TECHNICAL NOTE

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South Portuguese Population Data on the Loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and Gc

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ABSTRACT: Five South Portuguese Caucasian subpopulations were analyzed for the HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and Gc loci. Genotype distributions for these loci did not deviate from Hardy-Weinberg expectations. The allele and genotype frequencies found have been compared with previously published data from North and Central Portugal. A total of 11 out of 138 chi-square comparisons of allele frequencies between different Portuguese populations showed a certain degree of divergence. Alentejo, Algarve, Madeira Island and Azores Islands populations might be considered as different groups in a database. For forensic casework, a composite South Portuguese Caucasian population database was obtained for estimating multiple locus profile frequencies using the six PCR-based loci studied.

KEYWORDS: forensic science, DNA typing, population genetics, South Portugal, polymerase chain reaction, HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc

DNA profiling has rapidly become a routine technique in forensic laboratories. One of the PCR strategies that have been developed for forensic application is the reverse dot-blot analysis of HLA-DQA1 (1,2) and Polymarker (PM) systems (3). HLA-DQA1 is a well characterized PCR-based forensic system, located on chromosome 6. The PM system include five loci: on chromosome 19—Low Density Lipoprotein Receptor (LDLR), on chromosome 4—Glycophorin A (GYPA), on chromosome 11—Haemoglobin G Gammaglobin (HBGG), on chromosome 7—D7S8 and on chromosome 4—Group Specific Component (Gc).

For estimating multiple locus genetic profile in identity testing it is necessary to compile allele/genotype data from subpopulations that can allow a relevant estimate of any potential differences in subgroups appropriate to forensic casework analysis.

Substantial population data for HLA-DQA1 and PM loci exist in American (1–5), Asiatic (6–13), Australian (14,15) and European populations (16–21). There also have been some subpopulation data generated (22–31), in which different populations in relatively smaller geographical regions or ethnic groups were compared.

The South Portuguese Caucasian population can be divided into five regions: Estremadura, Alentejo, Algarve, Madeira Island and Azores Islands, based on specific culture and historical aspects. Figure 1 shows the geographic location of these regions. Some of these populations are relatively isolated and may offer some insight into genetic variation among subgroups.

This paper provides population genetic data on six PCR-based loci in South Portuguese Caucasian subpopulations and a composite South Portuguese Caucasian population and compares data with North and Central Portugal (28–31).

Materials and Methods

Population Samples

Whole blood samples were collected in EDTA tubes from a total of 514 healthy unrelated South Portuguese Caucasian individuals obtained from paternity investigation casework. Five different South Portugal regions (Fig. 1) were studied, namely Estremadura (including Ribatejo) (n = 187), Alentejo (n = 89), Algarve (n = 31), Madeira Island (n = 149) and Azores Islands (n = 58). All individuals and their parents were natives to their respective regions.

Blood samples were air-dried on cotton cloth and DNA was extracted by the Chelex method, previously described by Singer-Sam et al. (32).

Amplification and Typing

The DNA samples were amplified and typed for HLA-DQA1 by using the Amplitype HLA-DQA1 Forensic DNA Amplification and Typing Kit (Perkin Elmer Corporation, Norwalk, CT). For PM loci (LDLR, GYPA, HBGG, D7S8 and Gc), DNA samples were amplified and typed by using the Amplitype PM PCR Amplification and Typing Kit (Perkin Elmer Corporation, Norwalk, CT), according to the manufacturer's protocol. Amplification by PCR was carried out in a GeneAmp PCR System 2400 (Perkin-Elmer). One to 10 ng of DNA were used for PCR.

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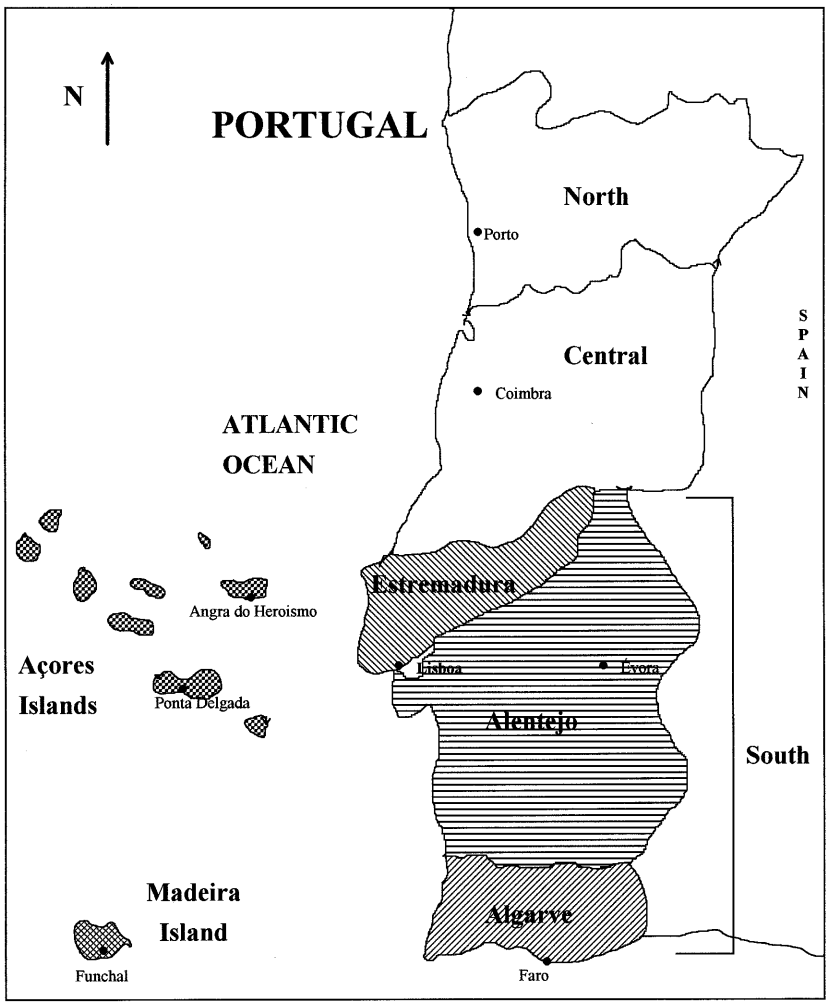

FIG. 1—*Geographic location of the Portuguese regions: North, Central and South (Estremadura, Alentejo, Algarve, Madeira and Azores Islands).*

Statistical Analysis

The distribution of the different genotype and allele frequencies for HLA-DQA1 and PM loci in the five different Portuguese subpopulation samples and for the total South Portugal sample was determined.

Departure from Hardy-Weinberg expectations was tested for each locus using conventional Pearson's chi-square method (χ^2). An exact significance probability test proposed by Sokal and Rohlf (33) was also performed.

Heterogeneity in allele frequencies for each locus was estimated for all pairwise comparisons of the populations groups by means of two-way RxC contingency table test and using a conventional χ^2 statistical parameter. Sampled populations were also compared with published data from North Portugal (28,29) and Central Portugal (30,31).

The potential usefulness of the studied markers for forensic studies in the Portuguese population was assessed by calculating the power of discrimination (PD), the chance of exclusion (CE) and the heterozygosity value (H).

The power of discrimination for each locus was calculated from genotype data, according to the formula given by Fisher (34). The chance of exclusion was calculated according to the formula given by Ohno et al. (35). The unbiased heterozygosity value was calculated using the formula described by Nei and Roychoudhury (36).

Results and Discussion

Genotype and Allele Frequencies

Genotype and allele frequencies for the HLA-DQA1 and PM loci in the five different Portuguese subpopulations and for the combined South Portugal population are shown in Tables 1–4.

The genotype frequencies for all loci did not deviate from Hardy-Weinberg expectations based on exact probability tests (Tables 1 and 3). By using the conventional χ^2 method, just three slight deviations from the Hardy-Weinberg equilibrium were observed due to an excess of heterozygotes at Gc in Estremadura and Alentejo samples and a deficit of heterozygotes at HLA-DQA1 in Alentejo. It should be noted that there would still be little impact for forensic purposes using these allele frequencies assuming independence, particularly because the exact probabilities did not show any deviations. In what concerns the combined South Portugal population, the genotype frequencies for the six loci did not deviate from Hardy-Weinberg expectations based on the conventional χ^2 method and on the exact probability tests.

The allele frequencies for the HLA-DQA1 and PM loci showed to be consistent with other European Caucasian populations.

Comparing the five Portuguese subpopulations, while Estremadura had the lowest value for HBGG allele C, Algarve had the highest values for the LDLR allele B, the HBGG allele C and the

Gc allele B. These data suggest that Algarve may have some African admixture as we will discuss later on.

Forensic Statistical Parameters

The forensic statistical parameters of the studied loci are presented in Table 5. In all five Portuguese subpopulations, HLA-DQA1 locus had a high heterozygosity ($H > 0.79$) and a high power of discrimination ($PD > 0.91$). For the PM system, the higher power of discrimination was obtained for the Gc locus, specifically, in the Algarve region ($PD = 0.786$), while the heterozygosity was just slightly higher ($H = 0.634$).

The combined power of discrimination for HLA-DQA1 and PM in the South Portugal population was 0.999; the combined chance of exclusion for the six loci studied in the same combined population was 0.885, while the mean heterozygosity value was 0.562, ranging from 0.477 for D7S8 to 0.809 for HLA-DQA1 (Table 6).

By the analysis of the statistical parameters obtained (PD, CE and H), it is shown that these markers are very useful for forensic application in all five subpopulations and in the combined South Portugal Caucasian population studied.

Comparison between Populations

Comparisons were performed between samples from the five Portuguese regions, and also with other Portuguese populations (28–31). A total of 11 out of 138 chi-square test performed were significant (Table 7). Differences were observed for LDLR, GYPA and HBGG systems, mainly, when Alentejo and Algarve populations were compared with other Portuguese subpopulations. No significant differences were found for HLA-DQA1, D7S8 and Gc systems. When the total South Portugal population was compared with North and Central Portugal populations, no significant differences in allele frequencies were observed. These results suggest that a weighted construct of the South Portugal population to the actual population percentages would not be substantially different.

According to the results on HLA-DQA1 and PM loci, some Portuguese subpopulation data might be considered for estimating DNA profile frequencies. Alentejo, Algarve, Madeira and Azores Islands populations could be considered as independent groups in a database. However, the small number of differences observed had no statistical significance when the corrections for the multiple

TABLE 1—*Observed genotype frequency distributions and Hardy-Weinberg equilibrium for HLA-DQA1 locus in the South Portuguese Caucasian populations.*

Genotype	Estremadura (n = 182)	Alentejo (n = 81)	Algarve (n = 31)	Madeira Island (n = 149)	Azores Islands (n = 57)	South Portugal (n = 500)
1.1–1.1	0.011	0.025	0.032	0.027	0.052	0.024
1.1–1.2	0.033	0.049	0.032	0.040	0.052	0.040
1.1–1.3	0.028	0.062	0.032	0.040	0.018	0.036
1.1–2	0.055	0.012	0.032	0.060	0.000	0.042
1.1–3	0.039	0.062	0.065	0.027	0.070	0.044
1.1–4	0.099	0.074	0.161	0.121	0.105	0.106
1.2–1.2	0.017	0.025	0.032	0.034	0.018	0.024
1.2–1.3	0.033	0.037	0.000	0.040	0.018	0.032
1.2–2	0.039	0.000	0.032	0.060	0.035	0.038
1.2–3	0.028	0.025	0.065	0.000	0.035	0.022
1.2–4	0.071	0.161	0.194	0.067	0.123	0.098
1.3–1.3	0.006	0.049	0.032	0.013	0.018	0.018
1.3–2	0.039	0.012	0.000	0.013	0.035	0.024
1.3–3	0.033	0.000	0.000	0.013	0.018	0.018
1.3–4	0.065	0.049	0.000	0.047	0.052	0.052
2–2	0.043	0.025	0.000	0.047	0.035	0.038
2–3	0.043	0.062	0.032	0.040	0.035	0.044
2–4	0.126	0.111	0.065	0.114	0.105	0.114
3–3	0.022	0.025	0.000	0.034	0.018	0.024
3–4	0.077	0.049	0.129	0.094	0.123	0.086
4–4	0.093	0.086	0.065	0.067	0.035	0.076
χ^2	3.332	26.591	26.623	20.038	10.602	20.084
p	0.999	0.032	0.055	0.170	0.780	0.169
p*	0.864	0.800	0.420	0.426	0.111	0.091

* Exact test probability.

TABLE 2—*HLA-DQA1 observed allele frequencies in the South Portuguese Caucasian populations.*

Allele	Estremadura (n = 182)	Alentejo (n = 81)	Algarve (n = 31)	Madeira Island (n = 149)	Azores Islands (n = 57)	South Portugal (n = 500)
1.1	0.137	0.154	0.194	0.171	0.175	0.158
1.2	0.118	0.160	0.194	0.138	0.149	0.139
1.3	0.104	0.130	0.048	0.091	0.088	0.099
2	0.195	0.123	0.081	0.191	0.140	0.169
3	0.132	0.123	0.145	0.121	0.158	0.131
4	0.313	0.309	0.339	0.289	0.289	0.304

TABLE 3—*Observed genotype frequency distributions and Hardy-Weinberg equilibrium for PM loci in the South Portuguese Caucasian populations.*

Locus	Genotype	Estremadura (n = 187)	Alentejo (n = 89)	Algarve (n = 31)	Madeira Island (n = 149)	Azores Islands (n = 58)	South Portugal (n = 514)
LDLR	AA	0.166	0.169	0.097	0.148	0.207	0.162
	AB	0.508	0.584	0.419	0.503	0.603	0.525
	BB	0.326	0.247	0.484	0.349	0.190	0.313
	χ^2	0.302	2.577	0.028	0.314	2.287	2.843
	p	0.582	0.108	0.867	0.575	0.130	0.092
	*p**	0.653	0.136	1.000	0.611	0.188	0.104
GYPA	AA	0.262	0.360	0.193	0.295	0.224	0.280
	AB	0.508	0.528	0.581	0.443	0.448	0.490
	BB	0.230	0.112	0.226	0.262	0.328	0.230
	χ^2	0.039	1.268	0.662	2.021	0.609	0.165
	p	0.844	0.260	0.416	0.155	0.435	0.685
	*p**	0.884	0.365	0.485	0.189	0.594	0.724
HBGG	AA	0.305	0.225	0.323	0.154	0.259	0.243
	AB	0.449	0.506	0.355	0.537	0.517	0.486
	AC	0.005	0.022	0.032	0.000	0.000	0.008
	BB	0.235	0.236	0.226	0.275	0.190	0.241
	BC	0.005	0.011	0.064	0.034	0.034	0.022
	CC	0.000	0.000	0.000	0.000	0.000	0.000
	χ^2	1.514	0.516	2.005	6.196	2.546	3.396
	p	0.679	0.915	0.571	0.102	0.467	0.335
	*p**	0.241	0.834	0.279	0.068	1.000	0.791
D7S8	AA	0.390	0.416	0.516	0.356	0.293	0.381
	AB	0.428	0.438	0.323	0.503	0.500	0.453
	BB	0.182	0.146	0.161	0.141	0.207	0.166
	χ^2	2.189	0.327	2.425	0.404	0.001	1.286
	p	0.139	0.568	0.119	0.525	0.993	0.257
	*p**	0.168	0.648	0.211	0.606	1.000	0.268
Gc	AA	0.086	0.045	0.000	0.054	0.035	0.058
	AB	0.043	0.135	0.161	0.107	0.103	0.091
	AC	0.337	0.427	0.387	0.309	0.310	0.344
	BB	0.011	0.000	0.032	0.040	0.034	0.021
	BC	0.219	0.123	0.226	0.181	0.172	0.188
	CC	0.304	0.270	0.194	0.309	0.345	0.298
	χ^2	8.069	8.746	4.356	1.390	1.249	5.012
	p	0.045	0.033	0.226	0.708	0.741	0.171
	*p**	0.070	0.393	0.295	1.000	1.000	0.088

* Exact test probability.

TABLE 4—*PM observed allele frequencies in the South Portuguese Caucasian populations.*

Locus	Allele	Estremadura (n = 187)	Alentejo (n = 89)	Algarve (n = 31)	Madeira Island (n = 149)	Azores Islands (n = 58)	South Portugal (n = 514)
LDLR	A	0.420	0.461	0.306	0.399	0.509	0.424
	B	0.580	0.539	0.694	0.601	0.491	0.576
GYPA	A	0.516	0.624	0.484	0.517	0.448	0.525
	B	0.484	0.376	0.516	0.483	0.552	0.475
HBGG	A	0.532	0.489	0.516	0.423	0.517	0.490
	B	0.463	0.494	0.435	0.560	0.466	0.495
	C	0.005	0.017	0.048	0.017	0.017	0.015
D7S8	A	0.604	0.635	0.677	0.607	0.543	0.608
	B	0.396	0.365	0.323	0.393	0.457	0.392
Gc	A	0.275	0.326	0.274	0.262	0.241	0.276
	B	0.142	0.129	0.226	0.185	0.172	0.161
	C	0.583	0.545	0.500	0.554	0.586	0.563

typing were considered. Although there were differences in allele frequencies at some loci in some of the subgroups, an estimate of a six locus profile would be rare in all subgroups. More data are needed to determine whether or not the hypothesis that substantial differences in allele frequencies could affect forensic estimates.

One of the possible reasons for the significant differences observed between Alentejo, Algarve and all the other subpopulations could be related to historical events. Alentejo and Algarve populations probably reflect the Arab occupation influence in Portugal until the 13th century, allied with a high degree of African migration during the 16th and 17th centuries, which might result in a potential African admixture. The observed differences between

TABLE 5—*Statistical parameters of forensic value for HLA-DQA1 and PM loci in the South Portuguese Caucasian subpopulations.*

Locus	Statistical Parameters	Estremadura	Alentejo	Algarve	Madeira Island	Azores Islands
HLA-DQA1	PD	0.934	0.937	0.919	0.937	0.938
	CE	0.614	0.622	0.577	0.623	0.625
	H	0.805	0.813	0.793	0.812	0.818
LDLR	PD	0.618	0.622	0.577	0.614	0.623
	CE	0.184	0.187	0.167	0.182	0.185
	H	0.488	0.500	0.432	0.481	0.504
GYPA	PD	0.624	0.607	0.621	0.624	0.620
	CE	0.187	0.179	0.187	0.187	0.186
	H	0.501	0.472	0.508	0.501	0.499
HBGG	PD	0.631	0.648	0.684	0.644	0.647
	CE	0.193	0.249	0.243	0.206	0.208
	H	0.504	0.519	0.551	0.509	0.520
D7S8	PD	0.613	0.604	0.585	0.612	0.621
	CE	0.182	0.178	0.171	0.182	0.186
	H	0.480	0.466	0.444	0.479	0.501
Gc	PD	0.742	0.747	0.786	0.765	0.749
	CE	0.297	0.300	0.340	0.318	0.304
	H	0.566	0.583	0.634	0.593	0.573

PD = power of discrimination, CE = chance of exclusion, H = heterozygosity.

TABLE 6—*Power of discrimination, change of exclusion and heterozygosity values for the six loci in the combined South Portugal Caucasian population.*

Statistical Parameters	HLA-DQA1	LDLR	GYPA	HBGG	D7S8	Gc	Combined Values
PD	0.937	0.619	0.624	0.646	0.612	0.755	0.999
CE	0.622	0.185	0.187	0.206	0.182	0.309	0.885
H	0.809	0.489	0.499	0.515	0.477	0.581	0.562

PD = power of discrimination, CE = chance of exclusion, H = heterozygosity.

Madeira and the Azores Islands could be seen as a consequence of different colonizing populations together with different degrees of isolation.

For forensic purposes, a combined South Portuguese Caucasian population database including the five subpopulations studied can be generated for estimating multiple locus profile frequencies on HLA-DQA1 and PM loci.

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TABLE 7—*Chi-square comparisons of allele frequencies between different Portuguese populations.*

	HLA-DQA1	LDLR	GYPA	HBGG	D7S8	Gc
Estremadura/Alentejo	5.787	0.821	5.638**	2.458	0.475	1.493
Estremadura/Algarve	9.275	2.838	0.220	8.701**	1.201	3.088
Estremadura/Madeira	2.530	0.287	0.000	9.312**	0.007	2.258
Estremadura/Azores	3.776	2.835	1.626	1.573	1.370	0.945
Estremadura/Central Portugal	5.242	0.100	1.069	3.276	0.650	4.706
Estremadura/North Portugal	9.540	1.433	0.005	3.551	0.100	0.200
Alentejo/Algarve	4.496	4.487*	3.704*	2.231	0.365	3.350
Alentejo/Madeira	5.122	1.719	5.152*	1.990	0.356	3.701
Alentejo/Azores	2.090	0.647	8.738**	0.236	2.459	2.822
Alentejo/Central Portugal	2.779	0.321	1.762	0.040	1.704	0.848
Alentejo/North Portugal	7.118	0.032	4.980*	0.258	0.799	0.752
Algarve/Madeira	6.661	1.873	0.222	4.812	1.068	0.757
Algarve/Azores	2.954	6.708**	0.206	1.476	3.013	1.315
Algarve/Central Portugal	6.121	3.253	1.114	2.716	2.290	2.773
Algarve/North Portugal	6.885	5.287*	0.166	1.434	1.522	3.566
Madeira/Azores	2.167	4.069*	1.567	3.066	1.426	0.360
Madeira/Central Portugal	6.418	0.610	0.940	1.713	0.708	5.469
Madeira/North Portugal	3.945	2.613	0.007	3.909	0.140	2.857
Azores/Central Portugal	1.566	1.808	3.820	0.470	0.254	4.488
Azores/North Portugal	1.408	0.471	1.294	0.114	0.733	1.532
Central Portugal/North Portugal	7.918	0.639	0.986	0.520	0.190	3.298
South Portugal/Central Portugal	4.676	0.059	0.873	0.146	1.077	5.531
South Portugal/North Portugal	7.896	1.581	0.113	0.852	0.219	1.298

* Deviation at $p < 0.05$ level.
** Deviation at $p < 0.01$ level.

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