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The distribution of HLA DQA1 and D1S80 (pMCT118) alleles and genotypes in the populations of Galicia and Central Portugal

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Summary. Two South-West European populations (Galicia and Central Portugal) have been studied for the HLA DQA1 and D1S80 systems. The allele and genotype frequencies found have been compared with other previously published data. The distribution of the observed genotypes is in Hardy-Weinberg equilibrium for both systems. In the D1S80 system, no significant differences were found between both populations, although in the HLA DQA1 system the allele DQA1*0301 is twice as frequent in the Galician population. Other populations that have been compared showed a certain degree of divergence for the HLA DQA1 system. The combined chance of exclusion for both systems is 0.84 in Galicia and 0.85 in Central Portugal, and the combined power of discrimination is 0.993 in the 2 populations studied.

Key words: DNA polymorphisms – Population study – PCR – HLA DQA1 – D1S80

Zusammenfassung. Zwei südwesteuropäische Populationen (Galizien und Zentral-Portugal) wurden auf die Systeme HLA DQA1 und D1S80 untersucht. Die Frequenzen der Allele und der Genotypen wurden mit anderen vorher pulbizierten Daten verglichen. Die Verteilung der beobachteten Genotypen ist bei beiden System im Hard-Weinberg-Gleichgewicht. Im System D1S80 wurden keine signifikanten Unterschiede zwischen beiden Populationen gefunden, obwohl im HAL DQA1-System das Allel DQA1 0301 in der Bevölkerung Galiziens doppelt so häufig ist. Andere Populationen, die verglichen worden sind, zeigen einen bestimmten Grad von Unterschiedlichkeit im HLA DQA1-System. Die kombinierte Ausschlußchance für beide Systeme ist 0,84 in Galizien und 0,85 in Zentral-Portugal und die kombinierte Diskriminations-Kraft ist 0,993 in beiden untersuchten Populationen.

Schlüsselwörter: DNA-Polymorphismen – Populationsstudie – PCR – HLA DQA1 – D1S80

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Introduction

Typing of DNA polymorphisms for forensic purposes is increasingly being performed using the polymerase chain reaction (PCR).

Although variable number of tandem repeat polymorphisms (VNTRs) are highly informative, their analysis as restriction fragment length polymorphisms (RFLPs) fail to detect a large proportion of mutations and has other limitations such as sensitivity, analysis time and the difficulty in typing highly degraded samples. PCR analysis of DNA polymorphisms has the additional advantage of distinguishing discrete alleles at each polymorphic locus avoiding the problems of database construction, 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 including minisatellites and microsatellites. HLA DQA1 [1] and D1S80 (pMCT118) [2, 3] are probably the most used PCR systems in forensic laboratories and so more population data are availabe.

This paper presents data on the frequencies of HLA DQA1 and D1S80 in 2 populations, from Galicia (NW Spain) and from the Coimbra area (Central Portugal). Additional aims were to compare the results obtained with other population data, to test whether or not the allele frequencies conform to Hardy-Weinberg expectations and to obtain some statistics of medico-legal interest such as the allelic diversity value, the power of discrimination and the chances of exclusion in paternity cases.

Material and methods

Samples. Blood samples were obtained from healthy unrelated individuals from Galicia and the Coimbra area (Fig. 1). DNA was extracted from EDTA blood using the phenol-chloroform procedure described by Valverde et al. [4].

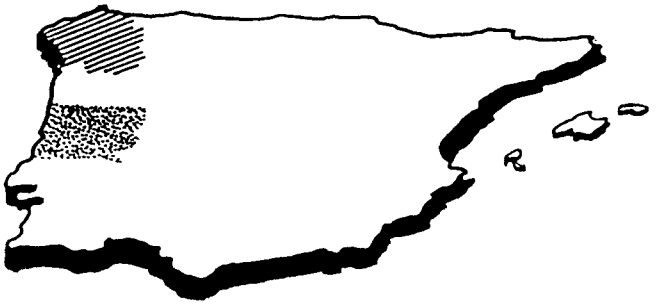

Fig. 1. Geographical areas of population study. (▨) Galicia; (▩) Coimbra area (Central Portugal)

Primers. The oligonucleotides used as primers for HLA DQA1 amplification were GH26 (5'GTGCTGCAGGTGTAAACTTGTACCAG3') and GH27 (5'CACGGATCCGGTAGCAGCGGTAGAGTTG3') which flank the second exon of the DQA1 locus and yield a fragment of 242 bp.

Amplification of D1S80 (pMCT 118) was achieved using the primers 5'GAAACTGGCCTCCAAACACTGCCCGCCG3' and 5'GTCTTGTTGGAGATGCACGTGCCCCTTGC3' described by Kasai et al. [3]. These primers amplify a 16 base pair repeating unit, the different alleles containing 14–40 repeating units.

Oligonucleotides were synthesized by the phosphoramidite method in a 380 A DNA synthesizer and purified through an OPC column (Applied Biosystems, Foster City, CA).

Amplification of DNA. PCR amplification of HLA DQA1 was performed by a slight modification to previously described procedures [5].

Each sample amplified contained 1–10 ng DNA, 10 m*M* Tris-HCl (pH 8.3), 2.5 m*M* $MgCl_2$, 50 m*M* KCl, 0.01% gelatin, 2.5 units of AmpliTaq DNA Polymerase (Cetus, Emeryville, USA), 188 μ*M* of each dNTP and 0.2 μ*M* of each primer. The volume of each sample was 50 μl. The cycling reaction was performed in a programmable heat block (DNA Thermal Cycler 9600, Perkin-Elmer/Cetus) set at 94°C for 1 min (denature), 60°C for 30 sec (anneal), and 72°C for 30 sec (extend). After 32 cycles, the samples were incubated for an additional 7 min at 72°C.

PCR amplification of D1S80 was achieved by the method described by Budowle et al. [6] with slight modifications. Each amplified sample contained 1–10 ng DNA, 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin, 2.5 units of AmpliTaq DNA Polymerase, 1 μ*M* of each primer and 200 μ*M* of each dNTP, in a total volume of 50 μl. The PCR was carried out for 30 cycles, 1 min at 95°C for denaturation, 1 min at 65°C for primer annealing, and 8 min at 70°C for primer extension.

Typing. HLA DQA1 alleles were analyzed using dot-blot with HLA DQα AmpliType (Cetus) and the protocols provided by the manufacturer.

For D1S80 typing was performed using PhastGels 8–25 (Pharmacia-LKB) and silver staining with the conditions described in Barros et al. [7].

Results and discussion

Allele and genotype frequencies

HLA DQA1 allele and genotype frequencies are shown in Tables 1 and 2. In both Galician and Portuguese populations, as found in other Caucasian populations, the most frequent allele is the DQA4 (0.3343 in Galicia and 0.312 in Coimbra). The allele with the lowest frequency is DQA1*0301 in Galicia (0.0871) and the DQA1*0102 and DQA1*0103 in the Coimbra area (0.1160), the frequency of the DQA1*0301 allele in the Portuguese population being twice the frequency observed in the Galician sample. The genotype DQA1 3,3 was not observed in the Portuguese population.

Table 1. Allele and genotype frequencies of the HLA DQA1 system in the population of Galicia (NW Spain)

Genotype	Observed		Expected	
	n	%	*n*	%
1.1–1.1	9	5.1	5.9	3.3
1.1–1.2	12	6.7	12.8	7.2
1.1–1.3	7	3.9	6.2	3.5
1.1–2	2	1.1	6.8	3.8
1.1–3	6	3.4	5.7	3.2
1.1–4	20	11.2	21.7	12.2
1.2–1.2	6	3.4	6.9	3.7
1.2–1.3	6	3.4	6.7	3.9
1.2–2	5	2.8	7.3	4.1
1.2–3	7	3.9	6.1	3.4
1.2–4	28	15.7	23.4	13.1
1.3–1.3	2	1.1	1.6	0.9
1.3–2	5	2.8	3.5	1.9
1.3–3	1	0.5	2.9	1.7
1.3–4	11	6.2	11.4	6.4
2–2	2	1.1	1.9	1.1
2–3	3	1.7	3.2	1.8
2–4	18	10.1	12.4	6.9
3–3	1	0.5	1.3	1.3
3–4	12	6.7	10.4	5.8
4–4	15	8.4	19.9	11.2

Allele	Allele[a]	Frequency
DQA 1.1	DQA1*0101	0.1826 ± 0.0410
DQA 1.2	DQA1*0102	0.1966 ± 0.0421
DQA 1.3	DQA1*0103	0.0955 ± 0.0312
DQA 2	DQA1*0201	0.1039 ± 0.0323
DQA 3	DQA1*0301	0.0871 ± 0.0299
DQA 4[b]	DQA1*0501	0.3343 ± 0.0500
	*0401	
	*0601	

χ^2, 13.3124; *P*, 0.57818; *d.f.*, 15

[a] World Health Organisation nomenclature

[b] Includes DQA 4.1, 4.2, 4.3

$n = 178$

D1S80 allele frequencies are shown in Table 3. Very similar results were obtained in both populations, the alleles 24 and 18 being the most frequent. The alleles observed ranged between 16 and 37 repeats.

Hardy-Weinberg equilibrium

A prerequisite for the application of any genetic marker in forensic cases is that the system be tested for deviation from Hardy-Weinberg equilibrium.

Table 2. Allele and genotype frequencies of the HLA DQA1 system in the population of Coimbra (Central Portugal)

Genotype	Observed		Expected	
	n	%	*n*	%
1.1–1.1	3	2.4	3.2	2.6
1.1–1.2	5	4.0	4.6	3.7
1.1–1.3	6	4.8	4.6	3.7
1.1–2	3	2.4	5.3	4.2
1.1–3	7	5.6	6.6	5.2
1.1–4	13	10.4	12.5	10.0
1.2–1.2	1	0.8	1.7	1.3
1.2–1.3	2	1.6	3.4	2.7
1.2–2	5	4.0	3.8	3.1
1.2–3	5	4.0	4.8	3.8
1.2–4	10	8.0	9.1	7.2
1.3–1.3	2	1.6	1.7	1.3
1.3–2	2	1.6	3.8	3.1
1.3–3	7	5.6	4.7	3.8
1.3–4	8	6.4	9.1	7.2
2–2	3	2.4	2.2	1.7
2–3	7	5.6	5.4	4.3
2–4	10	8.0	10.3	8.2
3–3	0	0	3.4	2.7
3–4	15	12.0	12.8	10.2
4–4	11	8.8	12.2	9.7

Allele	Allele[a]	Frequency
DQA 1.1	DQA1*0101	0.1600 ± 0.0464
DQA 1.2	DQA1*0102	0.1160 ± 0.0405
DQA 1.3	DQA1*0103	0.1160 ± 0.0405
DQA 2	DQA1*0201	0.1320 ± 0.0428
DQA 3	DQA1*0301	0.1640 ± 0.0468
DQA 4[b]	DQA1*0501	0.3120 ± 0.0586
	*0401	
	*0601	

χ^2, 9.5290; *P*, 0.84828; *d.f.*, 15

[a] World Health Organisation nomenclature

[b] Includes DQA 4.1, 4.2, 4.3

$n = 125$

Table 3. D1S80 allele frequencies in Galicia and Coimbra Area (Central Portugal)

Allele	Frequency in	
	Galicia ($n = 109$)	Coimbra Area ($n = 110$)
16	0.0000	0.0045
17	0.0092	0.0045
18	0.2982	0.2909
19	0.0000	0.0045
20	0.0367	0.0364
21	0.0275	0.0500
22	0.0413	0.0364
23	0.0183	0.0227
24	0.3394	0.3364
25	0.0367	0.0364
26	0.0000	0.0136
27	0.0229	0.0091
28	0.0229	0.0364
29	0.0642	0.0545
30	0.0092	0.0318
31	0.0367	0.0227
32	0.0046	0.0000
33	0.0092	0.0000
34	0.0092	0.0000
35	0.0000	0.0000
36	0.0092	0.0045
37	0.0046	0.0045

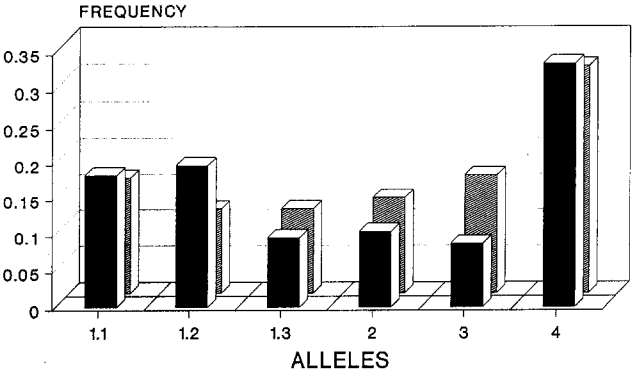

Fig. 2. Comparison of data from Galicia with Central Portugal for HLA DQA1 system. (■) Galicia; (▨) Portugal (Coimbra)

In the HLA DQA1 system conventional calculations can be carried out as the number of alleles is relatively small (Tables 1 and 2).

For the D1S80 system 18 alleles were observed, giving 253 possible genotypes and therefore a reliable estimation of Hardy-Weinberg expectations is not possible unless a larger sample is studied.

A less sensitive but informative approach to test for deviations in cases like this might be the binning approach suggested by Brenner and Morris [8] and used in other population studies [9]. Using a 6 allele model, no significant deviations between the expected and the observed values were found in either of the 2 populations. The validity of this was supported by repeating the chi square test after regrouping of alleles into new bins as described by Rans et al. [9]. Again, no significant differences were found (data not shown).

Alleles consisting of more than 37 repeats that have been reported in the literature were not detected in this work.

Comparison of population studies

Figures 2 and 3 show a qualitative comparison between the 2 populations studied carried out using a 2-way R × C contingency table test comparing allele distributions for population sample homogeneity and using chi-square and the likelihood ratio chi-square (G) as methods.

Although quantitative comparisons with single markers has to be performed cautiously, both populations ap-

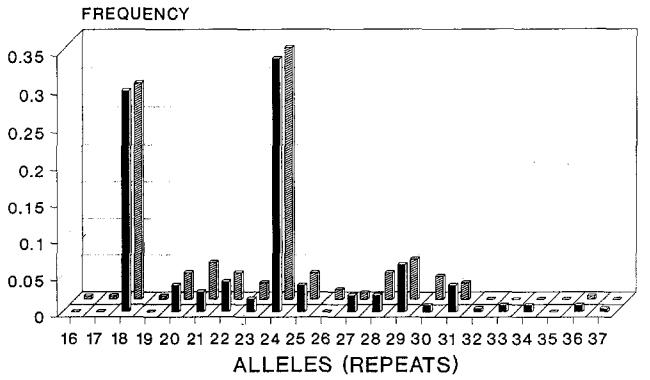

Fig. 3. Comparison of data from Galicia with Central Portugal for D1S80 system. (■) Galicia; (▨) Portugal (Coimbra)

Table 4. Comparison of different populations for the HLA DQA1 and D1S80 systems

Population	χ^2	*P* value
HLA DQA1		
Galicia-Coimbra	15.410	0.0087
Galicia-Cataluña [15]	3.726	0.5895
Galicia-Caucasian USA [10]	12.244	0.0316
Galicia-Black USA [10]	13.144	0.0221
Galicia-Hispanic USA [10]	32.985	0.0000
Galicia-Australian [16]	18.859	0.0020
Galicia-Caucasian UK [17]	30.524	0.0000
Galicia-Afrocaribbean UK [17]	25.528	0.0001
Galicia-Asian UK [17]	18.796	0.0021
Galicia-Finnish [18]	13.410	0.0198
Coimbra-Cataluña	5.896	0.3165
Coimbra-Caucasian USA	6.565	0.2550
Coimbra-Black USA	31.835	0.0000
Coimbra-Hispanic USA	17.327	0.0039
Coimbra-Australian	10.488	0.0625
Coimbra-Caucasian UK	16.475	0.0056
Coimbra-Afrocaribbean UK	44.517	0.0000
Coimbra-Asian UK	15.819	0.0074
Coimbra-Finnish	15.458	0.0086
D1S80		
Galicia-Coimbra	17.908	0.5935
Galicia-Finland [12]	41.050	0.0024
Galicia-Caucasian USA [12]	23.420	0.2193
Coimbra-Finland	25.615	0.0818
Coimbra-Caucasian USA	10.956	0.8588

pear to be very similar for D1S80 ($\chi^2 = 17.90$, d.f. = 20, $P = 0.59$, G = 22.02) while for HLA DQA1 significant differences were observed ($\chi^2 = 15.41$, d.f. = 5, $P < 0.01$, G = 15.49).

For HLA DQA1 (Table 4) significant differences were observed ($P < 0.01$) between the population of Galicia and Coimbra, US Hispanics, Australian, Caucasian UK, Afrocaribbean UK, Asian UK populations, and between the population of Coimbra and Black USA, US Hispanics, Caucasian UK, Afrocaribbean UK, Asian UK, and Finnish populations.

The main differences were observed between the Galician population and the population of US Hispanics [10].

Few differences between populations were observed for the D1S80 system (Table 4). Only a few populations were compared since the D1S80 population studies usually show the population data in a qualitative way [9, 11] making a quantitative comparison impossible. In any case a quantitative comparison of our data with other population samples [12] shows that the distribution of alleles in Caucasians is similar.

It is necessary to kept in mind that the absence of significant differences between 2 populations only demonstrates that the test used failed to detect may significant differences.

As with other VNTRs typed using SLPs, population heterogeneity does not seem to be a problem for D1S80 and probably closely related populations could use the same database.

The problem of population heterogeneity may be different for markers in coding DNA with possible strong selective influences. The results in HLA DQA1 support this fact and the 2 populations studied, although being very close geographically, show a certain degree of divergence in their HLA DQA1 results.

Table 5. Forensic value of HLA DQA1 and D1S80 system using some statistical parameters

Statistics	Population	
	Galicia	Coimbra Area
HLA DQA1		
CE	0.593	0.620
PD	0.921	0.932
h	0.791	0.809
D1S80		
CE	0.604	0.613
PD	0.920	0.907
h	0.787	0.793

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

Other statistical parameters

Other statistics of genetic and medico-legal interest are shown in Table 5. The combined chance of exclusion is 0.84 in the Galician population and 0.85 in the Portuguese population. The combined discrimination power is 0.99 in the Galician Portuguese populations, calculated following the method of Fisher [13]. The allelic diversity values (h) were calculated as described by Nei and Roychoudhury [14] and were similar to other populations studied.

In conclusion we have presented the allele and genotype frequencies of HLA DQA1 and D1S80 in 2 Iberian

populations and the results show that these markers are very useful for forensic application.

Both populations seem to be in Hardy-Weinberg equilibrium for the 2 systems, and the binning approach is, in our opinion, a valid approach to prove Hardy-Weinberg equilibrium in preliminary studies.

Only very distant populations show divergences for D1S80, but both populations studied here show a significant difference for HLA DQA1, the DQA1*0103 allele being twice as frequent in the Portuguese sample.

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