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HLA class I and II polymorphisms in Azores show different settlements in Oriental and Central islands

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Azores; HLA class I; HLA class II; HLA polymorphism

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Abstract: Human leucocyte antigen-A, -B, -Cw, -DRB1, -DQA1 and -DQB1 polymorphisms were examined in the Azorean population. The data were obtained at high-resolution level, using polymerase chain reaction (PCR) with sequence-specific primer, PCR-sequence-specific oligonucleotides and sequence-based typing. The most frequent allele in each locus was: A*0201 (24.5%), B*510101 (9.8%), Cw*0401 (14.8%), DRB1*070101 (18.3%), DQA1*0201 (17.4%) and DQB1*0301 (19.4%). The predominant extended haplotype was A*0202-B*1503-Cw*0202-DRB1*090102-DQA1*0303-DQB1*0202 (1.9%), which was found to be absent in the Portuguese mainland. The present study corroborates historical sources that say the Azores were populated not only by Portuguese but also by other Europeans, mostly Flemish people. Despite dendrogram analysis showing some remote Asian genetic affinities, the lack of specific alleles and haplotypes from those populations does not allow us to conclude for direct influence. Haplotype and allele frequencies in Azores show no homogeneous distribution between Oriental and Central islands of this archipelago. The Oriental islands harbour several haplotypes already found in mainland Portugal and identified as Mediterranean and European. The Central group of islands on the contrary clearly shows an influence of north Europeans (most probably derived from a well-documented Flemish settlement), with much less affinity to mainland Portugal.

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The highly polymorphic human leukocyte antigen (HLA) system, located in the short arm of chromosome 6, consists of a closely linked set of genes important in transplantation, disease and anthropological studies (1). HLA allele frequencies and haplotypic patterns have great importance as markers to determine genetic relatedness and the degree of admixture between populations (2).

The Azores Islands (Portugal), located in the middle of the North Atlantic Ocean, were found to be uninhabited by Portuguese in the first-half of the 15th century and were officially populated in 1439. The Archipelago is constituted by nine islands subdivided into three groups (Oriental: Santa Maria and São Miguel; Central: Terceira, Graciosa, São Jorge, Pico and Faial and Occidental: Flores and Corvo).

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During the 15th and 16th centuries, the islands played a significant role in sea traffic between America and Europe (3) being important for commerce and slave trade between Africa, America and Europe. This central and strategic position between the three continents brought to Azores people from different origins, mainly Portuguese but also Jews, Moorish prisoners, Sub-Saharan slaves, Dutch (Flemish), French, Italians, English and Spaniards (4). Some historic registries also mention the selling of Caribbean Indians to the Azores. According to several authors, the first settlers of the Oriental and Central groups had different origins. The Oriental group was probably settled first, mostly by people from different regions of mainland Portugal (5, 6) while the settlement of Central islands is known to be strongly influenced by Flemish people. By 1490, there were 2000 Flemings living on Central group islands (7).

Few studies have been done to characterize the Azorean population genetic profile (8, 9), but only one used HLA-generic class I and class II typing in Terceira Island (10). With the recent developments in DNA typing, HLA can be studied at a high-resolution level, for example, by sequencing. This high-resolution throughput allows an additional amount of information with great relevance to a more-specific HLA characterization, important in population's comparisons and disease studies (11, 12). Unfortunately, high-resolution HLA allele typing data is still scarce, making comparisons difficult. Previous HLA generic typing indicates that the Azorean population most likely contains an admixture of high frequency Caucasoid, Mongoloid and, to a lesser degree, Sub-Saharan HLA genes (10). In the later study, it was proposed that a Mongoloid population already existed in the Azores before the Portuguese discovery. Y-chromosome and mitochondrial DNA show major similarities to Portugal mainland and Europe and some genetic influence from Sub-Saharan Africans (8, 13) but failed to detect any Asiatic contribution to the present day gene pool.

The main goal of the present work is to present a clear genetic profile of the Azorean based on high-resolution typing of HLA-A, -B, -Cw, -DRB1, -DQA1 and -DQB1 loci. Allele and haplotype frequencies of this population and their relatedness to Portuguese mainland and other world populations will be evaluated. We expect that these comparisons may also contribute to clarify the origin of Azorean population and especially the Asian genetic influence previously reported (10).

Materials and methods

Population samples and HLA typing

The study population consisted of a total of 102 healthy unrelated individuals from the archipelago of Azores. Blood samples were collected after informed consent, from donors whose parents and grandfathers were born and living in the Azores (Az1). To analyse internal differences in the archipelago for HLA-A, -B and -DRB1 loci, this sample was further subdivided into Azores Oriental group of islands (AzOriental, $n = 43$) and Azores Central group (AzCentral, $n = 59$). The AzOriental comprise Santa Maria and São Miguel Islands, and AzCentral is constituted by Terceira, Graciosa, São Jorge, Faial and Pico Islands. Genomic DNA was isolated from whole blood containing ethylenediaminetetraacetic acid using a phenol-chloroform procedure and frozen at -20°C until use. All subjects were typed for HLA-A, -B and -DRB1 by sequence-based typing according to Kurz et al. (14) and Pozzi et al. (15) for HLA-A and -B loci, and amplifying and sequencing exon 2 of HLA-DRB1 locus with group-specific primers used in sequence-specific oligonucleotide probe (SSOP) typing (16). Typing procedures and allele identification methodology were as previously outlined (17).

Data analysis

To get a bigger sample for comparisons, we added data from Terceira Island already published (Az2, $n = 129$) (18) and typed by sequence-specific primer and SSOP for HLA-A, -B, -Cw, -DRB1, -DQA1 and -DQB1. These samples have been collected only with the information that ancestors were from Azores with no specification of the island's origin. Because of that, we used Az1 and Az2 samples for a global Azores analysis.

Basic genetic parameters (allele and haplotype frequencies, gene diversity and Hardy–Weinberg equilibrium) at the six loci were estimated with ARLEQUIN v2.000 (19). The same population genetic software was employed to calculate Ewes-Watterson's, Slatkin's and Chakraborty's selective neutrality statistical tests to examine the presence of selective forces influencing allelic diversity at each locus. Linkage disequilibrium (LD) (D) and relative LD (D′) between two alleles at two different loci and their level of significance (P) was calculated according to Weir (20).

The Azores data was compared to mainland Portugal (17) and several populations which are available at the same typing resolution (www.allelefrequencies.net) (12, 21–29). An analysis of molecular variance (AMOVA) was performed with these populations based on Euclidean distances between all pairs of haplotypes (30). Comparative analysis of our data set with other populations available in the literature was achieved using the software included in the PHYLIP v.3.6 package (31). First, SEQBOOT was used to perform a bootstrap analysis from gene frequency data. The program generates multiple data sets resampled from the original data. Distance matrices from each replicate data set were generated using GENDIST and used as input to NEIGHBOR to produce neighbor-joining trees.

A single consensus-bootstrapped tree was obtained with CONSENSE. The topology was visualized with TREEVIEW (32). Various dendrograms were made with different groups of loci depending on the level of HLA resolution typing available for the comparisons. Principal coordinate analysis using HLA-A, -B and -DRB1 allele frequencies was carried out on the MultiVariate Statistical Package MVSP3 for Windows (Kovach Computing Services, Anglesey, Wales, UK; http://www.kovcomp.com/mvsp).

Results

Table 1 summarizes the allele frequencies of HLA-A, -B, -Cw class I loci and HLA-DRB1, -DQA1 and -DQB1 class II loci in the Azores population. A total of 33 HLA-A, 53 HLA-B, 23 HLA-Cw, 40 HLA-DRB1, 14 HLA-DQA1 and 16 HLA-DQB1 alleles were found. The population is in Hardy–Weinberg equilibrium at each loci except HLA-B ($P < 0.02$). Overall, the heterozygosity was high (HLA-A: 0.90; HLA-B: 0.96; HLA-Cw: 0.92; HLA-DRB1: 0.94; HLA-DQA1 and HLA-DQB1: 0.89). Ewes-Watterson, Slatkin's and Chakraborty's tests of selective neutrality yielded non-significant results for HLA-A, -B and -DRB1 suggesting that selection was acting on these three loci.

An AMOVA between Azores and mainland Portugal, with HLA-A, -B and -DRB1 loci, showed that only a residual genetic variation found can be attributed to differences among populations, the remaining is because of within population differences. An exact test of population differentiation showed significant results between Azores and mainland Portugal ($P = 0.0057$) but no differences within the Azores islands.

HLA-A locus

The most frequent of these alleles is by far HLA-A*0201 found in 24.5% of the samples, which is similar to Portugal mainland. Three alleles follow with frequencies ranging between 10 and 11% (A*0101, A*0301 and A*2402), slightly higher than in mainland Portugal (7–8%). Two alleles specific of the sub-Sahara region were found (*0202 and *0225). Surprisingly, group A*24 (with four alleles in Azores) is not polymorphic in mainland Portugal, where it is represented only by B*2402.

HLA-B locus

HLA-B*5101 (9.8%) was the most frequent, an allele that appears with diverse values in Europe (3–23%). Other alleles show frequencies varying from 5 to 8% (B*0702, B*3501, B*4402, B*4403, B*1801 and B*1402). Allele B*3801 (4.6%), despite having similar frequencies with several Europeans populations, is rare in Portugal (0.3%). Otherwise, B*3502 (1.1%) has a much lower prevalence than in Portugal, where it is found at the world highest prevalence (5%).

HLA-Cw locus

Alleles Cw*0401 (15%) are the most common, with frequency higher than Europeans and similar to several sub-Saharan populations. The second most frequent allele, HLA-Cw*0701 (12.4%), has similar frequencies in sub-Sahara but lower among Caucasians. Alleles HLA-Cw*0602, Cw*1203, Cw*0202 and Cw*0702 appear in 8–10% of the samples with similar (Cw*0602 and Cw*0702) and lower (Cw*1203 and Cw*0202) frequencies than those found in European populations.

HLA-DRB1 locus

Allele DRB1*0701 (18.3%) is the most common, showing higher frequency than in other Europeans with the exception of some Spanish populations (21). Alleles DRB1*0301, DRB1*0101, DRB1*1301 and DRB1*1501 appear in 7–8% of the samples, with similar frequencies found in Portugal (DRB1*1301 and DRB1*1501) and European (DRB1*0101 and DRB1*0301) populations.

HLA-DQA1 locus

DQA1*0201 (17.4%) is the most common allele, presenting a frequency similar to Europeans (and even Chinese) but higher than Sub-Saharans. Allele DQA1*0505 (13.2%) is absent in many populations (e.g., Spain and Cameroon), is found at similar frequencies in Tunisia but has a much higher frequency in Italy (30%). DQA1*0501 (12.8%) in Azores has similar frequencies to some sub-Saharan populations (e.g., Cameroon, Congo and Uganda) being always lower than in Europeans.

HLA-DQB1 locus

DQB1*0301 (19.4%) and DQB1*0202 (17.3%) are the most common alleles in the Azores and have frequencies rather similar or slightly higher to other Europeans (21). DQB1*0501, DQB1*0201 and DQB1*0603 alleles also have values similar to other European populations (10–11%). Amazingly, DQB1*0203 (3.5%) is a very rare allele in other world populations.

Haplotype frequencies

The exact test of LD between each of the 15 pairs of loci was statistically significant except for HLA-A and -DQB1. The most

HLA-A, -B, -C, -DRB1, -DQA1 and -DQB1 alleles frequencies in the Azorean population

HLA-A alleles ($n = 231$)	Frequency	HLA-B alleles ($n = 231$)	Frequency	HLA-C alleles $n = 129$	Frequency	HLA-DRB1 alleles ($n = 231$)	Frequency	HLA-DQA1 alleles ($n = 129$)	Frequency	HLA-DQB1 alleles ($n = 129$)	Frequency
A*0101	0.102	B*0702	0.076	Cw*0102	0.012	DRB1*0101	0.076	DQA1*0101	0.112	DQB1*0201	0.058
A*0201	0.245	B*0705	0.002	Cw*0202	0.078	DRB1*0102	0.039	DQA1*0102	0.109	DQB1*0202	0.173
A*0202	0.022	B*0720	0.002	Cw*0303	0.054	DRB1*0103	0.015	DQA1*0103	0.097	DQB1*0203	0.035
A*0205	0.022	B*0801	0.035	Cw*0304	0.016	DRB1*0106	0.002	DQA1*0104	0.016	DQB1*0301	0.194
A*0206	0.002	B*1302	0.013	Cw*0401	0.148	DRB1*0301	0.084	DQA1*0105	0.008	DQB1*0302	0.101
A*0225	0.002	B*1401	0.015	Cw*0404	0.004	DRB1*0302	0.002	DQA1*0201	0.174	DQB1*0303	0.05
A*0301	0.106	B*1402	0.05	Cw*0501	0.062	DRB1*0304	0.015	DQA1*0202	0.004	DQB1*0304	0.012
A*0302	0.007	B*1501	0.028	Cw*0602	0.097	DRB1*0401	0.03	DQA1*0301	0.089	DQB1*0402	0.047
A*1101	0.065	B*1503	0.015	Cw*0701	0.124	DRB1*0402	0.03	DQA1*0302	0.019	DQB1*0501	0.112
A*1112	0.002	B*1516	0.002	Cw*0702	0.078	DRB1*0403	0.017	DQA1*0303	0.058	DQB1*0502	0.019
A*2301	0.041	B*1517	0.004	Cw*0704	0.019	DRB1*0404	0.022	DQA1*0401	0.05	DQB1*0503	0.016
A*2302	0.002	B*1518	0.002	Cw*0802	0.062	DRB1*0405	0.013	DQA1*0501	0.128	DQB1*0601	0.004
A*2402	0.11	B*1539	0.013	Cw*1202	0.008	DRB1*0407	0.013	DQA1*0505	0.132	DQB1*0602	0.054
A*2403	0.004	B*1801	0.056	Cw*1203	0.089	DRB1*0408	0.002	DQA1*0601	0.004	DQB1*0603	0.101
A*2417	0.002	B*2703	0.002	Cw*1401	0.012	DRB1*0701	0.183			DQB1*060401	0.012
A*2418	0.002	B*2705	0.026	Cw*1402	0.016	DRB1*0801	0.037			DQB1*0609	0.012
A*2501	0.013	B*2708	0.002	Cw*1502	0.054	DRB1*0803	0.007				
A*2601	0.033	B*3501	0.074	Cw*1503	0.012	DRB1*0804	0.002				
A*2602	0.002	B*3502	0.011	Cw*1505	0.004	DRB1*0806	0.002				
A*2605	0.002	B*3503	0.03	Cw*1601	0.027	DRB1*0901	0.028				
A*2901	0.009	B*3504	0.004	Cw*1602	0.008	DRB1*1001	0.009				
A*2902	0.048	B*3508	0.002	Cw*1701	0.012	DRB1*1101	0.048				
A*3001	0.007	B*3527	0.013	Cw*1703	0.004	DRB1*1102	0.02				
A*3002	0.017	B*3533	0.002			DRB1*1103	0.013				
A*3101	0.011	B*3701	0.004			DRB1*1104	0.033				
A*3102	0.004	B*3801	0.046			DRB1*1111	0.002				
A*3201	0.035	B*3806	0.004			DRB1*1201	0.024				
A*3206	0.007	B*3901	0.02			DRB1*1301	0.076				
A*3301	0.015	B*3906	0.004			DRB1*1302	0.024				
A*3303	0.002	B*4001	0.017			DRB1*1303	0.009				
A*6601	0.007	B*4002	0.017			DRB1*1305	0.004				

A*6801	0.039	B*4005	0.002	DRB1*1310	0.003
A*6802	0.013	B*4101	0.004	DRB1*1322	0.003
		B*4202	0.004	DRB1*1401	0.008
		B*4402	0.067	DRB1*1404	0.002
		B*4403	0.054	DRB1*1406	0.004
		B*4405	0.002	DRB1*1501	0.073
		B*4501	0.009	DRB1*1502	0.007
		B*4901	0.039	DRB1*1601	0.017
		B*5001	0.037	DRB1*1602	0.002
		B*5101	0.098		
		B*5108	0.007		
		B*5132	0.002		
		B*5201	0.007		
		B*5301	0.011		
		B*5401	0.002		
		B*5501	0.011		
		B*5601	0.004		
		B*5701	0.028		
		B*5703	0.004		
		B*5706	0.002		
		B*5801	0.013		
		B*6701	0.002		

Human leucocyte antigen (HLA)-A, -B and -DRB1 allele frequencies were calculated from 231 samples. HLA-C, -DQA1 and -DQB1 were calculated from 129 samples.

Table 1

representative extended haplotypes and A-B-DRB1 haplotypes (Azores Central and Oriental) are summarized in Table 2. Table 3 summarizes the most frequent A-B-DRB1 haplotypes found in Azores Central and Oriental islands. Table 4 summarizes the most

Most common human leucocyte antigen (HLA)-A-B-C-DRB1-DQA1-DQB1 extended haplotypes and HLA-A-B-DRB1 haplotypes in the Azorean population as estimated by maximum likelihood

Haplotypes ≥0.008	Frequency
A*0101-B*0801-Cw*0202-DRB1*0102-DQA1*0101-DQB1*0501	0.008
A*0101-B*1402-Cw*0802-DRB1*0102-DQA1*0101-DQB1*0501	0.012
A*0101-B*1402-Cw*0802-DRB1*0407-DQA1*0501-DQB1*0203	0.008
A*0101-B*5701-Cw*0701-DRB1*0701-DQA1*0201-DQB1*0303	0.008
A*0201-B*0702-Cw*0702-DRB1*0101-DQA1*0101-DQB1*0501	0.016
A*0201-B*0702-Cw*0702-DRB1*1501-DQA1*0102-DQB1*0302	0.008
A*0201-B*1539-Cw*0303-DRB1*0401-DQA1*0301-DQB1*0302	0.008
A*0201-B*4402-Cw*0501-DRB1*1301-DQA1*0103-DQB1*0603	0.012
A*0201-B*4901-Cw*0701-DRB1*1102-DQA1*0505-DQB1*0301	0.008
A*0201-B*5001-Cw*0602-DRB1*0701-DQA1*0201-DQB1*0202	0.012
A*0201-B*5101-Cw*1401-DRB1*090102-DQA1*0302-DQB1*0303	0.008
A*0201-B*5101-Cw*1502-DRB1*0701-DQA1*0201-DQB1*0202	0.012
A*0201-B*5701-Cw*0602-DRB1*0701-DQA1*0201-DQB1*0303	0.008
A*0202-B*1503-Cw*0202-DRB1*090102-DQA1*0303-DQB1*0202	0.019
A*0205-B*4901-Cw*0701-DRB1*1102-DQA1*0505-DQB1*0301	0.008
A*0301-B*0702-Cw*0702-DRB1*1501-DQA1*0102-DQB1*0602	0.012
A*0301-B*2705-Cw*0202-DRB1*0404-DQA1*0301-DQB1*0302	0.008
A*0301-B*4001-Cw*0701-DRB1*0301 – DQA1*0501-DQB1*0201	0.008
A*1101-B*1801-Cw*0501-DRB1*0301-DQA1*0501-DQB1*0201	0.008
A*1101-B*4402-Cw*0501-DRB1*0403-DQA1*0301-DQB1*0304	0.008
A*2402-B*3503-Cw*1203-DRB1*1101-DQA1*0505-DQB1*0301	0.008
A*2402-B*3901-Cw*1203-DRB1*1301-DQA1*0103-DQB1*0603	0.008
A*2402-B*5101-Cw*0202-DRB1*1101-DQA1*0505-DQB1*0301	0.008
A*2601-B*3801-Cw*1203-DRB1*1301-DQA1*0103-DQB1*0603	0.008
A*2902-B*4403-Cw*1601-DRB1*0701-DQA1*0201-DQB1*0202	0.016
A*3201-B*1401-Cw*0802-DRB1*0701-DQA1*0201-DQB1*0202	0.012
A*3201-B*1402-Cw*0802-DRB1*0801-DQA1*0401-DQB1*0402	0.008
HLA A-B-DRB1 haplotypes ≥1	
A*0101-B*0801-DRB1*0301	0.013
A*0101-B*1402-DRB1*0102	0.013
A*0201-B*0702-DRB1*0101	0.015
A*0201-B*1801-DRB1*1104	0.013
A*0201-B*4402-DRB1*1301	0.013
A*0201-B*5101-DRB1*0701	0.02
A*0202-B*1503-DRB1*0901	0.011
A*0301-B*0702-DRB1*1501	0.013
A*0301-B*3501-DRB1*0101	0.011
A*2402-B*0702-DRB1*1501	0.015
A*2902-B*4403-DRB1*0701	0.024

Table 2

Most common human leucocyte antigen (HLA)-A-B-DRB1 haplotypes in Azores Oriental and Central groups as estimated by maximum likelihood (only frequencies above 0.017 or haplotypes common to Oriental and Central Azores Islands are summarized). Haplotypes' possible origin is according to Sanchez-Velasco (26), Arnaiz-Villena (33), Goméz-Casado (34) and www.allelefrequencies.net (21)

Haplotypes	Possible origin	Azores Oriental	Azores Central
A*0101-B*0801-DRB1*0301	Pan European	0.026	
A*0101-B*0801-DRB1*0401	?	0.026	
A*0101-B*1402-DRB1*0102	Mediterranean		0.018
A*0201-B*0702-DRB1*0101	?		0.018
A*0201-B*1302-DRB1*0701	Central European	0.013	0.018
A*0201-B*1801-DRB1*1104	Mediterranean	0.026	
A*0201-B*4002-DRB1*1501	?		0.018
A*0201-B*4402-DRB1*0401	3.8% in Irish		0.027
A*0201-B*4402-DRB1*0701	North-Western European	0.026	
A*0201-B*4403-DRB1*0701	North-Western European		0.018
A*0201-B*5101-DRB1*0701	?	0.026	
A*0301-B*1801-DRB1*1104	?		0.018
A*0301-B*4403-DRB1*0701	?		0.027
A*1101-B*4402-DRB1*1201	?		0.018
A*1101-B*5101-DRB1*0101	?		0.018
A*2301-B*3501-DRB1*0101	?		0.018
A*2402-B*0702-DRB1*1501	?	0.038	
A*2402-B*0801-DRB1*0301	?		0.018
A*2601-B*1401-DRB1*0701	?		0.018
A*2902-B*0702-DRB1*0103	?		0.018
A*2902-B*4001-DRB1*0301	?		0.018
A*2902-B*4403-DRB1*0701	Western European	0.038	
A*2902-B*5701-DRB1*0701			0.018
A*3002-B*1801-DRB1*0301	Iberia/North African	0.026	
A*3201-B*4901-DRB1*0701		0.026	
A*3301-B*1402-DRB1*0102	Mediterranean	0.038	0.018

Table 3

common two loci haplotypes with statistical significance LD found in Azores.

Only two of the nine most frequent (≥1.2%) extended haplotypes found in Azores, A*2902-B*4403-Cw*1601-DRB1*0701-DQA1*0201-DQB1*0202 (1.6%), considered of Western European origin, and A*0201-B*5101-Cw*1502-DRB1*0701-DQA1*0201-DQB1*0202 (1.2%) have the corresponding A-B-DRB1 partial haplotype in mainland Portugal with frequencies 0.7 and 1.7%, respectively. One of these extended haplotypes was not found in mainland Portugal: A*0301-B*0702-Cw*0702-DRB1*1501-DQA1*0102-DQB1*0602 considered of North African/Western European origin (21).

Some three-loci haplotypes found in Azores were identified before as Pan European (A*0101-B*0801-DRB1*0301: 1.3%), Mediterranean (A*0201-B*1801-DRB1*1104: 1.3%) and North African/Western European (A*0301-B*0702-DRB1*1501: 1.3%) (21). Six of the 11 most frequent three-loci haplotypes were common to mainland Portugal, including the most frequent A*0201-B*5101-DRB1*0701 (2% in Azores and 0.7% in mainland Portugal).

The most frequent A-B-DRB1 haplotypes estimated from donors whose parents and grandfathers were born and living on the same island (Az1 samples, Table 3) do not show a homogeneous distribution in the Oriental and Central Azores islands. Only two of 26 have a distribution common to both the groups of islands: the Central European A*0201-B*1302-DRB1*0701, which is nevertheless absent from mainland Portugal, and the Mediterranean A*3301-B*1402-DRB1*0102, which exists in mainland Portugal.

The Western European haplotype A*2902-B*4403-DRB1*0701 was found only on the Oriental islands, at a frequency of 3.8%. The same happens with the pan-European A*0101-B*0801-DRB1*0301 (present at 2–6%), the Mediterranean A*0201-B*1801-DRB1*1104

Most common human leucocyte antigen (HLA)-A, -B ($n = 231$): HLA-A, -DRB1 ($n = 231$); HLA-B, -DRB1 ($n = 231$); HLA-A, -Cw ($n = 129$); HLA-B, -Cw ($n = 129$); HLA-DRB1, -DQB1 ($n = 129$); HLA-DRB1, -DQA1 ($n = 129$) and HLA-DQA1, -DQB1 ($n = 129$) two-loci haplotypes in the Azores population as estimated by maximum likelihood (only frequencies above 0.01 with statistical significance linkage disequilibrium are summarized)

Haplotypes	Frequency	SD	D	D′	*P*
A*0101-B*0801	0.021	0.007	0.017	0.742	<0.001
A*0101-B*1402	0.013	0.006	0.008	0.345	<0.05
A*0101-B*5101	0.02	0.01	0.010	0.443	<0.05
A*0101-B*5701	0.01	0.005	0.007	0.313	<0.05
A*0201-B*4001	0.011	0.004	0.006	0.284	<0.05
A*0201-B*4402	0.029	0.009	0.012	0.543	<0.05
A*0201-B*4901	0.017	0.005	0.008	0.335	<0.05
A*0201-B*5101	0.044	0.008	0.02	0.872	<0.001
A*0202-B*1503	0.011	0.005	0.01	0.454	<0.001
A*0301-B*0702	0.02	0.007	0.012	0.536	<0.001
A*0301-B*3501	0.03	0.009	0.022	0.964	<0.001
A*1101-B*3501	0.015	0.007	0.01	0.457	<0.001
A*1101-B*4402	0.013	0.004	0.009	0388	<0.001
A*2402-B*0702	0.017	0.007	0.009	0.391	<0.05
A*2402-B*1501	0.012	0.004	0.009	0.394	<0.001
A*2402-B*3503	0.011	0.006	0.008	0.329	<0.05
A*2402-B*3801	0.011	0.007	0.006	0.267	<0.05
A*2601-B*3801	0.01	0.004	0.009	0.377	<0.001
A*2902-B*4403	0.025	0.006	0.023	1	<0.001
A*3301-B*1402	0.015	0.007	0.014	0.63	<0.001
A*0101-DRB1*0102	0.013	0.004	0.009	0.392	<0.001
A*0101-DRB1*0301	0.019	0.008	0.011	0.475	<0.05
A*0201-DRB1*0401	0.015	0.007	0.008	0.354	<0.05
A*0201-DRB1*0402	0.016	0.006	0.009	0.383	<0.05
A*0202-DRB1*0901	0.011	0.006	0.009	0.401	<0.001
A*0301-DRB1*0101	0.02	0.008	0.012	0.5	<0.05
A*0301-DRB1*0404	0.011	0.005	0.008	0.369	<0.001
A*0301-DRB1*0801	0.013	0.006	0.009	0.373	<0.05
A*0301-DRB1*1104	0.011	0.004	0.007	0.318	<0.05
A*2301-DRB1*0701	0.015	0.009	0.008	0.346	<0.05
A*2402-DRB1*1302	0.01	0.003	0.008	0.340	<0.001
A*2402-DRB1*1501	0.017	0.007	0.009	0.391	<0.05
A*2601-DRB1*1301	0.011	0.005	0.009	0.378	<0.001
A*2902-DRB1*0701	0.032	0.01	0.023	1	<0.001
A*3201-DRB1*0701	0.017	0.007	0.011	0.475	<0.05
B*0702-DRB1*0101	0.013	0.006	0.007	0.229	<0.05
B*0702-DRB1*1501	0.034	0.007	0.029	0.949	<0.001
B*0801-DRB1*0301	0.019	0.005	0.017	0.549	<0.001
B*1302-DRB1*0701	0.011	0.005	0.008	0.28	<0.001
B*1401-DRB1*0701	0.016	0.006	0.012	0.412	<0.001
B*1402-DRB1*0102	0.028	0.005	0.026	0.848	<0.001
B*1503-DRB1*0901	0.011	0.004	0.010	0.345	<0.001
B*1801-DRB1*0301	0.016	0.008	0.011	0.37	<0.001

B*1801-DRB1*1104	0.017	0.007	0.015	0.491	<0.001
B*3501-DRB1*0101	0.026	0.009	0.021	0.684	<0.001
B*3801-DRB1*1301	0.022	0.008	0.018	0.603	<0.001
B*4402-DRB1*0401	0.011	0.007	0.009	0.287	<0.001
B*4402-DRB1*1201	0.015	0.006	0.014	0.449	<0.001
B*4403-DRB1*0701	0.04	0.008	0.030	1	<0.001
B*5701-DRB1*0701	0.024	0.009	0.019	0.623	<0.001
A*0101-Cw*0602	0.035	0.007	0.025	0.781	<0.001
A*0101-Cw*0701	0.028	0.014	0.015	0.469	<0.05
A*0101-Cw*0802	0.019	0.009	0.013	0.406	<0.05
A*0101-Cw*1402	0.011	0.004	0.009	0.281	<0.001
A*0201-Cw*0401	0.04	0.017	0.003	0.009	<0.001
A*0201-Cw*0702	0.042	0.012	0.023	0.719	<0.05
A*0202-Cw*0202	0.019	0.008	0.017	0.531	<0.001
A*0205-Cw*0701	0.015	0.005	0.012	0.375	<0.001
A*0301-Cw*0401	0.047	0.012	0.032	1	<0.001
A*1101-Cw*0401	0.022	0.006	0.012	0.375	<0.05
A*1101-Cw*0501	0.016	0.008	0.011	0.344	<0.05
A*1101-Cw*0602	0.019	0.008	0.013	0.406	<0.05
A*2402-Cw*0202	0.023	0.008	0.014	0.438	<0.05
A*2402-Cw*0303	0.03	0.009	0.025	0.781	<0.001
A*2402-Cw*1203	0.026	0.014	0.016	0.5	<0.05
A*2601-Cw*1203	0.017	0.01	0.014	0.438	<0.001
A*2902-Cw*1601	0.019	0.006	0.018	0.563	<0.001
A*3201-Cw*0802	0.019	0.007	0.017	0.531	<0.001
B*0702-Cw*0702	0.066	0.018	0.06	1	<0.001
B*0801-Cw*0701	0.031	0.013	0.027	0.45	<0.001
B*1402-Cw*0802	0.047	0.013	0.043	0.717	<0.001
B*1501-Cw*0303	0.027	0.014	0.026	0.433	<0.001
B*1539-Cw*0303	0.019	0.011	0.019	0.317	<0.001
B*3501-Cw*0401	0.058	0.011	0.047	0.783	<0.001
B*4402-Cw*0501	0.043	0.016	0.038	0.633	<0.001
B*5001-Cw*0602	0.043	0.013	0.039	0.65	<0.001
B*5101-Cw*1502	0.047	0.013	0.041	0.683	<0.001
DRB1*0101-DQB1*0501	0.054	0.013	0.046	0.39	<0.001
DRB1*0102-DQB1*0501	0.031	0.015	0.027	0.229	<0.001
DRB1*0301-DQB1*0201	0.035	0.013	0.03	0.254	<0.001
DRB1*0301-DQB1*0203	0.016	0.006	0.013	0.11	<0.001
DRB1*0304-DQB1*0201	0.016	0.006	0.015	0.127	<0.001
DRB1*0401-DQB1*0302	0.012	0.005	0.009	0.076	<0.05
DRB1*0402-DQB1*0302	0.031	0.012	0.028	0.237	<0.001
DRB1*0404-DQB1*0302	0.023	0.011	0.021	0.178	<0.001
DRB1*0405-DQB1*0302	0.016	0.007	0.014	0.119	<0.001
DRB1*0701-DQB1*0202	0.15	0.036	0.118	1	<0.001
DRB1*0701-DQB1*0303	0.028	0.009	0.019	0.161	<0.001
DRB1*0801-DQB1*0402	0.043	0.007	0.041	0.347	<0.001

Table 4 **continued overleaf**

Continued

Haplotypes	Frequency	SD	D	D′	*P*
DRB1*090102-DQB1*0202	0.02	0.008	0.016	0.136	<0.001
DRB1*090102-DQB1*0303	0.011	0.006	0.009	0.076	<0.001
DRB1*1101-DQB1*0301	0.05	0.01	0.041	0.347	<0.001
DRB1*1102-DQB1*0301	0.027	0.01	0.023	0.195	<0.001
DRB1*1103-DQB1*0301	0.016	0.011	0.013	0.11	<0.001
DRB1*1104-DQB1*0301	0.027	0.01	0.021	0.178	<0.001
DRB1*1201-DQB1*0301	0.019	0.01	0.015	0.127	<0.001
DRB1*1301-DQB1*0603	0.089	0.015	0.081	0.686	<0.001
DRB1*1401-DQB1*0503	0.012	0.004	0.012	0.102	<0.001
DRB1*0101-DQA1*0101	0.054	0.016	0.046	0.331	<0.001
DRB1*0301-DQA1*0501	0.054	0.018	0.044	0.317	<0.001
DRB1*0304-DQA1*0501	0.027	0.012	0.025	0.18	<0.001
DRB1*0401-DQA1*0301	0.012	0.008	0.009	0.065	<0.05
DRB1*0402-DQA1*0301	0.031	0.01	0.029	0.209	<0.001
DRB1*0403-DQA1*0301	0.016	0.008	0.014	0.101	<0.001
DRB1*0404-DQA1*0301	0.019	0.012	0.017	0.122	<0.001
DRB1*0405-DQA1*0303	0.016	0.006	0.015	0.108	<0.001
DRB1*0701-DQA1*0201	0.171	0.023	0.139	1	<0.001
DRB1*0801-DQA1*0401	0.043	0.015	0.041	0.295	<0.001
DRB1*0901-DQA1*0302	0.016	0.006	0.015	0.108	<0.001
DRB1*0901-DQA1*0303	0.023	0.011	0.022	0.158	<0.001
DRB1*1101-DQA1*0505	0.047	0.011	0.040	0.288	<0.001
DRB1*1102-DQA1*0505	0.027	0.015	0.024	0.173	<0.001
DRB1*1103-DQA1*0505	0.012	0.005	0.01	0.072	<0.001
DRB1*1104-DQA1*0505	0.016	0.012	0.011	0.079	<0.05
DRB1*1201-DQA1*0505	0.016	0.007	0.012	0.086	<0.001
DRB1*1301-DQA1*0103	0.089	0.015	0.082	0.59	<0.001
DRB1*1302-DQA1*0102	0.023	0.009	0.021	0.151	<0.001
DRB1*1401-DQA1*0104	0.012	0.008	0.012	0.086	<0.001
DRB1*1501-DQA1*0102	0.05	0.015	0.042	0.302	<0.001
DRB1*1601-DQA1*0102	0.012	0.01	0.01	0.072	<0.001
DQA1*0501-DQB1*0201	0.058	0.014	0.051	0.481	<0.001
DQA1*0201-DQB1*0202	0.136	0.026	0.106	1	<0.001
DQA1*0303-DQB1*0202	0.023	0.008	0.013	0.123	<0.05
DQA1*0501-DQB1*0203	0.035	0.011	0.03	0.283	<0.001
DQA1*0505-DQB1*0301	0.132	0.017	0.106	1	<0.001
DQA1*0301-DQB1*0302	0.074	0.016	0.065	0.613	<0.001
DQA1*0303-DQB1*0302	0.019	0.009	0.014	0.132	<0.05
DQA1*0201-DQB1*0303	0.031	0.011	0.022	0.208	<0.001
DQA1*0301-DQB1*0304	0.012	0.007	0.011	0.104	<0.001
DQA1*0401-DQB1*0402	0.047	0.016	0.044	0.415	<0.001
DQA1*0101-DQB1*0501	0.097	0.025	0.084	0.792	<0.001
DQA1*0102-DQB1*0502	0.016	0.01	0.013	0.123	<0.001
DQA1*0104-DQB1*0503	0.016	0.004	0.015	0.142	<0.001

DQA1*0102-DQB1*0602	0.047	0.01	0.041	0.387	<0.001
DQA1*0103-DQB1*0603	0.093	0.022	0.083	0.783	<0.001
DQA1*0102-DQB1*0604	0.012	0.006	0.01	0.094	<0.001
DQA1*0102-DQB1*0609	0.012	0.006	0.01	0.094	<0.001

Table 4

(2–6%, roughly the same as in Portugal), the Iberian/Paleo North African A*3002-B*1801-DRB1*0301 (2.6%, but 1.7% in Portugal), the North-Western European A*0201-B*4402-DRB1*0701 (2.6%) and the North African/Western European A*0301-B*0702-DRB1*1501 (1–3%) (Table 3). Other haplotypes already found in mainland Portugal in residual frequencies appear only in the Oriental group (A*0201-B*5101-DRB1*0701: 2.6% and A*2402-B0702-DRB1*1501: 3.8%). Three haplotypes are an exception to this situation: the North-Western European A*0201-B*4403-DRB1*0701 haplotype (Azores Central: 1.8% and Portugal mainland: 3.1%), the Mediterranean haplotype A*3301-B*1402-DRB1*0102 (Azores Central: 1.8%; Azores Oriental: 3.8% and Portugal mainland: 2.1%) and the haplotype A*0201-B*4402-DRB1*1301 exclusive from the Central islands and found before in the South of Portugal at a similar frequency (1%).

The haplotypes A*0101-B*1402-DRB1*0102 (Mediterranean) and A*0201-B*0702-DRB1*0101 (0.3% in Northern Ireland) were present exclusively on the Central group of islands at a frequency of 1.8% each. The Central European haplotype A*0201-B*1302-DRB1*0701 is present in Azores (1.8%) but is absent in mainland Portugal. The two most frequent haplotypes in Central Azores (A*0301-B*4403-DRB1*0701, 2.8% and A*0201-B*4402-DRB1*0401, 2.7% each) were found in Northern Ireland at 0.5 and 3.8%, respectively.

The most frequent haplotypes found in mainland Portugal have similar frequencies (A*2902-B*4403-DRB1*0701 and A*2301-B*4403-DRB1*0701), lower frequencies (A*0201-B*4403-DRB1*0701; A*3301-B*1402-DRB1*0102; A*3002-B*1801-DR*0301 and A*0201-B*0702-DRB1*1501) or are absent (A*0101-B*4403-DRB1*0701; A*0201-B*1801-DRB1*0301; A*2301-B*4901-DRB1*1302; A*6801-B*4501-DRB1*0405 and A*2402-B*5101-DRB1*0701) in the Azores.

Phylogenetic analyses

A phylogenetic tree constructed with HLA-A, -B and -DRB1 allele frequencies (Fig. 1A) clusters Azores to Asian and Native American populations. The same analysis without Asian and Ameridian populations groups Azores to other Europeans rather than Portugal and North Africans. When considering the two Azorean subpopulations (Oriental and Central), analysed only with European and African populations, the former emerges more similar to Portugal and North Africans, but the later clusters to other Europeans (Fig. 1B). A principal coordinate analysis based on HLA-A, -B and -DRB1 allele frequencies shows the Azorean in close proximity to Europeans (Fig. 2).

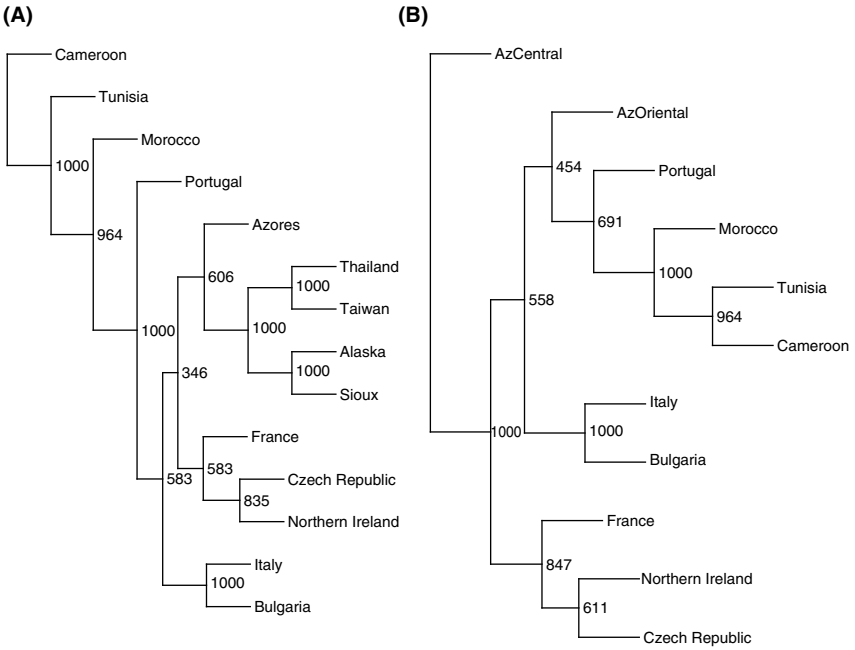

Fig. 1. **Neighbor-joining (NJ) dendrogram showing the comparative position of the Azores with other populations typed with similar resolution.** Numbers above branches are node support after the bootstrap technique implemented in PHYLIP package program BOOT. For references of the populations used, see *Data analysis*. (A) Dendrogram constructed with standard genetic distances calculated using human leucocyte antigen (HLA)-A, -B and -DRB1 allele frequencies. (B) Dendrogram constructed with standard genetic distances calculated using HLA-A, -B and -DRB1 allele frequencies, considering the two subpopulations Azores Oriental (AzOriental) and Azores Central (AzCentral), analysed only with European and African populations.

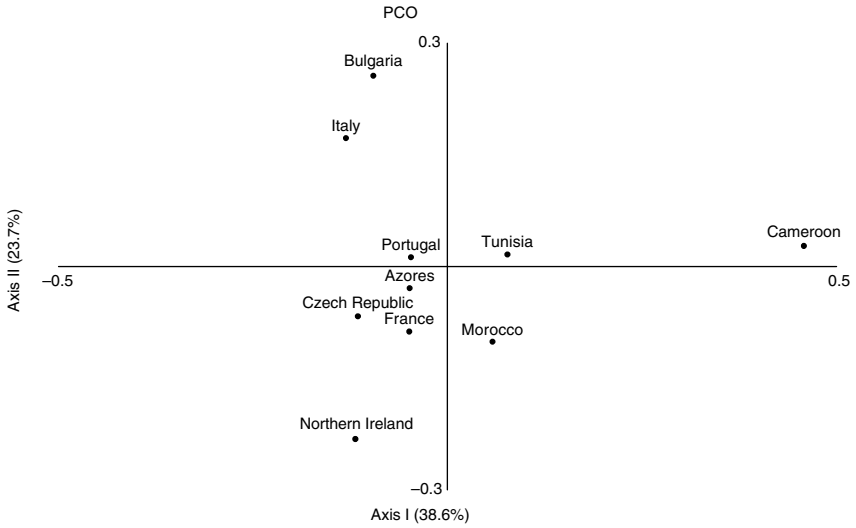

Fig. 2. **Principal coordinates analysis using human leucocyte antigen (HLA)-A, -B, and -DRB1 allele frequencies.** For references of the populations used see *Data analysis*.

Discussion

During its settlement after 1432, the Azores experienced the influence of various peoples, especially Portuguese, Dutch (Flemish), Spaniards, Africans, Jewish and Italians (4, 35, 36). The important role played by Azores in sea traffic between America and Europe during the 15th and 16th century was one of the principal reasons for a settlement involving people from several origins.

The present study describes HLA-A, -B, -Cw, -DRB1, -DQA1 and -DQB1 high-resolution allele frequencies and estimated haplotypes in Azores. This high-resolution throughput shows that several HLA-generic groups typed before by serology (10) were in fact composed of a variety of groups and alleles (Table 1). This additional and much more complete information is highly relevant for a detailed HLA structure characterization. Unfortunately, many world populations do not have HLA allele and haplotype high-resolution characterization, making difficult the comparison with the present Azores data. However, websites such as www.allelefrequencies.net have been of great help, because it is bringing together in one source the data for HLA, and other immunogenetic loci, in worldwide populations.

A dendrogram based on HLA-A, -B and -DRB1 allele frequencies clusters Azoreans to Asian and Amerindians (Fig. 1A), a result not consistent with those obtained when class I and class II allele frequencies are used (data not shown). In fact, the data shows that the Azoreans are more similar to other Europeans than to mainland Portuguese. Otherwise, if we look at the haplotype content prevailing in Azores, we cannot find a common stratus with the known haplotypes specific to Asiatic or Ameridian populations. Thus, our results are not in agreement with the previous report of high-frequency Mongoloid haplotypes in Azores (10), and we could not find the most common haplotypes of Amerindians (24, 25). The low-resolution haplotypes previously found at high frequencies (2% each) and considered as of Mongoloid possible origin (10) were found at much lower frequencies (A*0201-B*5001-DRB1*0701: 0.9%; A*2402-B*4402-DRB1*1102: 0.2% and A*2902-B*4901-DRB1*0701: 0.2%). Two of these haplotypes were also present in Madeira Island and South Portugal (37) in higher frequencies than previously reported in Azores (10). The Azores similar to Asians shown by the dendrogram analysis reflects similar allele content rather than a real genetic influence of those populations.

The haplotype and allele frequencies found in Azores reflect the influence of Portuguese and other Europeans in the settlement of this archipelago. Several haplotypes are common to mainland Portugal (e.g., A*0101-B*0801-DRB1*0301, A*0201-B*5101-DRB1*0701 and A*0201-B*1801-DRB1*1104), reflecting the weight of the Portuguese in the Azorean settlement process. However, many haplotypes are absent from mainland Portugal (e.g., A*0301-B*0702-DRB1*1501, A*0101-B*1402-DRB1*0102, A*0201-B*0702-DRB1*0101 and A*0201-B*4402-DRB1*1301), some of those with a clear European origin. Only two of the nine most frequent (>1.2%) extended haplotypes (Table 2) found in Azores have their partial A-B-DRB1 haplotypes common to mainland Portugal. The haplotypes not common to mainland Portuguese could be brought to Azores by Flemish and other European peoples involved in settling these Atlantic islands. The same conclusions were reported on the HFE gene mutation frequency in the Central islands of the Azores archipelago. The frequencies identified on the C282Y or the H63D mutations in the group of individuals under investigation were similar to those previously reported in the groups which possibly populated the Azores (38). None of the HLA three-loci A-B-DRB1 haplotypes found in West Africa Guiné-Bissau population

(unpublished data) was found in Azores in spite of the sub-Saharan input of slaves into the Portuguese Atlantic Islands. African specific alleles (e.g., A*0202, A*0225, B*1503, B*1516, B*2703, B*4202 and B*5703) were found but mostly at low frequencies (39). Another study performed in the Central Group of islands identified also HLA-B*2703 which was first described in African populations and later in other populations with known or possible African admixture (40). This probably indicates a minor genetic influence left by the sub-Saharan slave trade which is also in agreement with recent Y-chromosome findings and mtDNA data (8, 9).

Diversity between Azores islands

HLA-A, -B and -DRB1 allele and haplotype frequencies calculated from Az1 samples show a clear heterogeneity between Azores Central and Oriental islands. A dendrogram based on allele frequencies separate these two groups and cluster Central islands to Asiatic and Amerindian populations (data not shown). This similarity of Central islands to Asians should not reflect a real genetic influence of those populations as referred above. On the contrary, Oriental islands have more affinity to mainland Portugal in this analysis. A phylogenetic tree constructed only with European and Sub-Saharan populations (Fig. 1B) clarifies the differences between these two groups of Azores islands. The Oriental group again shows its connection to Portugal and North Africans, but the Central group emerges more similar to other Europeans. These results are in agreement with the different haplotype profiles found in the two groups of islands, because only two of the 26 most frequent tree loci haplotypes are common to both the groups. The most frequent haplotypes found in one group are absent in the other (Table 3). Azores Oriental presents several high-frequency haplotypes common to mainland Portugal contrary to what happens in the Central group of islands which presents only three of those haplotypes. Several most frequent haplotypes found in Central islands have unknown origin probably because of a lack of HLA characterization on several European populations, from where they could be introduced. Further HLA high-resolution studies on Dutch, Belgium and other central European populations should clarify the origin of some of these haplotypes.

Both the dendrogram analysis and the haplotype content clearly point to a different settlement of both the groups of islands from the Archipelago. This observation corroborates historical sources that say, the Azores were populated not only by Portuguese, especially on Oriental group, but also by other Europeans, mostly Flemish people in Central Islands.

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