HLA-DQA1 SUBTYPING BY PCR FOLLOWED BY A COMBINED SSO/RFLP METHOD OF DETECTION. DISTRIBUTION OF ALLELES AND GENOTYPES IN TWO SPANISH POPULATIONS.

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INTRODUCTION.

The HLA-DQA1 locus has become a well defined genetic marker of routine use in forensic haemogenetics. Forensic HLA-DQA1 genotyping has been greatly facilitated by the development of a PCR based Kit (Amplitype HLA-DQalpha, Perkin-Elmer, Cetus Corporation). However this method allows to distinguish only 6 of the 8 alleles identified in the HLA-DQA1 locus.
In this article we present a method for the genetic characterization of the HLA-DQA1 locus by employing PCR coupled with hybridization to sequence-specific oligonucleotide probes, for the detection of the 6 most common alleles, followed by an allele-specific restriction fragment length polymorphism analysis (endonucleases Fok I and Rsa I) for HLA-DQA1*4 subtyping [1].
The method has been applied to determine the HLA-DQA1 allele and genotype frequencies in two spanish populations.

MATERIALS AND METHODS.

Blood Samples and DNA extraction

DNA was extracted from blood of 206 unrelated individuals living in Madrid and 211 unrelated autochthonous individuals from the Basque Country using the chelex resin extraction approach [2].

Amplification and "Dot-Blot" Typing

The amplification of the HLA-DQA1 locus by PCR using biotinylated primers and the analysis of the alleles by hybridization to filter strips carrying immovilized allele specific oligonucleotide DNA probes were performed using the Amplitype HLA-DQalpha Forensic DNA Amplification and Typing Kit (Perkin-Elmer Cetus).

Endonuclease digestion and electrophoresis

All samples carrying the allele 4 were digested using two restriction enzymes: Rsa I and Fok I [1]. The digested fragments were separated by vertical discontinuous polyacrylamide (11%T, 3%C) gel electrophoresis using the Tris-chloride/Tris-glycine buffer system [3]. After electrophoresis the digested DNA fragments were detected by silver stain [4]. The specific electrophoretic patterns of the different HLA-DQA1 alleles after Rsa I and Fok I digestions are shown in table 1 (according to reference 1).

	ALLELES							
	*0101	*0102	*0103	*0201	*0301	*0401	*0501	*0601
Rsa I	201, 39	201, 39	230	230	201	198	198	230
Fok I	245	245	245	196, 49	245	196	242	196

Table 1. Electrophoretic patterns (in base pairs) of HLA-DQA1 alleles after Rsa I and Fok I digestion . (Adopted from reference 1).

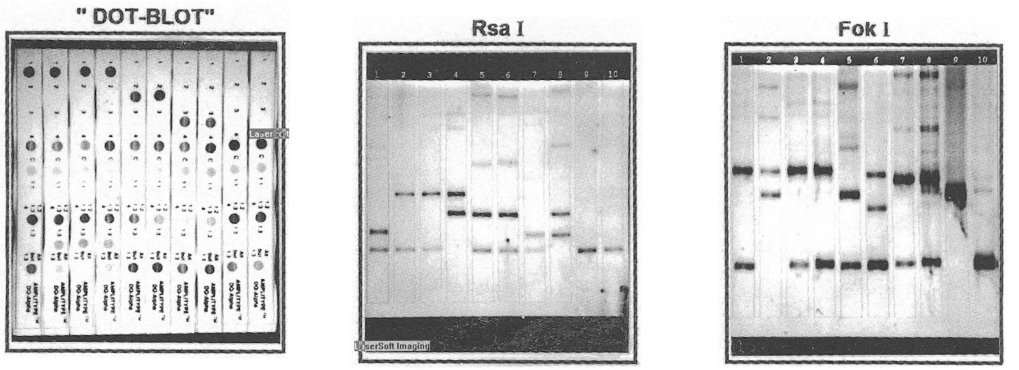

Fig. 1. HLA-DQA1 phenotypes analyzed by PCR and a combined SSO/RFLP method of detection samples: 1. HLA-DQA1*0102/*0401, 2. *0103/*0501, 3. *0103/*0401, 4. *0103/*0601, 5. *0201/*0501, 6. *0201/*0401, 7. *0301/*0501, 8.*0301/*0601, 9. *0501/*0501 and 10. *0401/*0401.

	ALLELES							
	*0101	*0102	*0103	*0201	*0301	*0401	*0501	*0601
Madrid	0.160	0.153	0.087	0.167	0.109	0.022	0.298	0.002
B. C.	0.187	0.173	0.102	0.216	0.104	0.033	0.182	0.002

	PD	h	Heterozy.	X^2 (df)	p
Madrid	0.936	0.816	81.07%	25.69 (18)	0.10<p<0.20
B. C.	0.949	0.835	79.15%	23.27 (20)	0.20<p<0.30

Table 2. HLA- DQA1 allele frequencies from 206 individuals resident in Madrid and from 211 autochthonous individuals from the Basque Country (B. C.). The power of discrimination (PD), allelic diversity value (h), heterozygosity and chi-square (X^2) are also shown.

RESULTS AND DISCUSSION.

Figure 1 illustrates 10 HLA-DQA1 different phenotypes (all of them carrying the HLA-DQA1*4 allele) as analyzed by PCR followed by a combined SSO/RFLP method of detection. As can be seen, the analysis of HLA-DQA1 homoduplexes after Rsa I and Fok I digestion by discontinuous polyacrylamide gel electrophoresis under non-denaturing conditions allowed, according to the polymorphic patterns described by Ju et al. (1991) (Table 1), a clear distinction of the HLA-DQA*0401, *0501 and *0601 alleles. It is necesary to point out, for a correct interpretation of the electrophoretic patterns, that the electrophoretic mobility of the Rsa I and Fok I digests is determined not only by size but also by conformational features. For instance, the 230 bp fragment obtained after Rsa I digestion of the HLA-DQA1*0103 allele showed lower mobility than the 230 bp fragments of the HLA-DQA1*0601 and HLA-DQA1*0201 alleles (Fig. 1, compare lanes 3, 4 and 5). In the same way, the 245 bp fragment obtained after Fok I digestion of the HLA-DQA1*0102 and *0103 alleles showed lower mobility than the 245 bp fragment of the HLA-DQA1*0301 (Fig. 1, compare lanes 1, 3 and 8).

The allele frequencies for HLA-DQA1 in a population sample of 206 unrelated individuals living in Madrid and in a population sample of 211 unrelated individuals autochthonous from the Basque Country are shown in table 2. No evidence of deviations from the Hardy-Weinberg equilibrium was observed in this two populations when the observed and expected genotypes numbers were compared by means of the Chi-squared test (Table 2). The allele frequencies of these two spanish population samples were compared by a 2-way RxC contingency table to determine whether significant differences could be observed. The data showed significant differences between the two spanish populations analyzed (Chi-square=17.11; P=0.017; df=7). The major differences in allele distributions were obsreved for the HLA-DQA1*0501 allele (Madrid: 29.8%, Basque Country: 18.2%) and the HLA-DQA1* 0201 allele (Madrid: 16.7%, Basque Country: 21.6%).

On the other hand, the comparison of the HLA-DQA1 allelic data presented here with those reported in other Caucasians populations [5-10] showed that the Basque Country (autochthonous) data were significantly different from the rest of the Caucasian populations, while the Madrid (resident) data only show significant differences when compared to the Finland, British and USA data.

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REFERENCES

[1].Ju LY, Gu XF, Larger E, Krishnamoorthy R, Charrom D (1991). Electrophoresis 12:270-273
[2].Singer-Sam J, Tanguay RL, Riggs AD (1989). Amplifications 3:11
[3].Alonso A, Martín P, Albarrán C, Sancho M (1993). Int J Leg Med 105:311-314
[4].Blum H, Beier H, Gross HJ (1987). Electrophoresis 8:93-99
[5].De Stefano F, Casarino L, Mannucci A, Delfino L, Canale M, Ferrara GB (1992). Forensic Sci. Int. 55:59-66
[6].Kloosterman AD, Daselaar P, Budowle B, Riley EL (1992). The third Int. Symp. on Human Identification.
[7].Sajantila A, Ström M, Budowle B, Tienari PJ, Ehnholm C, Peltonen L (1991). Int J Leg Med 104:181-184
[8].Sullivan KM, Gill P, Lingard D, Lygo JE (1992). Int J Leg Med 105:17-20
[9].Schneider PM, Veit A, Rittner C (1991). DNA-Tecnology and its forensic aplication. Spriger-Verlag.
[10].Helmuth R, Fildes N, Blake E, Luce MC, Chimera J, Madej R, Gorodezky C, Stoneking M, Schmill N, Klitz W, Higuchi R, Erlich HA (1990). Am J Hum Genet 47:515-523.