA1	0.890	0.936

*Deviation at $p < 0.05$ level.

($p = 0.032$) between Pyrenees and Teruel, for the D7S8 locus between the Pyrenees and U.S. Caucasians, and for the Gc ($p = 0.009$) and DQA1 ($p = 0.012$) loci between the Teruel and US Caucasians (Table 5).

Whether or not these population differences at these loci are real or due to sampling remains to be seen. Although Table 5 shows differences for some loci between the sample populations, the allele frequencies are not substantially different (see Tables 1 and 3), from practical point of view. However, the homogeneity analyses are an example how statistics can detect significant differences between populations, and, yet, if either population was used as a database for human identity testing, the resulting DNA profile frequency estimates would not be substantially different.

Other statistical parameters calculated as PD, CE, and h are shown in Table 6. The combined chance of exclusion (%) was 89.08 in the Pyrenean population and 88.45 in the Teruel population. The combined discrimination power (%) was 99.99 in the Pyrenean population and 99.97 in the Teruel population. The heterozygosity values (%) ranged from 50.19 for D7S8 to 82.54 for HLA-DQA1 in the Pyrenean population and from 51.52 for D7S8 to 82.42 for HLA-DQA1 in the Teruel population. These results show that the PM and HLA-DQA1 loci are very useful for forensic identity purposes and are appropriate for the general case situation for the respective regions in Spain.

TABLE 5—*G statistic (p values) for homogeneity test between Pyrenees and Teruel populations and between U.S. Caucasians.*

Locus	Pyren/Teruel	Pyren/U.S.	Teruel/U.S.
LDLR	0.356 ± 0.015	0.357 ± 0.015	1.000 ± 0.000
GYPA	0.838 ± 0.012	0.167 ± 0.012	0.307 ± 0.015
HBGG	0.048 ± 0.007*	0.506 ± 0.016	0.374 ± 0.015
D7S8	0.032 ± 0.006*	0.002 ± 0.001*	0.333 ± 0.015
Gc	0.249 ± 0.015	0.059 ± 0.008	0.009 ± 0.003*
HLA-DQA1	0.427 ± 0.016	0.139 ± 0.011	0.012 ± 0.003

* = Deviation at $p < 0.005$ level.

TABLE 6—*Statistical parameters of medico-legal interest.*

	PD%	CE%	H%
LDLR			
Pyrenees	59.11	18.75	50.47
Teruel	64.50	18.62	52.10
GYPA			
Pyrenees	61.32	18.72	50.31
Teruel	65.46	18.69	52.26
HBGG			
Pyrenees	66.46	19.34	50.47
Teruel	68.12	19.59	54.15
D7S8			
Pyrenees	56.01	18.68	50.19
Teruel	63.48	18.53	51.52
Gc			
Pyrenees	77.52	32.76	62.34
Teruel	75.48	29.52	60.44
HLA DQ A1			
Pyrenees	92.39	62.51	82.54
Teruel	92.97	62.36	82.42

PD = Power of discrimination.
CE = Chance of exclusion.
H = Heterozygosity.

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