TECHNICAL NOTE

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Population Data on the Loci LDLR, GYPA, HBGG, D7S8, and GC in Three Southwest European Populations

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ABSTRACT: Three Southwest European populations: Galicia (NW Spain), a mixed Spanish population from the rest of Spain (outside Galicia), and a population sample from the Coimbra area (Centre of Portugal) have been studied for the Low Density Lipoprotein Receptor (LDLR), Glycophorin A (GYPA), Hemoglobin G Gammaglobin (HBGG), D7S8 and Group Specific Component (GC). The allele and genotype frequencies found have been compared with other previously published data. All loci meet Hardy-Weinberg expectations in the three sampled populations. There was no evidence of association in any of the three population samples, between the five loci studied. No significant differences were found with Caucasian populations, nevertheless, significant differences were observed between our three population studies and the US SW Hispanic and African populations. The AmpliType PM DNA test greatly facilitates DNA testing in forensic laboratories, providing quick results and a good discrimination power from a single test.

KEYWORDS: forensic science, population studies

One of the most important developments in the field of human identity testing is the use of DNA typing to analyze biological evidence.

Although Variable Numbers of Tamdem Repeat Polymorphisms (VNTRs) are highly informative, their analysis as Restriction Fragment Length Polymorphisms (RFLPs) requires a relatively long time to carry out and also has other limitations such as its lower sensitivity and its difficulty in typing highly degraded samples. By using the Polymerase Chain Reaction (PCR) these disadvantages are overcome. PCR also has the additional advantage of being able to distinguish discrete alleles at each polymorphic locus, thereby avoiding the problems of database construction, the estimation of gene frequencies and the statistical evaluation of the results.

The number of highly polymorphic systems which can be analyzed using PCR is continuously being increased, not only in coding DNA but mainly in repetitive DNA.

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Several genetic loci, that are amenable to PCR, now can be analyzed using commercially available kits. The AmpliType® HLA DQα PCR Amplification and Typing kit (Perkin-Elmer) was the first PCR-based test applied to forensic casework analysis (1–3). Six alleles are able to be detected using the reverse dot blot format and sequence specific oligonucleotide (SSO) probes.

The AmpliType PM® PCR Amplification and Typing kit (Perkin-Elmer) is the second commercially available product for forensic casework analysis based on the same reverse dot blot typing technology (4). With this kit, the loci HLADQA1, LDLR (Low Density Lipoprotein Receptor) (5), GYPA (Glycophorin A) (6), HBGG (Hemoglobin G Gammaglobin) (7), D7S8 (8,9) and GC (Group Specific Component) (10) are amplified in a multiplex fashion. The last five loci listed are typed simultaneously in a single reverse dot blot strip containing immobilized allele specific probes; HLADQA1 must be typed in a separate strip. The chromosomal locations, PCR product size and number of alleles for each loci are given in Table 1.

This paper presents data on the frequencies of these markers, except HLADQA1, in three populations: from Galicia (NW Spain), a mixed Spanish population from the rest of Spain (outside Galicia), and a population sample from the Coimbra area (Centre of Portugal). Additional aims were to test whether or not the allele frequencies conform to Hardy-Weinberg expectations, to compare the results obtained with other population data and to obtain some statistical parameters of medico-legal interest such as the allelic diversity value, the power of discrimination and the chance of exclusion in paternity cases.

This paper also investigates the usefulness of the five loci LDLR, GYPA, HBGG, D7S8, and GC included in the AmpliType PM®

TABLE 1—*AmpliType® PM genetic marker characteristics.*

Locus	Chromosome	PCR product (bp)	N° Alleles
DQA1	6	239/242	6
LDLR	19	214	2
GYPA	4	190	2
HBGG	11	172	3
D7S8	7	151	2
GC	4	138	3

(Perkin-Elmer) system in order to determine their potential in the forensic casework.

Materials and Methods

Samples

Blood samples were taken from a total of 334 healthy unrelated individuals: 143 were from Galicia (NW Spain), 132 from the rest of Spain and 119 from Coimbra (Centre of Portugal). Blood was aliquoted (700 μL) and stored at −20°C before typing. Control bloodstains (from 1 to 10 μL) up to one year old and single hair roots were also used.

DNA Extraction

DNA was extracted from whole blood following a phenol-chlorophorm method (11). Briefly, the samples were incubated in 50 mM Tris-HCl, 150 mM NaCl and 100 mM $EDTANa_2$, with the addition of SDS (1.25%) and 0.3 mg/mL proteinase K, and precipitated with absolute ethanol after two extractions with phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1), respectively. DNA was quantified using a Perkin Elmer 552 UV/VIS spectrophotometer.

DNA from bloodstains and hair roots was extracted using the Chelex extraction procedure of Singer-Sam et al. (12).

DNA Amplification

PCR amplification was performed using the AmpliType® PM PCR Amplification and Typing Kit (Perkin Elmer) following the manufacturer's recommended protocol. The amplification reaction contained 40 μL of AmpliType® PM PCR Reaction Mix, 40 μL of AmpliType® Primer Set and 20 μL of DNA (1ng/μL).

Amplification was carried out in a Perkin Elmer GeneAmp® 9600 thermal cycler for 32 cycles: 30 s at 95°C for denaturation, 30 s at 63°C for primer annealing, and 30 s at 72°C for primer extension. After 32 cycles, the samples were incubated an additional 10 min at 72°C.

Verification of PCR Amplification

The presence and size of AmpliType® PM PCR products was verified by electrophoresis through agarose gels (3% NuSieve® and 1% SeaKem®) in 0.5X TBE buffer (44.5 mM Trisborate, 1 mM EDTA) containing 0.5 μg/mL ethidium bromide. The Gibco BRL 123 bp ladder was used as molecular weight marker. The gel was run at 115 V for approximately 1 hour. Native polyacrylamide gels (PhastGels High Density, Pharmacia) run in the Phast-System (Pharmacia-LKB) were also used. Detection of the PCR amplified products was made with the silver staining method of Heukeshoven and Dernick (13) (Fig. 1).

DNA Typing

Typing of these five loci was performed by reverse dot blot with ASO (Allele Specific Oligonucleotides) probes, using the AmpliType® PM PCR Amplification and Typing Kit and following the manufacturer's recommended protocol. The amplified PCR products were hybridized to DNA probe strips and specifically bound amplified DNA was visualized upon the enzymatic conversion of a colorless substrate into a blue colored precipitate (Fig. 2).

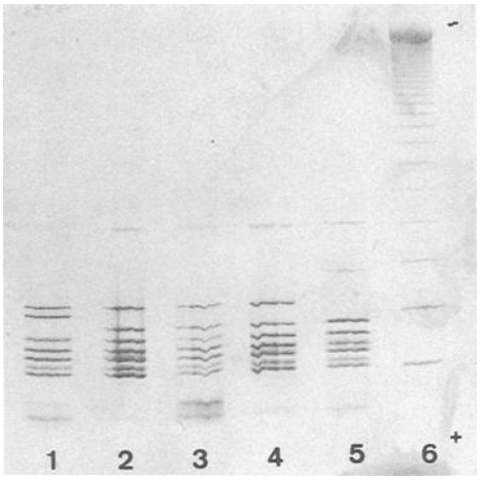

FIG. 1—*Native polyacrylamide gel showing AmpliType® PM PCR products. The following bands are present: 242/239 bp (HLA DQA1), 214 bp (LDLR), 190 bp (GYPA), 172 bp (HBGG), 151 bp (D7S8) and 138 bp (GC). Lane 1–4: HLA DQA1 heterozygote. Lane 5: HLA DQA1 homozygote. Lane 6: GIBCO BRL 123 bp ladder.*

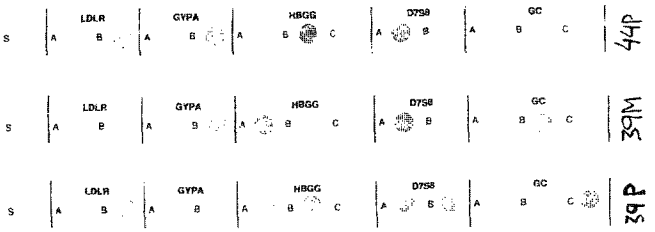

FIG. 2—*LDLR, GYPA, HBGG, D7S8 and GC phenotypes of three samples using Amplitype® PM DNA Probe Strips.*

Statistical Analysis

Hardy-Weinberg equilibrium was tested for with conventional Pearson's chi-square method (χ^2), and with the Exact Test proposed by Guo and Thompson (14) and based on conventional Monte Carlo methods. For the Monte Carlo method used in this study, the number of batches was 100 and the size of each batch 170, as proposed by Guo and Thompson (14).

The Test for nonrandom association of alleles, (s_k^2) Test, was performed by computing the confidence interval of the observed variance (s_k^2) of the number of heterozygous loci. Following Chakraborty (15):

$$s_k^2 = (N_1 + 4N_2)/n - [(N_1 + 2N_2)/n]^2$$

where N_0 = frequencies of double homozygotes, N_1 = frequencies of single heterozygotes and N_2 = frequencies of double heterozygotes, and the variance of s_k^2 may be written as:

$$\mathrm{Var}(s_k^2) \approx \left\{ \sum_j h_j - 7 \sum_j h_j^2 + 12 \sum_j h_j^3 - 6 \sum_j h_j^4 + 2\left[\sum_j h_j(1 - h_j) \right]^2 \right\}/n$$

with h_j being the estimated panmitic heterozygosities at each locus (16).

Using this variance and assuming that the sampling distribution of s_k^2 approximates normality, the upper 95% confidence limit for s_k^2 is:

$$L \cong \Sigma h_j - \Sigma h_j^2 + 2\{\text{var } s_k^2\}^{1/2}$$

according to Brown et al. (16).

Comparison of population data was carried out using a two-way RxC contingency table test comparing allele distributions for population sample homogeneity and using a conventional chi-square as statistical parameter.

The potential usefulness of the five markers was assessed by calculating the heterozygosity value (h), the power of discrimination (PD) and the chance of exclusion (CE).

The heterozygosity value was calculated using the formula:

$$h = 2n(1 - \Sigma x_i^2)/(2n - 1)$$

where x_i = allele frequencies and n = total number of individuals sampled, as described by Nei and Roychoudhury (17).

The PD (the probability that two individuals chosen at random from a given population have different phenotypes) was calculated using the formula:

$$PD = 1 - \sum_{i=1}^{n} p_i^2$$

where p_i = expected phenotype frequencies (18).

The CE (the probability of excluding a falsely accused man as the father in paternity analysis) was calculated using the formula:

$$CE = \sum_{i=1}^{n} p_i(1 - p_i)^2(1 - p_i + p_i^2) + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} p_i p_j (p_i + p_j)(1 - p_i - p_j)^2$$

where p_i, p_j = respective gene frequencies (19).

Results and Discussion

Figure 3 shows the geographic location of the sampling. The three populations studied have different characteristics. The Galician population (NW Spain) is a well defined population from both the cultural and genetic points of view. It has been very well characterized from the genetic point of view, not only for classical groups (20), but also for DNA polymorphisms (11,21). The Galician population is an unstratified and panmitic population, with an immigration rate near zero during the last 15 centuries.

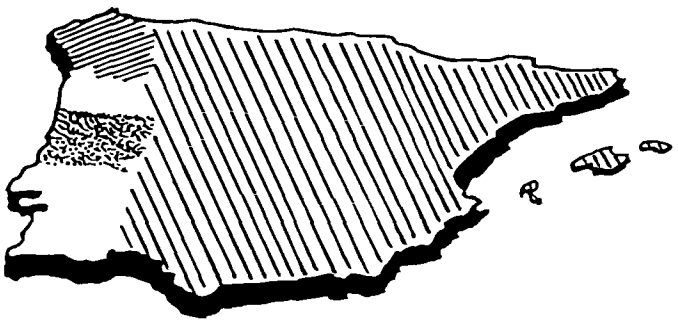

FIG. 3—*Geographical areas of population study.* ▤ *Galicia;* ▨ *Rest of Spain;* ▩ *Coimbra area.*

Central Portugal is a population that is not so well defined, but, nevertheless, unstratified. It has had some migrations which include Arabic invasions of the Iberian peninsula.

The Spanish population is considered to be a stratified population which reflects the six centuries of Arab rule in Southern Spain, while at the same time, there are some well defined populations like the Basque population in Northern Spain.

The distributions of phenotypes and allelic frequencies from the population survey in Galicia, the rest of Spain, and the Coimbra area are shown in Tables 2–3. The analysis of the observed and the expected values for each population gave a non-significant P value, based on the χ^2 Test and Exact Test, indicating that the three sampled populations do not deviate from Hardy-Weinberg equilibrium for the five loci.

Although the loci in this study are on different chromosomes (except for GYPA and GC, which are on different arms of chromosome 4), and, it might be expected that their frequencies are independent, analyses were performed to determine whether or not there were any detectable associations between any of the loci studied. Independence among the five loci was evaluated by examining whether or not the observed variance (s_k^2) of the number of heterozygous loci in the population sample was outside its confidence interval, under the assumption of independence using the procedure described by Brown et al. (16). Chakraborty (15) showed that this test is more powerful than the classical χ^2 Test. There was no evidence of association in any of the three population samples, between the five loci studied, using the s_k^2 criterion (Table 4). Taking into account the Hardy-Weinberg equilibrium and the s_k^2 Test, no evidence of substructure was found in the Spanish population sample.

Comparison of Population Studies

Comparisons were performed between Galicia, the sampled populations from the rest of Spain and Coimbra. Comparisons were

TABLE 2—*Observed and expected (in parenthesis) genotype frequencies.*

Genetic Marker	Genotype	Galicia N = 143	Spain N = 132	Coimbra N = 119
LDLR	AA	0.161 (0.159)	0.189 (0.193)	0.176 (0.187)
	AB	0.475 (0.479)	0.500 (0.493)	0.513 (0.491)
	BB	0.364 (0.362)	0.311 (0.314)	0.311 (0.322)
GYPA	AA	0.224 (0.233)	0.258 (0.258)	0.294 (0.312)
	AB	0.517 (0.499)	0.500 (0.499)	0.530 (0.493)
	BB	0.259 (0.268)	0.242 (0.243)	0.176 (0.195)
HBGG	AA	0.322 (0.350)	0.220 (0.228)	0.201 (0.230)
	AB	0.524 (0.471)	0.515 (0.495)	0.538 (0.483)
	AC	0.014 (0.012)	0.000 (0.003)	0.017 (0.016)
	BB	0.133 (0.159)	0.258 (0.270)	0.227 (0.254)
	BC	0.007 (0.008)	0.007 (0.004)	0.017 (0.017)
	CC	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
D7S8	AA	0.315 (0.313)	0.303 (0.332)	0.286 (0.326)
	AB	0.489 (0.493)	0.545 (0.488)	0.571 (0.490)
	BB	0.196 (0.194)	0.152 (0.180)	0.143 (0.184)
GC	AA	0.070 (0.076)	0.076 (0.076)	0.093 (0.124)
	AB	0.112 (0.097)	0.060 (0.086)	0.109 (0.104)
	AC	0.300 (0.303)	0.341 (0.314)	0.412 (0.353)
	BB	0.028 (0.031)	0.023 (0.024)	0.025 (0.022)
	BC	0.182 (0.192)	0.205 (0.177)	0.134 (0.147)
	CC	0.308 (0.301)	0.295 (0.323)	0.227 (0.250)

TABLE 3—*Allele frequencies and Hardy-Weinberg equilibrium.*

Locus	Allele	Galicia $N = 143$	Spain $N = 132$	Coimbra $N = 119$
LDLR	A	0.399	0.439	0.433
	B	0.601	0.561	0.567
		$\chi^2 = 0.009\ p = 0.924$	$\chi^2 = 0.029\ p = 0.864$	$\chi^2 = 0.231\ p = 0.631$
		Exact Test: 0.905	Exact Test: 0.903	Exact Test: 0.693
GYPA	A	0.483	0.508	0.559
	B	0.517	0.492	0.441
		$\chi^2 = 0.188\ p = 0.665$	$\chi^2 = 0.000\ p = 0.483$	$\chi^2 = 0.646\ p = 0.506$
		Exact Test: 0.723	Exact Test: 0.538	Exact Test: 0.635
HBGG	A	0.591	0.477	0.479
	B	0.399	0.519	0.504
	C	0.010	0.004	0.017
		$\chi^2 = 1.861\ p = 0.602$	$\chi^2 = 1.135\ p = 0.567$	$\chi^2 = 1.526\ p = 0.676$
		Exact Test: 0.614	Exact Test: 0.605	Exact Test: 0.623
D7S8	A	0.559	0.576	0.571
	B	0.441	0.424	0.429
		$\chi^2 = 0.007\ p = 0.934$	$\chi^2 = 1.793\ p = 0.180$	$\chi^2 = 3.305\ p = 0.069$
		Exact Test: 0.968	Exact Test: 0.324	Exact Test: 0.142
GC	A	0.276	0.277	0.353
	B	0.175	0.155	0.147
	C	0.549	0.568	0.500
		$\chi^2 = 0.553\ p = 0.907$	$\chi^2 = 2.188\ p = 0.534$	$\chi^2 = 2.640\ p = 0.451$
		Exact Test: 0.883	Exact Test: 0.653	Exact Test: 0.492

TABLE 4—*Test of significance for nonrandom association of alleles* (s_k^2).

Galicia		Spain		Coimbra	
s_k^2	Confidence Interval 95%	s_k^2	Confidence Interval 95%	s_k^2	Confidence Interval 95%
0.489	0.583	0.492	0.587	0.535	0.591
0.494	0.583	0.583	0.587	0.524	0.591
0.517	0.583	0.542	0.587	0.504	0.591
0.454	0.575	0.506	0.581	0.475	0.581
0.533	0.583	0.454	0.587	0.554	0.591
0.412	0.583	0.444	0.587	0.561	0.591
0.521	0.575	0.455	0.581	0.358	0.581
0.562	0.583	0.516	0.587	0.469	0.591
0.489	0.575	0.492	0.581	0.546	0.589
0.550	0.575	0.497	0.581	0.449	0.581

also made between these three groups, the Swiss population (22) and also with four population groups from the USA (23) (Table 5).

No significant differences were found in any of the three populations (Galicia, rest of Spain and Coimbra) for any of the five loci studied, with the only exception being the comparison of Galicia with the rest of Spain for the HBGG system ($P = 0.04$) which is not highly significant, indicating that a correlation exists between the Galician population and the population from the rest of Spain.

Likewise, a comparison of our data with data from the Swiss population and US Caucasian population showed that the distribution of alleles was similar. Again, small differences were observed for the HBGG system ($P = 0.025$ between Galicia and Switzerland, and $P = 0.015$ between Galicia and U.S. Caucasians) and GYPA ($P = 0.041$ between Galicia and U.S. Caucasians). However, if one corrects for multiple typing, then they are not significant. The reason for these minor differences found between the population of Galicia and other Caucasian populations may correspond to the characteristics of the Galician population that we have previously mentioned.

Nevertheless, significant differences for most of the systems were observed between our three populations studies and the US SW Hispanic and African populations; the main differences corresponding to the comparison with the African populations ($P < 0.001$).

As for the comparison with the US SE Hispanic population, no differences were found between this population and our population data, with the only exception being the comparison with Galicia for the HBGG system ($P = 0.005$).

Other Statistical Parameters

Other statistical parameters of genetic and medico-legal interest are shown in Table 6. The combined chance of exclusion was 0.703 in the Galician population, 0.698 in the Spanish population and 0.708 in the Coimbra population. The combined discrimination power was 0.995 in the Galician population, 0.995 in the Spanish population and 0.999 in the Coimbra population. The heterozygosity values (h) ranged from 0.475 for LDLR to 0.594 for GC in the Galician population, from 0.500 for LDLR and GYPA to 0.606 for GC in the Spanish population and from 0.490 for D7S8 to 0.604 for GC in the Coimbra population.

Forensic Usefulness

All forensic samples tested (bloodstains and hair roots) were unequivocally typed even when minute bloodstains (1 μL) and single hairs were used. From whole blood, the minimal amount of DNA with reliable results was 2 ng of purified DNA. These results are in agreement with the observations of Herring et al. (24).

The AmpliType PM test greatly facilitates DNA testing in forensic laboratories. This test has the advantage of providing results in less than four hours and of obtaining and of combining multiple gene frequency data from a single test. This provides a good discrimination power without increased sample consumption, which is specially valuable when the sample is a limiting factor.

TABLE 5—*Comparison of the five markers in different populations.*

	LDLR	GYPA	HBGG	D7S8	GC
Galicia	$\chi^2 = 0.98$	$\chi^2 = 0.44$	$\chi^2 = 10.02$	$\chi^2 = 1.22$	$\chi^2 = 2.74$
Spain	$p = 0.614$, 2 d.f.	$p = 0.802$, 2 d.f.	$p = 0.040$, 4 d.f.	$p = 0.544$, 2 d.f.	$p = 0.740$, 5 d.f.
Galicia	$\chi^2 = 0.81$	$\chi^2 = 3.26$	$\chi^2 = 7.37$	$\chi^2 = 2.07$	$\chi^2 = 5.19$
Coimbra	$p = 0.668$, 2 d.f.	$p = 0.196$, 2 d.f.	$p = 0.117$, 4 d.f.	$p = 0.355$, 2 d.f.	$p = 0.393$, 5 d.f.
Spain	$\chi^2 = 0.08$	$\chi^2 = 1.70$	$\chi^2 = 3.06$	$\chi^2 = 0.17$	$\chi^2 = 5.75$
Coimbra	$p = 0.962$, 2 d.f.	$p = 0.427$, 2 d.f.	$p = 0.547$, 4 d.f.	$p = 0.918$, 2 d.f.	$p = 0.332$, 5 d.f.
Galicia	$\chi^2 = 0.96$	$\chi^2 = 1.57$	$\chi^2 = 11.16$	$\chi^2 = 0.51$	$\chi^2 = 3.48$
Switzerland	$p = 0.616$, 2 d.f.	$p = 0.455$, 2 d.f.	$p = 0.025$, 4 d.f.	$p = 0.775$, 2 d.f.	$p = 0.627$, 5 d.f.
Galicia	$\chi^2 = 2.97$	$\chi^2 = 6.42$	$\chi^2 = 14.15$	$\chi^2 = 2.54$	$\chi^2 = 2.37$
U.S. Caucasian	$p = 0.226$, 2 d.f.	$p = 0.041$, 2 d.f.	$p = 0.015$, 5 d.f.	$p = 0.281$, 2 d.f.	$p = 0.796$, 5 d.f.
Galicia	$\chi^2 = 0.41$	$\chi^2 = 3.95$	$\chi^2 = 14.65$	$\chi^2 = 0.32$	$\chi^2 = 2.97$
U.S. SE Hispanic	$p = 0.815$, 2 d.f.	$p = 0.139$, 2 d.f.	$p = 0.005$, 4 d.f.	$p = 0.854$, 2 d.f.	$p = 0.705$, 5 d.f.
Galicia	$\chi^2 = 12.11$	$\chi^2 = 14.60$	$\chi^2 = 35.10$	$\chi^2 = 7.32$	$\chi^2 = 2.73$
U.S. SW Hispanic	$p = 0.002$, 2 d.f.	$p < 0.001$, 2 d.f.	$p < 0.001$, 4 d.f.	$p = 0.027$, 2 d.f.	$p = 0.741$, 5 d.f.
Galicia	$\chi^2 = 19.76$	$\chi^2 = 0.064$	$\chi^2 = 93.92$	$\chi^2 = 4.10$	$\chi^2 = 125.81$
U.S. African	$p < 0.001$, 2 d.f.	$p = 0.970$, 2 d.f.	$p < 0.001$, 5 d.f.	$p = 0.129$, 2 d.f.	$p < 0.001$, 5 d.f.
Spain	$\chi^2 = 0.57$	$\chi^2 = 1.31$	$\chi^2 = 1.31$	$\chi^2 = 0.29$	$\chi^2 = 5.86$
Switzerland	$p = 0.752$, 2 d.f.	$p = 0.520$, 2 d.f.	$p = 0.726$, 3 d.f.	$p = 0.864$, 2 d.f.	$p = 0.320$, 5 d.f.
Spain	$\chi^2 = 0.85$	$\chi^2 = 3.35$	$\chi^2 = 2.18$	$\chi^2 = 1.04$	$\chi^2 = 2.32$
U.S. Caucasian	$p = 0.654$, 2 d.f.	$p = 0.187$, 2 d.f.	$p = 0.702$, 4 d.f.	$p = 0.594$, 2 d.f.	$p = 0.803$, 5 d.f.
Spain	$\chi^2 = 0.76$	$\chi^2 = 2.21$	$\chi^2 = 5.48$	$\chi^2 = 0.69$	$\chi^2 = 9.52$
U.S. SE Hispanic	$p = 0.685$, 2 d.f.	$p = 0.331$, 2 d.f.	$p = 0.242$, 4 d.f.	$p = 0.707$, 2 d.f.	$p = 0.090$, 5 d.f.
Spain	$\chi^2 = 6.75$	$\chi^2 = 10.02$	$\chi^2 = 18.59$	$\chi^2 = 6.14$	$\chi^2 = 3.22$
U.S. SW Hispanic	$p = 0.034$, 2 d.f.	$p = 0.007$, 2 d.f.	$p < 0.001$, 4 d.f.	$p = 0.046$, 2 d.f.	$p = 0.666$, d.f.5
Spain	$\chi^2 = 28.03$	$\chi^2 = 0.45$	$\chi^2 = 105.51$	$\chi^2 = 1.17$	$\chi^2 = 129.21$
U.S. African	$p < 0.001$, 2 d.f.	$p = 0.799$, 2 d.f.	$p < 0.001$, 5 d.f.	$p = 0.557$, 2 d.f.	$p < 0.001$, 5 d.f.
Coimbra	$\chi^2 = 0.9$	$\chi^2 = 0.81$	$\chi^2 = 5.06$	$\chi^2 = 0.83$	$\chi^2 = 8.45$
Switzerland	$p = 0.638$, 2 d.f.	$p = 0.667$, 2 d.f.	$p = 0.281$, 4 d.f.	$p = 0.660$, 2 d.f.	$p = 0.133$, 5 d.f.
Coimbra	$\chi^2 = 0.59$	$\chi^2 = 1.21$	$\chi^2 = 6.83$	$\chi^2 = 1.57$	$\chi^2 = 6.96$
U.S. Caucasian	$p = 0.745$, 2 d.f.	$p = 0.546$, 2 d.f.	$p = 0.234$, 5 d.f.	$p = 0.455$, 2 d.f.	$p = 0.224$, 5 d.f.
Coimbra	$\chi^2 = 0.94$	$\chi^2 = 3.90$	$\chi^2 = 2.24$	$\chi^2 = 1.42$	$\chi^2 = 7.93$
U.S. SE Hispanic	$p = 0.625$, 2 d.f.	$p = 0.142$, 2 d.f.	$p = 0.691$, 4 d.f.	$p = 0.491$, 2 d.f.	$p = 0.160$, 5 d.f.
Coimbra	$\chi^2 = 7.33$	$\chi^2 = 5.44$	$\chi^2 = 13.59$	$\chi^2 = 6.99$	$\chi^2 = 6.47$
U.S. SW Hispanic	$p = 0.026$, 2 d.f.	$p = 0.066$, 2 d.f.	$p = 0.009$, 4 d.f.	$p = 0.030$, 2 d.f.	$p = 0.263$, 5 d.f.
Coimbra	$\chi^2 = 25.74$	$\chi^2 = 3.67$	$\chi^2 = 89.9$	$\chi^2 = 1.16$	$\chi^2 = 125.03$
U.S. African	$p < 0.001$, 2 d.f.	$p = 0.159$, 2 d.f.	$p < 0.001$, 5 d.f.	$p = 0.558$, 2 d.f.	$p < 0.001$, 5

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

Marker	Statistic	Galicia	Spain	Coimbra
LDLR	PD	0.614	0.621	0.620
	CE	0.182	0.186	0.185
	h	0.475	0.500	0.491
GYPA	PD	0.625	0.625	0.621
	CE	0.187	0.187	0.186
	h	0.517	0.500	0.493
HBGG	PD	0.631	0.613	0.649
	CE	0.196	0.192	0.209
	h	0.545	0.523	0.516
D7S8	PD	0.621	0.619	0.620
	CE	0.186	0.185	0.185
	h	0.489	0.545	0.490
GC	PD	0.764	0.752	0.765
	CE	0.317	0.306	0.317
	h	0.594	0.606	0.604

PD = Power of Discrimination
CE = Chance of Exclusion
h = heterozygosity

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