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Frequency of HLA DQA1 alleles in an Italian population

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Summary. A sample of 103 Italians was tested for HLA-DQA1 polymorphism using the polymerase chain reaction (PCR) and dot blot hybridization. Results were in Hardy-Weinberg equilibrium. The power of discrimination was 0.91 and the rate of exclusion 56.7. The frequencies of the DQA1*0201 and DQA1*0301 alleles were found to be significantly different from other Caucasian populations.

Key words: DNA polymorphism – PCR – Population genetics, HLA DQA1

Zusammenfassung. Eine Stichprobe von 103 Italienern wurde auf den HLA-DQ-α Polymorphismus untersucht: die Polymerasekettenreaktion (PCR) und die Dot-Blot-Hybridisierung fanden Anwendung. Die Resultate waren im Hardy-Weinberg Gleichgewicht. Der Diskriminationsindex des Systems beträgt 0.91 und die Ausschließungschance beträgt 0.57. Die Frequenzen der Allele DQA1*0201 und DQ A1*0301 waren signifikant unterschiedlich im Vergleich zu anderen europäischen Populationen.

Schlüsselwörter: DNA-Polymorphismus – PCR – Populationsgenetik, HLA DQA1

Introduction

The HLA class II genes DQ, DR and DP are located on the second exon of chromosome 6 encoding the NH2 terminal outer domain. These genes encode alpha and beta glycopeptides which form a protein expressed at the surface of B-lymphocytes, macrophages, thymic epithelium and activated T-cells. These proteins play an important role in the generation and regulation of the immune response.

HLA class II polymorphism was previously detected by immunological and molecular analysis. Nowadays the PCR technique (Saiki et al. 1985), combined with dot blot hybridization (Saiki et al. 1986), RFLP analysis (Maeda et al. 1989; Uryu et al. 1990; Ota et al. 1991), or the non-denaturing PAGE method (Ip et al. 1990, Barros et al. 1992) has superseded earlier methods, allowing direct investigation of the region.

Eight alleles have been reported at the HLA DQA1 locus using dot blot hybridization. According to the WHO nomenclature (Bodmer et al. 1990) the alleles are named as follows: DQA1*0101; DQA1*0102; DQA1*0103; DQA1*0201; DQA1*0301; DQA1*0401; DQA1*0501; DQA1*0601. The system shows a high Power of Discrimination (PD) ranging from 0.85 to 0.93, while the exclusion rate for paternity varies between 54.8% and 62.7% in all the populations studied (Helmuth et al. 1990, Tamaki et al. 1991). For these reasons, the system is now routinely applied in identity testing for forensic purposes. Gene frequencies are available for many different populations (Helmuth et al. 1990, Sajantila et al. 1991, Schneider et al. 1991, Tamaki et al. 1991, Comey and Budowle 1991) but such information has not yet been reported for Italians.

The aim of this study was therefore to assess the allelic frequencies of the HLA DQA1 system in an Italian population sample, determining PD value and heterozygosity rate and checking possible differences with other Caucasian populations.

Materials and methods

Extraction. DNA was extracted from fresh peripheral blood of 103 unrelated donors living in Ancona, using the Chelex method suggested by Walsh et al. (1991).

Amplification and allele analysis were carried out using the Amplitype HLA DQα Forensic DNA Amplification and Typing Kit (Cetus Corporation), which does not detect A1*0501 and A1*0601 alleles.

Amplification. Amplification was carried out as follows: 31 cycles at a denaturing temperature of 94°C for 1 min, annealing at 60°C for 30 s and extension at 72°C for 30 s followed by 1 cycle at a denaturing temperature of 94°C for 1 min, annealing at 60°C for 30 s and extension at 72°C for 420 s in an automatic DNA thermal cycler (Violet).

After each amplification, samples were tested on agarose gel containing ethidium bromide, to check the quality and quantity of amplification products.

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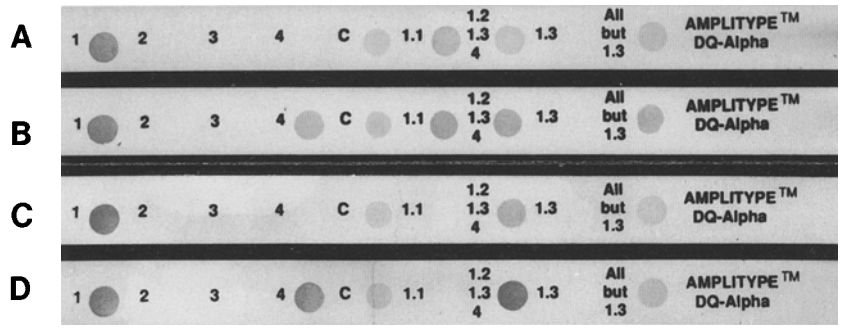

Fig. 1A–D. Examples of HLA-DQA1 genotypes: **A** = DQA1*0101/*0102; **B** = DQA1*0101/*0401; **C** = DQA1*0102/*0102; **D** = DQA1*0102/*0401

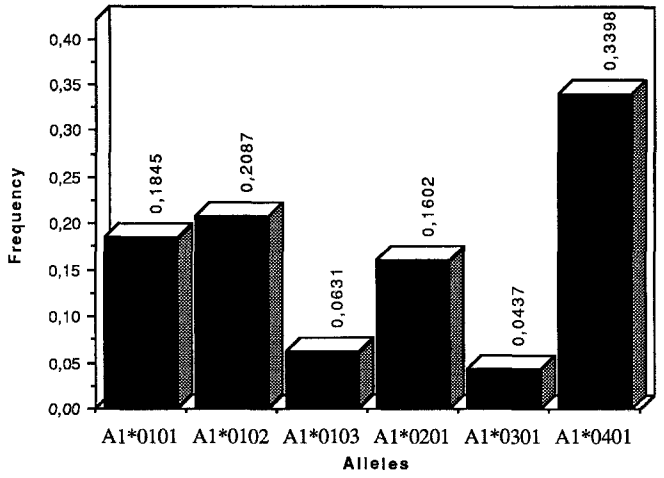

Fig. 2. Frequency distribution of HLA DQA1 alleles in a population sample of 103 unrelated Italians

Hybridization. Aliquots of 35 µl of DNA were denatured by incubation at 95°C for 7–8 min and added to the strips which had been previously soaked in a solution containing 5 × SSPE (3.6 *M* NaCl, 200 m*M* $NaH_2PO_4 \cdot H_2O$, 20 m*M* EDTA, pH 7.4), 0.5% w/v SDS and enzyme conjugate. After incubation at 55°C in a shaking water bath for 20 min at 70 rpm, the strips were rinsed three times in wash solution (2.5 × SSPE, 0.1% w/v SDS).

Color development. Color was developed in a solution containing 0.1 *M* sodium citrate (pH 5), 3% hydrogen peroxide and chromogen solution (3,3′, 5,5′-tetramethylbenzidine in 100% ethanol) at room temperature by orbital shaking at 50 rpm for 25 min.

Color development was stopped by washing the strips in deionized water. The strips were then air-dried, photographed and stored (Fig. 1). The results were fed into a spreadsheet (Excel) in which each cell was provided with a suitable algorithm to immediately update the entire data base (observed, expected, percentage values and gene frequencies) and the statistical evaluations (PD, allelic diversity, heterozygosity, exclusion rate).

Results

Figure 2 shows the allele frequency distribution. Allele DQA1*0401 was the most common (0.3398) and DQA1 *0103 and DQA1*0301 were the rarest (0.0631 and 0.0437 respectively). We found 19 genotypes in our study (Table 1).

The most frequent genotype was found to be DQA1* 0101/DQA1*0401 (17.47%), followed by DQA1*0102/ DQA1*0401 (14.56%). Genotypes DQA1*0103/DQA1* 0103 and DQA1*0301/DQA1*0301 were not observed.

Table 1. Observed and expected HLA DQA1 genotypes in 103 Italians

Genotypes	Observed		Expected	
	n	%	*n*	%
*0101/*0101	3	2.91	3.50	3.40
*0101/*0102	6	5.82	7.93	7.70
*0101/*0103	2	1.94	2.40	2.33
*0101/*0201	4	3.89	6.09	5.91
*0101/*0301	2	1.94	1.66	1.61
*0101/*0401	18	17.48	12.91	12.54
*0102/*0102	4	3.89	4.49	4.36
*0102/*0103	3	2.91	2.71	2.63
*0102/*0201	10	9.71	6.89	6.69
*0102/*0301	1	0.97	1.88	1.82
*0102/*0401	15	14.56	14.61	14.19
*0103/*0103	0	0.00	0.41	0.40
*0103/*0201	4	3.89	2.08	2.02
*0103/*0301	1	0.97	0.57	0.55
*0103/*0401	3	2.91	4.42	4.29
*0201/*0201	1	0.97	2.64	2.57
*0201/*0301	2	1.94	1.44	1.40
*0201/*0401	11	10.68	11.21	10.89
*0301/*0301	0	0.00	0.20	0.19
*0301/*0401	3	2.91	3.06	2.97
*0401/*0401	10	9.71	11.89	11.55
Total	103	100.00	103	100.00

Chi square: 8.92; $0.50 < P < 0.70$, $df = 11$ (Classes with less than 2 observations were pooled)

The chi square test, used to calculate the correlation between expected and observed genotypes, showed that there was no significant deviation from Hardy-Weinberg equilibrium ($0.50 < P < 0.70$). Allelic diversity (h) was found to be 0.78. Observed heterozygosity was 82.52%. The PD value for identity testing was calculated to be 0.91, while the power of exclusion for paternity determination was 56.7%. A statistical test was performed to compare our data with those reported for German, Finnish and two American-Caucasian populations (Table 2).

Discussion

Helmuth et al. (1990) found important differences in allele frequencies between Caucasian and non-Caucasian

Table 2. Determination of population sample homogeneity (2-way R × C contingency table)

Polulations	χ^2	20 *df*
Italians/Am-Caucasians (Helmuth et al. 1990)	42.92	$0.01 < P < 0.001$
Italians/Am-Caucasians (Comey and Budowle 1991)	25.43	$0.20 < P < 0.10$
Italians/Finns (Sajantila et al. 1991)	31.57	$0.05 < P < 0.02$
Italians/Germans (Schneider et al. 1991)	21.93	$0.50 < P < 0.30$

	A	B	C	D	E	F	G	H	I
1	Genotypes	Observed	%	Expected	%	Gen. Freq.		Genotypes	χ 2
2	0101-0101	3	2,91	3,50	3,40	*A1*0101*		0101-0101	0,0727
3	0101-0102	6	5,83	7,93	7,70	0,184466		0101-0102	0,4706
4	0101-0103	2	1,94	2,40	2,33			0101-0103	0,0661
5	0101-0201	4	3,88	6,09	5,91	*A1*0102*		0101-0201	0,7158
6	0101-0301	2	1,94	1,66	1,61	0,208738		0101-0301	0,0696
7	0101-0401	18	17,48	12,91	12,54			0101-0401	2,0044
8	0102-0102	4	3,88	4,49	4,36	*A1*0103*		0102-0102	0,053
9	0102-0103	3	2,91	2,71	2,63	0,063107		0102-0103	0,0302
10	0102-0201	10	9,71	6,89	6,69			0102-0201	1,4056
11	0102-0301	1	0,97	1,88	1,82	*A1*0201*		0102-0301	0,4109
12	0102-0401	15	14,56	14,61	14,19	0,160194		0102-0401	0,0103
13	0103-0103	0	0,00	0,41	0,40			0103-0103	0,4102
14	0103-0201	4	3,88	2,08	2,02	*A1*0301*		0103-0201	1,7655
15	0103-0301	1	0,97	0,57	0,55	0,043689		0103-0301	0,3286
16	0103-0401	3	2,91	4,42	4,29			0103-0401	0,4548
17	0201-0201	1	0,97	2,64	2,57	*A1*0401*		0201-0201	1,0215
18	0201-0301	2	1,94	1,44	1,40	0,339806		0201-0301	0,2162
19	0201-0401	11	10,68	11,21	10,89			0201-0401	0,0041
20	0301-0301	0	0,00	0,20	0,19	*Total*		0301-0301	0,1966
21	0301-0401	3	2,91	3,06	2,97	1		0301-0401	0,0011
22	0401-0401	10	9,71	11,89	11,55			0401-0401	0,3014
23									
24	Total	103	100	103	100			Σχ2	10,009

Fig. 3. Computer screen reproduction. Entering a value in each cell of column B automatically updates all values in other cells. Implemented functions are exemplified as follows: B24 = SUM(B2:B22); D2 = PRODUCT(F3; F3; B24); D3 = PRODUCT(2; F3; F6; B24); E2 = PRODUCT(D2; 100)/B24; F3 = SUM(B2 + B2 + B3 + B4 + B5 + B6 + B7)/SUM(B24 + B24); I2 = PRODUCT(B2 − D2)* (B2 − D2)/D2; I24 = SUM(I2:I22)

populations; the works of Sajantila et al. (1991), Schneider et al. (1991) and Comey and Budowle (1991) confirmed these earlier observations.

The distribution of HLA-DQA1 alleles in the Italian sample studied is not only different from that determined for non-Caucasians, as expected, but also shows some significant variations from the frequencies reported for Caucasians. Although the frequencies of alleles A1*0101, A1*0102, A1*0103, A1*0401 are similar to other Caucasian frequencies, those of alleles HLA-DQA1*0201 and HLA-DQA1*0301 are strikingly different. In Italians allele A1*0301 is the rarest, while it is the second most frequent in the sample reported by Helmuth et al. (1990) and the third in the Finnish population studied by Sajantila et al. (1991) and in the American Caucasians studied by Comey and Budowle (1991). Analysis of European samples [Sajantila et al. (1991); Schneider et al. (1991)] would seem to indicate a decreasing gradient of distribution from North to South for allele A1*0301, whereas the behaviour of allele A1*0201 is diametrically opposite. The statistical chi square test used to compare the genotypes of the samples investigated so far confirmed that homogeneity increases with the geographic proximity of the European ethnic groups: $0.05 < P < 0.02$ for Finns and $0.50 < P < 0.30$ for Germans. The 2 American Caucasian populations show a different trend so that the sample studied by Comey and Budowle ($0.20 < P < 0.10$) is more similar to our sample than that of Helmuth et al. ($0.01 < P < 0.001$). This probably reflects the different ethnic composition of American Caucasians.

The data base used (Fig. 3) is simple, useful and flexible, because it only requires the input of new observations for immediate updating of all genetic, comparative and statistical values.

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