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ORIGINAL ARTICLE

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HLA-DQA1 in autochthonous Basques: description of a genocline for the DQA1*0201 allele in Europe

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Abstract A total of 250 autochthonous Basque individuals were tested in the HLA-DQA1 system, and the allele frequency distribution found was significantly different from that of any other European population. The differences centre mainly on the alleles DQA1*0201 and DQA1*0501: for the former the Basques have the highest frequency described anywhere in the world (f = 0.210) and for the latter they have the lowest frequency in Europe (f = 0.204). For the allele DQA1*0201 a genocline is also described in Europe with the Basques and Finno-Ugric populations basically at the extremes. The genocline reflects the isolation of the Basque population since prehistoric times, and supports existing linguistic, archaeological and genetic data.

Key words HLA-DQA1 · Basques · Population genetics · Genocline

Introduction

The typing of DNA polymorphisms for the purposes of forensic science or population genetics has increased greatly with the development of the polymerase chain reaction (PCR). Amongh the systems amenable to amplification by PCR is HLA-DQA1 (Saiki et al. 1986). At the DNA level this is the most widely studied locus in the human genome, and the one for which most population studies are available. This locus has a high polymorphic level throughout the second exon which codes for the alpha chain of the molecule HLA-DQ, where 14 alleles have been identified (Bodmer et al. 1993), 8 of which can be distinguished with the technique used in this study.

According to the terminology adopted by the World Health Organization HLA Nomenclature Committee and the forensic nomenclature (in brackets), the eight alleles typed in this paper are DQA1*0101 (DQA 1.1), DQA1*0102 (DQA 1.2), DQA1*0103 (DQA 1.3), DQA1*0201 (DQA 2), DQA1*0301 (DQA 3), DQA1*0401 (DQA 4.2), DQA1*0501 (DQA 4.1) and DQA1*0601 (DQA 4.3).

We have analysed the polymorphism of the HLA-DQA1 locus in the Basque population, one of the European populations which stirs up most interest, due to its anthropogenetic peculiarities. The main characteristics which distinguish the Basques from the surrounding populations are their language (Basque is the only European language with no known relatives) and the frequencies of certain genes. Conventional biochemical polymorphisms, for which considerable data exist, show these differentiating genetic characteristics of the Basques. These studies have given rise to various explanations concerning the origin and evolution of the Basques (Bertranpetit and Cavalli-Sforza 1991; Calafell and Bertranpetit 1994; Manzano et al. 1996). It is therefore of great interest to analyse polymorphisms at the DNA level, such as HLA-DQA1, as there is at present little data on this with regard to the Basque population (Martín et al. 1994; Alonso et al. 1995; Esparza et al. 1995).

Material and methods

Population sample

Whole blood samples were obtained from 250 healthy unrelated Basque individuals from the Basque province of Gipuzkoa, a region situated in the centre of Basque territory and which has the highest proportion of Basque speaking individuals. These individuals had eight immediate forebears with Basque surnames, and their four grandparents were natives of the Basque Country.

DNA from 5 µl frozen blood was extracted using the Chelex 100 procedure described by Walsh et al. (1991). After extraction 20 µl of the supernatant from each sample was used for amplification.

Amplification and typing of HLA-DQA 1

Amplification by PCR was carried out in a programmable heat block (Linus Micro Thermal Cycler) in reaction volumes of 20 µl. The cycling reaction was performed using the Amplitype HLA

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DQα PCR Amplification and Typing Kit (Perkin-Elmer), using the corresponding proportions to the reaction volumes according to the manufacturer.

Before typing the presence of an amplified DQA1 DNA sequence was verified by subjecting 20% of each sample to horizontal electrophoresis on 1.5% (w/v) agarose gels in 1 × TBE (1.34 M Tris, 0.75 M H_3BO_3, 25 mM EDTA) and viewing the PCR product on a 302 nm transilluminator. The amplified DNA was typed using the Amplitype HLA DQα PCR Amplification and Typing Kit (Perkin-Elmer) following the recommended protocol. Using this reverse dot-blot method we found six alleles. Subsequent digestion of DQA1 PCR products using Rsa I and Fok I allowed a distinction between alleles DQA1*0401, DQA1*0501 and DQA1*0601, usually clustered in a DQA1*04 major allele group. Rsa I digestion distinguished DQA1*0601 from DQA1*0501 and DQA1*0401; Fok I digestion cuts DQA1*0401 and DQA1*0601 alleles but not DQA1*0501 (Harrington et al. 1991). Reactions were performed in total volumes of 15 µl, using 1 unit of enzyme with each sample and digesting at 37°C for 30 min in the appropriate incubation buffer. Digestion products were separated in acrylamide-piperazindiacrylamide gels (8% T, 3% C) as described by Wiegand et al. (1993), and were silver-stained following the procedure proposed by Bassam et al. (1991).

Statistical analysis

The allele frequencies for the locus HLA-DQA1 and the distribution of the different genotypes in the Basque population sample studied were determined. Standard errors were calculated as

$$\sqrt{f(1-f)/2n}$$

where f represents the frequency of each allele and n the number of individuals sampled.

The Hardy-Weinberg condition (HWE) was tested by a likelihood ratio test (G statistic) (Sokal and Rohlf 1969).

$$G = 2 \sum_{i=1}^{a} f_i \ln (f_i/f_i)$$

where f_i is the observed frequency of the i genotype and f_i the expected frequency in an "a" genotype system. To evaluate the empirical significance level 1000 random samples were generated as described in Deka et al. (1991).

An unbiased estimate of heterozygosity (allelic diversity) was calculated from the equation $h = 2n\ (1 - \Sigma p_i^2)/2n - 1$, where p = observed frequency of each allele and n = number of individuals (Nei and Roychoudhury 1974). Standard error was calculated as $\sqrt{h\ (1-h)/n}$ (Edwards et al. 1992). The power of discrimination was calculated from genotype data using the formula $PD = 1 - \Sigma P_j^2$, where P_j = observed frequency of each genotype (Fisher 1951). The chance of exclusion (CE) was calculated as described in Smouse and Chakraborty (1986). Heterogeneity in DQA1 allele distributions was estimated for all pairwise comparisons of the population groups by the χ^2 heterogeneity test. Degrees of freedom were estimated as (m–1)(n–1), where m is the number of populations and n the number of alleles with an absolute frequency greater than 5. Regression analyses were performed using SPSS v6.1 software.

Results and discussion

In the Basque population analysed 27 of the 36 possible genotypes were observed (Table 1). The remaining genotypes are classed as "others" in the calculation of frequencies. The allelic frequencies (Table 2) were highest for the three alleles DQA1*0102 (f = 0.210), DQA1*0201 (f = 0.210) and DQA1*0501 (f = 0.204). At the other extreme the rarest allele was DQA1*0601 (f = 0.002), which was found to be present in only one of the 250 individuals in the sample.

Table 1 Observed and expected distribution and frequencies of HLA-DQA1 genotypes in Basques (n = 250 individuals)

Genotype	Obs.	Exp.
0101,0101	9	6.40
0101,0102	19	16.80
0101,0103	4	6.72
0101,0201	13	16.80
0101,0301	8	8.80
0101,0401	1	1.60
0101,0501	17	16.32
0102,0102	6	11.03
0102,0103	9	8.82
0102,0201	25	22.05
0102,0301	18	11.55
0102,0401	3	2.10
0102,0501	19	21.42
0103,0103	2	1.76
0103,0201	10	8.82
0103,0301	3	4.62
0103,0401	2	0.84
0103,0501	10	8.57
0201,0201	13	11.03
0201,0301	8	11.55
0201,0401	3	2.10
0201,0501	19	21.42
0201,0601	1	0.21
0301,0301	4	3.03
0301,0501	10	11.22
0401,0501	1	2.04
0501,0501	13	10.40
Others	0	1.99
Total	250	250

Table 2 Observed distribution and frequencies ± standard errors of HLA-DQA1 alleles in Basques

Allele	Total	Frequency
*0101	80	0.160 ± 0.016
*0102	105	0.210 ± 0.018
*0103	42	0.084 ± 0.012
*0201	105	0.210 ± 0.018
*0301	55	0.110 ± 0.014
*0401	10	0.020 ± 0.006
*0501	102	0.204 ± 0.018
*0601	1	0.002 ± 0.002
n	500	

HWE was checked by the G statistic and no significant variation from the observed and expected values was found (G = 20.448; p = 0.999). Other statistics of genetic and legal/medical interest were also calculated, such as the chance of exclusion (CE = 0.6483), the power of discrimination (PD = 0.9436) and the allelic diversity value (h = 0.8267). With regard to this last statistic, the heterozygosity observed in the sample studied was 0.812.

The allele frequencies of the Basque population analysed in this study were compared with representative samples of European and North African populations using the χ^2 heterogeneity test (Table 3). In cases where several samples of the same population were published, we con-

Table 3 Comparison of the Basque population studied here with various European and North African populations, using the χ^2 heterogeneity test. (1) populations are compared with no grouping of alleles unless statistically necessary. (2) comparisons are made with alleles in three groups: DQA1*0201, DQA1*0501 and the rest (d.f. = 2). SP = Spain, PO = Portugal, GE = Germany. (*) $0.05 < p < 0.01$; (**) $p < 0.01$. n = number of chromosomes.

Populations	n	$\chi2(1)$	d.f.	p value (1)	$\chi2(2)$	p value (2)	Reference
Basque Country (SP)	292	3.70	5	0.5940	0.73	0.6952	Esparza et al. (1995)
Basque Country (SP)	422	5.55	6	0.4750	0.68	0.7123	Martín et al. (1994)
Madrid (SP)	412	14.39	6	0.0256*	11.41	0.0033**	Martín et al. (1994)
Barcelona (SP)	390	15.80	5	0.0075**	16.38	0.0003**	Crespillo et al. (1996)
Galicia (SP)	356	25.30	5	< 0.0001**	27.98	< 0.0001**	Lareu et al. (1993)
Andalusia (SP)	348	23.49	5	0.0003**	16.25	0.0003**	Sanz et al. (1994)
Valencia (SP)	214	25.93	5	< 0.0001**	6.42	0.0404*	Aler et al. (1996)
Portugal	212	16.68	5	0.0052**	8.92	0.0116*	Espinheira et al. (1994)
South Portugal	468	15.39	5	0.0088**	11.17	0.0038**	Espinheira et al. (1994)
Oporto (PO)	650	12.64	5	0.0270*	4.18	0.1239	Pinheiro & Pontes (1994)
France	220	1.86	5	0.8680	1.31	0.5190	Pascal et al. (1994)
Italy	2972	57.45	5	< 0.0001**	64.03	< 0.0001**	Presciuttini & De Stefano (1994)
Western Algeria	94	18.94	5	0.0020**	14.88	0.0006**	Djoulah et al. (1994)
Algeria	212	16.20	5	0.0063**	9.11	0.0105*	Arnaiz-Villena et al. (1995)
England	402	25.93	5	< 0.0001**	9.78	0.0075**	Sullivan et al. (1992)
Great Britain	354	34.17	6	< 0.0001**	8.47	0.0145*	Doherty et al. (1992)
Belgium	436	14.27	6	0.0268*	13.19	0.0014**	Decorte et al. (1994)
Netherlands	314	15.30	5	0.0092**	14.95	0.0006**	Kloosterman et al. (1993)
Switzerland	454	13.90	5	0.0163*	17.94	< 0.0001**	Kratzer et al. (1994)
Germany	604	16.61	5	0.0053**	20.66	< 0.0001**	Reinhold & Arnold (1994)
Munich (GE)	426	27.69	5	< 0.0001**	23.97	< 0.0001**	Weichhold et al. (1994)
Denmark	292	18.29	5	0.0026**	12.05	0.0024**	Cowland et al. (1995)
Norway	362	24.91	6	0.0004**	10.87	0.0044**	Rønningen et al. (1990)
Sweden	522	62.33	5	< 0.0001**	33.78	< 0.0001**	Allen et al. (1993)
Hungary	726	42.29	5	< 0.0001**	40.52	< 0.0001**	Woller et al. (1996)
Finland	224	33.64	5	< 0.0001**	31.66	< 0.0001**	Sajantila et al. (1991)
Russia	78	32.68	5	< 0.0001**	14.93	0.0006**	Kurth et al. (1992)

sidered the largest one. The results show that there are statistically significant differences between the Basque population and practically all the European populations. However no significant differences were found with other Basque population samples (Martín et al. 1994; Esparza et al. 1995). Significant differences were also found when our Basque population was compared with Berbers (χ^2 = 16.20; d.f. = 5; p = 0.0063), a group which some authors have linked genetically with the Basques on the basis of analyses of various loci in the HLA system (Arnaiz-Villena et al. 1995). A principal component analysis performed with six alleles from the HLA-DQA1 locus (not shown) displayed a location of the Berbers distant from the Basques.

The differences found when comparing the Basques with other European populations can be attributed to the alleles DQA1*0201 and DQA1*0501. This can be seen in Table 3, where these comparisons are set out using the same statistical method, but grouping all alleles except DQA1*0201 and DQA1*0501. In populations where no distinction was made between alleles DQA1*0401, DQA1*0501 and DQA1*0601, comparisons were established by grouping these three alleles. Practically all the comparisons show significant differences. When we analysed the frequencies of these two alleles we found that the Basque population had the highest value for allele DQA1*0201 (f = 0.210) in the range of variation described in Europe i.e. 0.208 (Pascal et al. 1994) – 0.058 (Sajantila et al. 1991), and the lowest for DQA1*0501 (f = 0.204), with a range of variation in Europe from 0.215 (Rønningen et al. 1990) to 0.381 (Presciuttini and De Stefano 1994). Furthermore, going beyond the European scope of this article, a comparison with more than 100 population samples published to date shows that the frequency of allele DQA1*0201 in the Basques is the highest described anywhere in the world. The range of frequencies is from 0.208 (Pascal et al. 1994) to 0.000 (Gao et al. 1992).

In order to test for a possible spatial structuring of frequencies for alleles DQA1*0201 and DQA1*0501, a representation of the geographical variation of allele frequencies was drawn up for the Basques and representative samples of European and North African populations. For this analysis three samples of Basques were combined, as there were no statistically significant differences between them. While for the frequency of allele DQA*0501 no spatial structuring was found, that of allele DQA1*0201 has a distribution pattern related to geographical distance, fitting a regression line when the distance in kilometres is taken from the Basque Country (Fig. 1). However no sat-

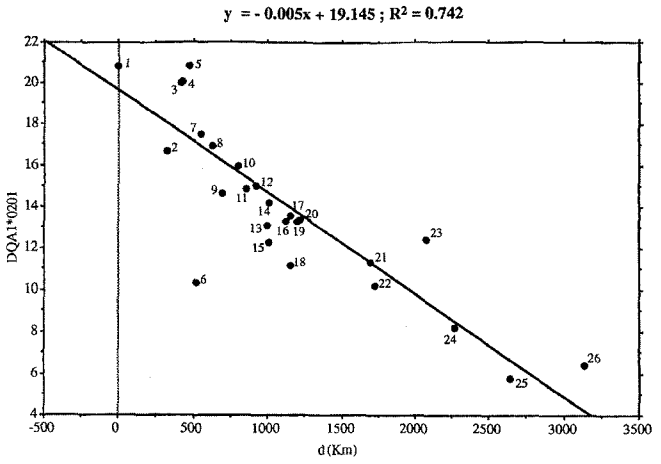

Fig. 1 Regression analysis between DQA1*0201 allele frequency and distance from the Basque Country to different European populations (see references in Table 3). 1 = Basque Country (pooled); 2 = Madrid (SP); 3 = Barcelona (SP); 4 = Valencia (SP); 5 = France; 6 = Galicia (SP); 7 = Oporto (PO); 8 = Portugal; 9 = Andalusia (SP); 10 = South Portugal; 11 = West Algeria; 12 = Switzerland; 13 = Belgium; 14 = England; 15 = Algeria; 16 = Great Britain; 17 = Germany; 18 = Netherlands; 19 = Italy; 20 = Munich (GE); 21 = Denmark; 22 = Hungary; 23 = Norway; 24 = Sweden; 25 = Finland; 26 = Russia. Regression coefficient (r) = 0.861

isfactory fit was obtained in longitude or latitude. Figure 1 shows that the regression line obtained between the frequency of allele DQA1*0201 and the distance in kilometres from the Basque Country (y = –0.005x + 19.145) has a value of $R^2 = 0.742$. Analysis of the geographical distribution of allele DQA1*0201 indicates that the highest frequency is found among Basques, and there is a gradual decrease in all directions away from the Basque Country, at the rate of around 2% every 400 km. The opposite pole to the Basques in terms of the frequency of this allele is found in the most distance area towards the North East, corresponding to populations which are linguistically Finno-Ugric (Fig. 1).

This gradient does not seem to reflect any adaptive effect with regard to latitute or diseases. In spite of the fact that the frequency of some alleles of the HLA-DQA1 system has been associated with various diseases such as insulin-dependent diabetes mellitus (Khalil et al. 1990) no specific association was found for allele DQA1*0201. Furthermore there was not the longitudinal gradient so frequently attributable to an East West demic diffusion (Cavalli-Sforza et al. 1993). Therefore the geographical structuring of this allele may reflect other evolutionary processes, such as the genetic isolation of the Basques from the surrounding populations since prehistoric times. This effect has also been pointed out by Calafell and Bertranpetit (1994) in their principal component analysis of Europe where they indicated that "the opposite pole to the Basques in terms of genetic characteristics is always the most distant point to them, shifting according to the area considered". The two ends of the gradient detected in this work may reflect in part a differential ethnic origin, as the populations at each end are considered as European outliers in various genetic analyses (Cavalli-Sforza et al. 1993). The data presented in this study support the argument (put forward in previous studies via multivariate analysis of a high number of classical polymorphisms; Bertranpetit and Cavalli-Sforza 1991; Calafell and Bertranpetit 1994; Manzano et al. 1996) that Basques are genetically differentiated.

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